ACUPUNCTURE – CONCEPTS AND PHYSIOLOGY

Edited by **Marcelo Saad**

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Preface

Acupuncture is growing in popularity world-wide. Since it started to be scientifically studied in the 1970's, acupuncture is conquering position as an efficient and safe therapeutic method. The amount of cumulated scientific evidence is already enough to guarantee a highly detached status for acupuncture among other complementary therapies. In many countries, acupuncture is well integrated into the conventional health system. This scenario would seem to be very favorable and the value of acupuncture would thus seem well consolidated. However, there are several factors that could shake this structure.The mechanisms of action of acupuncture are not entirely clear. Although we have many pieces of this puzzle, it is still not complete neither entirely mounted. Another debilitating element is the absence of a convincing model of sham acupuncture for a control group in clinical trials. All this facts, allied to inappropriate prejudice and unfamiliarity, reinforce the false notion that acupuncture works mainly due to placebo effect. There is still the issue of the absence of a universal consensus about the degree in which acupuncture can be independent to the traditional chinese medicine.

However, acupuncture can also be self-sustained. Currently, it is practiced in more than 160 countries and regions. The UNESCO (United Nations Education, Scientific and Cultural Organization) inscribed acupuncture on its List of the Intangible Cultural Heritage of Humanity in 2010. There cannot be larger acclamation than this one.

Acupuncture and related techniques are useful tools for treating a spectrum of diseases. But there are still many areas of controversy. We hope this book can contribute to guide the advance of this ancient medical art.

In the present work, the reader will find texts written by authors from different parts of the world. The chapters cover strategic areas to collaborate with the consolidation of the knowledge in acupuncture. The book doesn't intend to solve all the questions regarding this issue. The main objective is to share elements to make acupuncture more and better offered at health systems worldwide.

This book contains information about Basic Concepts of Acupuncture; Research about Physiologic Effects; New Proposed Concepts; and Current Scenario.

I hope the reading of this work will be useful and pleasant.

With Best Regards

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Part 1

Acupuncture Basic Concepts

1

Traditional Theory

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1. Introduction

Traditional Chinese medicine, including herbal medicine and acupuncture, as one of the most important parts in complementary and alternative medicine (CAM).
Recent research showed that complementary and alternative medicine could contribute to primary health care. Traditional Chinese medicine(TCM) has evolved a system that aims to cure illness by penetrating the skin at specific points. This system is called acupuncture, derived from the Latin words "acus" and "punctura" meaning "needle" and "pricked", respectively.
TCM can be characterized as holistic with emphasis on the integrity of the human body and the close relationship between human and its social and natural environment. Therefore, it studies, through synthesis and analysis, the interaction between the dynamic activities of various ports of the human body and their relationship to the external environment. So understanding the fundamental theory is necessary for learning about the various branches of traditional Chinese medicine.

2. Fundamental theories

2.1 Theory of Yin-Yang and five elements

The theory of Yin-Yang holds that the world is material and that this material world evolves constantly as the result of the mutual action of two opposing material forces.
What is Yin-Yang? At its origin, the terms were used to designate the two slopes of a mountain. The sunny side was Yang and the shady side was Yin. Later, their meaning broadened to include all opposites. All natural events and states of being are rooted in Yin and Yang all aspects of the natural world could be seen as having a dual aspect, for example, day and night, brightness and darkness, movement and stillness, heat and cold, etc. within the field of medicine different parts of the body are classified as either Yin or Yang. For example, the upper and exterior parts of the body belong to Yang and the lower and interior parts to Yin; the hands belong to Yang; while the feet to Yin; the five Zang organs pertain to Yin; the six Fu organs to Yang.
The Yin-Yang nature of a phenomenon is not absolute but relative. This relativity is reflected in two ways. On one hand, under certain conditions Yin may change into Yang and vice versa, and on the other, any phenomenon may be infinitely divided into its Yin and Yang aspects, reflecting its own inner Yin-Yang relationship. Day, for example, is Yang, while night is Yin. However, each can be further classified as follows: morning is Yang within Yang, afternoon is Yin within Yang, the first half of the night Yin within Yin, and the

second half of the night Yang with Yin. This differentiation of the natural world into its opposite parts can be carried out infinitely.
Therefore it can be seen that Yin and Yang are at the same time opposite in nature and yet interdependent. They both oppose and complement each other, and exist within all natural phenomena. Traditional Chinese medicine applies the Yin-Yang principles of interconnection and continuous transformation to the human body to explain its physiology and pathology and to guide clinical diagnosis and treatment.

2.2 The theory of the five elements

The five elements refer to five categories in the natural world, namely wood, fire, earth, metal and water. According to the theory wood, fire, earth, metal and water are the basic substances that constitute the material world. These substances are not only of the relations with generation and restriction but set in a state of constant motion and change for example wood promotes fire, fire promotes earth, earth promotes metal, metal promotes water, and water, in turn, promotes wood. (Fig.1)
As far as the relationship of generation of each of the five elements is concerned, it is composed of two aspects--promoting and being promoted. The element that promotes is called the mother, while the element that is promoted is called the child. Hence, the relation of promoting and being promoted among the five elements is also known as that of mother and child. Take fire for example, since fire produces earth, it is called the mother of earth; on the other hand it is produced by wood, so it is called the child of wood. Restriction connotes bringing under control or restraint.
The Chinese people recognized that wood, fire, earth, metal and water were the indispensable in their daily lives as well as having different natures. In light of the theory of

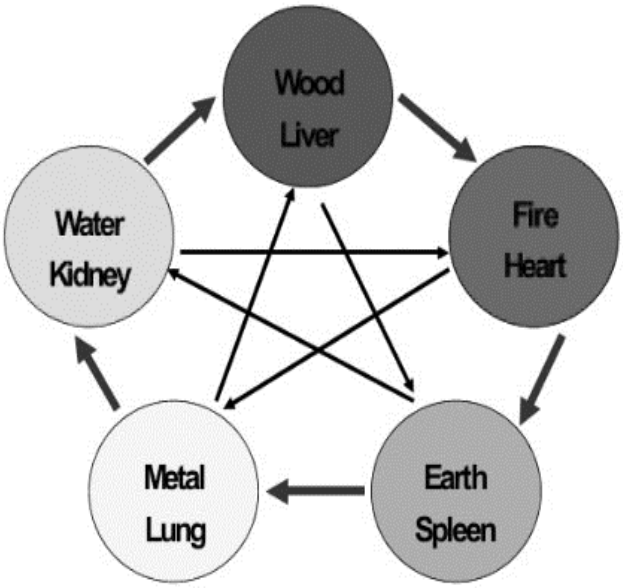

Fig. 1. The five elements

five elements, traditional Chinese medicine has made a comprehensive comparison and study of all kinds of things and phenomena in nature as well as in the Zang-Fu organs, tissues, physiology and pathology of the human body, classified them respectively to one of the five elements, i.e., wood, fire, earth, metal and water, in accordance with their different properties, functions and forms, thus expounding the physiology, pathology of the human body and the correlation between man and his natural surroundings. The following is a table showing the classification of things according to the theory of the five elements.

2.3 The theory of Zang-Fu system

In traditional Chinese medicine (TCM), the internal organs of the human body are divided into two groups: "Five Zang organs", "Six Fu organs" .The five Zang organs include the heart, the liver, the spleen, the lung and the kidney. Preserving vital substances is their characteristic common. The six Fu-organs consist of the gallbladder, the stomach, the large intestine, the small intestine, the bladder and the triple energizer (San Jiao) of which the characteristic in common is transmitting and digesting water and food.

2.3.1 Five zang organs

2.3.1.1. Heart

The heart is situated in the thorax and the main physiological functions of heart are: dominating the blood and vessels, opening into the tongue and, supporting the mind. The blood circulation relies on the cooperation between the heart and the vessels, dominating the blood and vessels means that the heart is the motive force for blood circulation, whilst the vessels are the physical structures which contain and circulate the blood. With the heart being of primary importance. Supporting the mind means thinking is related to the physiological functions of the heart. The tongue is connected to the Heart Meridian internally, and via this connection the heart dominates the sense of taste and the speech. So disorders of the heart will reflect on the tongue.

2.3.1.2. Lung

The lung, situated in the thorax and main physiological functions are: controlling respiration, dominating Qi dispersing and descending, supporting skin and hair, communicates with the throat and opens into the nose. Its meridian connects with the large intestine.

Controlling respiration respiration means that the lung is a respiratory organ through which the clean Qi (the air) from the exterior and the Qi from the interior can be mingled.

Whereas dominating the Qi of the whole body means that the function of lung in respiration greatly influences the functional activities of the whole body and is closely related to the formation of pectoral Qi. Dysfunction of the lung in descending may lead to the upward reversal of lung Qi bringing on the symptoms such as cough and shortness of breath.

The skin and hair are warmed and nourished by defensive Qi and body fluid distributed by the lung. The nose is the pathway for respiration. The respiratory and olfactory functions of the nose depend on lung Qi. When lung Qi is normal, the respiration will be free,and if lung Qi is abnormal will lead to shortness of breath and vibration of the ala nasi. Since the throat is also a gateway of respiration, when the lung is diseased, it could cause pathological changes in the throat, such as hoarse voice and aphonia.

2.3.1.3. Spleen

The spleen is situated in the Middle Energizer. Its main physiological functions of spleen involves: the transportation and transformation of water and food on one hand, and of dampness on the other, controlling all the blood of the body and keeping it circulating normally within the vessels, transports and transforms the essence of food and drink to nourish the muscles and the four limbs. The mouth is the aperture of the spleen, for this reason, the lips reflect the condition of the spleen's function of transporting and transforming water and food. When the spleen is healthy, there will be ample Qi and blood and the lips will be red and lustrous.

2.3.1.4. Liver

The liver is situated in the right hypochondriac region and main physiological functions are storing and regulating blood, supporting the free flow of Qi, controlling the tendons and opening into the eyes. The liver has the function of storing blood and regulating its amount in circulation, normal adult's liver can provide, at least, 1000-2000 milliliters of blood so as to keep enough blood for the heart to pump out. The liver is responsible for supporting the free flow of Qi, through this harmonious functional activity of all the Zang-Fu organs including itself.

Dysfunction of the liver, therefore, is often accompanied by emotional changes such as mental depression or over-excitement, because in addition to the heart, emotional activity is closely related to the liver Qi. The liver also nourish the tendons of the whole body to maintain their normal activities of tendon that is the joints and muscles and so the movement of the limbs. The liver has control on the digestion through secretion and excretion of bile. The liver is opening into the eyes, means that whether the eyes' visual sense functions well or not is mainly dependent on the nourishment by the blood stored in the liver.

2.3.1.5. Kidney

The kidneys are located at both sides of the lumbus, which is therefore described as "the home of the kidney, its main functions are: to store congenital and acquired essence and control human reproduction , dominate water metabolism in other words kidney regulates the distribution of water and helps maintain fluid balance in the body and the reception of Qi which means that the kidney assists the lung in its function of receiving and descending the Qi. In other words, respiration depends not only on the descending function of the lung, but also on the kidney's function of reception and control, kidney produce marrow to fill up the brain, opening into the ears which means the hearing function of ears relies on the nourishment by the essential Qi of the kidney. The ears therefore pertain to the kidney and kidney also control anterior and posterior orifices. Anterior orifice" refers to the urethra and genitalia which have the function of urination and reproduction. "Posterior orifice" refers to the anus which has the function of excreting the feces. Decline or deficiency of kidney Qi, therefore, may give rise to frequency of micturition, enuresis, oliguria and anuria, seminal emission, impotence, premature ejaculation and infertility in reproduction, and prolonged diarrhea with prolapse of rectum or constipation.

2.3.2 Six Fu organs

2.3.2.1 The Gallbladder

The gallbladder is attached to the liver and main function is to store bile and continuously excrete it to the intestines to aid the stomach and spleen in digestion failure to aid, resulting

in abdominal distention and loose stool. Since the bile is bitter fluid, and yellow in color, upward reversal of gallbladder Qi may give rise to a bitter taste in the mouth. The liver and gallbladder together have the function of suportting the free flow of Qi. Similarly, the relation of the liver to emotional changes such as: fear and palpitations, insomnia and dream-disturbed sleep is shared by the gallbladder.

2.3.2.2 Stomach

The stomach is located in the epigastrium and the main physiological function of stomach is reservoir of food and drink, then food and drink are fermenting and grinding by action of the stomach so disturbance of these functions of the stomach will cause poor appetite, capacity for only small amount of food, and pain in the epigastric region. Of course, only in cooperation with the spleen's function digesting and transforming food and drink can be performed successfully by the stomach's function of receiving, to receive, digest and transform the food and drink. Sufficient stomach Qi make all the five Zang organs to be full of vigor, while the deficiency of stomach Qi leads to their weakness.

2.3.2.3 Small Intestine

The small intestine is located in the abdomen main physiological functions are reception and continue digestion and absorbs essential substance and part of the water from the food, transmitting the residue of the food to the large intestine and of the water to the bladder.

2.3.2.4 The large intestine

The large intestine is located in the abdomen and the main function of the large intestine is to receive the waste material sent down from the small intestine, absorb its fluid content, and form the remainder into feces to be excreted. Pathological changes of the large intestine will lead to dysfunction in this transportation function, resulting in loose stools or constipation.

2.3.2.5 The Bladder

The bladder is located in the lower abdomen and the main function of the bladder is the temporary storage of urine, which is discharged from the body through Qi activity with assistance of the kidney Qi. Dysfunction of the bladder will lead to symptoms such as anuria, urgency of micturition and dysuria; and the failure of the bladder to control urine may lead to incontinency and enuresis.

2.3.2.6 San Jiao (Triple Energizer)

The "Sanjiao" is a large Fu organ containing all the internal organs, and also used to locate the body parts. The physiological functions of Sanjiao control the activities of the Qi of the human body. Triple Energizer is a collective name of the upper, middle and lower Jiao (energizer) :the upper Jiao is that the portion of the body cavity above the diaphragm which houses the heart and the lung. The middle Jiao is the portion between the diaphragm and umbilicus which houses the spleen and the stomach. The lower Jiao is the portion below the umbilicus which houses the liver, the kidney, the bladder, the intestines. Pathologically, diseases due to an abnormality of upper, or middle, or lower Jiao are manifestations of dysfunctions of the Zang-Fu organs within it.

2.4 The theory of essence, qi, blood and body fluids

The four basic substances of life are essence, Qi, Blood and body fluids, they are the material bases which maintain the normal activities of the human body.

2.4.1 Essence

Essence (Jing) is a fundamental material of the human body and the material basis for various physiological functions of the human body. The congenital essence is received from one's parents, and is stored in the kidney; it is also known as "the prenatal essence", serving to promote the growth, development maturity, and reproduction of the body, thus the congenital essence is also called the "reproductive essence. The acquired essence is derived through the functions of the Zang-Fu organs from the nutritive substance of food and drink to nourish the body. In turn, it serves as the material basis for the functional activities of the Zang-Fu organs. Consequently, it is called "the essence of the Zang-Fu organs. The essence stored in the kidney includes both the congenital essence and the acquired one. The two are interdependent on and promote each other. Before birth, the congenital essence prepares the material basis for the acquired one; and after birth, the acquired essence continues to replenish the congenital.

2.4.2 Qi

Qi is described as the basic particles which constitute the universe and produce everything in the world through their movements and changes. Qi in its physiological sense refers to the motive force or energy (which is produced by the basic particles) required for various functional processes.

Certain qualitative terms differentiate Qi in the human body according to its source, function, and distribution. The terms are: primary Qi (Yuan Qi), pectoral Qi (Zong Qi), nutrient Qi (Ying Qi) and defensive Qi (Wei Qi). In terms of their source they may be further classified into congenital Qi and acquired Qi. Primary Qi, which is derived from congenital essence and inherited from the parents, is referred to as the congenital Qi. After birth, pectoral Qi, nutrient Qi, and defensive Qi are all derived from the refined essence of food, and are therefore known as the acquired Qi.

2.4.2.1 Primary Qi (Yuan Qi)

Derived from the congenital essence, primary Qi needs to be supplemented and nourished by the Qi obtained after birth from the essence of food and water. Primary Qi takes root in the kidney and spreads to the entire body via the triple energizer (San Jiao). It stimulates and promotes the functional activities of the Zang-Fu organs and the associated tissues.

2.4.2.2 Pectoral Qi (Zong Qi)

Pectoral Qi is formed by the combination of the clean Qi (Qing Qi) which is inhaled by the lung, and the essential Qi of the food and drink which is transformed by the spleen and stomach. Pectoral Qi is stored in the chest. Its main functions are: the one is to promote the lung's function of controlling respiration, so the strength or weakness of speech and respiration are related to the quality of pectoral Qi; and the other is to promote the heart's function of dominating the blood and vessels, so the circulation of Qi and blood, and the coldness and warmth and the motor ability of the four limbs and body trunk are all closely associated with the pectoral Qi.

2.4.2.3 Nutrient Qi (Ying Qi)

Derived from the essential Qi of the food and drink transformed by the spleen and stomach, nutrient Qi circulates in the vessels. Its primary function is both to produce blood and to circulate with it to provide further nourishment.

2.4.2.4 Defensive Qi (Wei Qi):

Defensive Qi is also derived from the Qi of food essence, but unlike nutrient Qi it circulates outside the vessels. Defending the body against exogenous pathogenic factors is its principal function, hence the name defensive Qi.Other functions includ: defend the body against exogenous pathogenic factors, control the opening and closing of the pores, moisten the skin and hair, readjust body temperature, and warm up the Zang-Fu organs.

2.4.2.5 Function of Qi

The physiological activities of the Zang-Fu and meridians, the circulation of blood and distribution of body fluid, are all dependent on the promoting and stimulating effect of Qi.

2.4.3 Blood

Blood is a red liquid circulating in the vessels, and is a vital nutrient substance in the body. The fundamental substances required in blood formation originate from the essence of food and drink produced by the spleen and stomach, these two organs are regarded as the source of Qi and blood. Blood circulates throughout the body, passing through the five Zang and six Fu organs in the interior, and the skin, muscles, tendons and bones on the exterior. In this way blood nourishes and moistens the various organs and tissues of the body. Qi and Blood are the foundation for human mental activities. Deficiency of blood, therefore, may lead to the mental disorders. An example is deficiency of heart or liver blood resulting in the mental restlessness, with symptoms such as palpitation, insomnia and dream-disturbed sleep.

2.4.4 Body fluid

Body fluid is formed from food and drink after its digestion and absorption by the spleen and stomach. The transportation, distribution and excretion of body fluid principally rely on the spleen's function of transportation, the lung's function of dispersing and descending and regulating water passages, and the kidney's function of controlling urination and separating the clear and the turbid. Of the three, the kidney is the most important. Body fluid moistens and nourishes various parts of the body.

3. The meridian-collateral system

The meridians (Jing) and collaterals (Luo) are pathways in which Qi and blood circulates. The meridians are the major channels of the system and they run lengthwise within the interior of the body. They bring the body into an organic whole to carry on the systematic activities. Even though the five Zang and six Fu organs, and all the tissues and orifices each perform their own physiological activities, they need another structure to integrate their functions to maintain the body as an organic whole. The integration is accomplished mainly by the network of meridians, of which the four functions are involved with the meridian Qi.
The collaterals are the branches of the meridians and they run crosswise from the meridians either on or just below the body surface. Since they are distributed over the entire body, the meridians and collaterals link together the Zang-Fu and other organs, the orifices of the body, the skin, muscles and bones. They form a specific network which communicates with the internal organs and limbs and connects the upper to the lower and the exterior to the interior of the body.

The meridian system consists of the twelve regular meridians: the three Yin meridians of the hand, the three Yin meridians of the foot, the three Yang meridians of the hand and the three Yang meridians of the foot, they the chief pathways of Qi and blood so are called regular meridians. There are eight extra meridians: namely Du, Ren, Chong, Dai, Yinqiao, Yangqiao, Yinwei and Yangwei meridians. The eight extra meridians are not directly related to any of the internal organs.They are interlaced with the twelve regular meridians, helping reinforce the communication between and adjustment of the twelve regular meridians.

3.1 The routes of the twelve regular meridians

The twelve regular meridians start and terminate at given parts, run along the regular routes and meet in a specified sequence. They are, moreover, associated with the Zang-Fu organs. Though we say there are twelve regular meridians, in fact these twelve meridians are doubled when counting symmetrically on both the left and right sides of the body.

3.1.1 The Lung Channel of Hand Taiyin

The Lung Channel originates in the middle portion of the body cavity [1] and runs downwards internally to connect with the Large Intestine [2]; turning back, it passes upward through the diaphragm [3]; to enter its pertaining organ, the Lung [4]; from the internal zone between the lungs and the throat [5] it emerges to the surface of the body under the clavicle. Descending, the Lung Channel then runs along the medial aspect of the upper arm [6] to reach the elbow crease. From there, it runs along the anterior portion of the forearm [7] passes above the major artery of the wrist, and emerges at the radial side of the tip of the thumb [8]; another section of the Lung Channel branches off just above the wrist and runs directly to the radial side of the tip of the index finger [9] to connect with the Large Intestine Channel (See.Fig. 2).

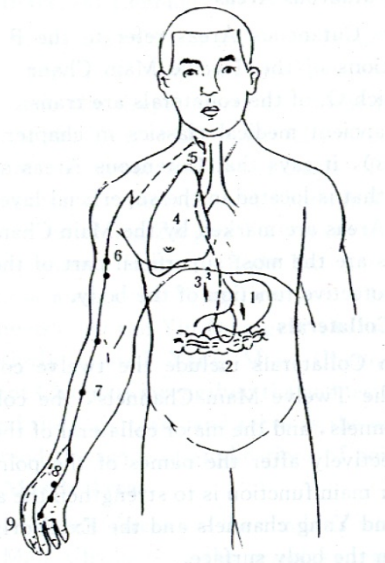

Fig. 2. The Lung Channel of Hand Taiyin

3.1.2 The large Intestine Channel of hand Yangming

The large Intestine Channel begins at the tip of the index finger, and runs upwards along the radial side of the index finger [1] and between the thumb and the index finger. It passes through the depression between the tendons of the thumb [2] and then continues upwards along the lateral aspect of the forearm to the lateral side of the elbow. From there, it ascends along the anterior border of the upper arm [3] to the highest point of the shoulder [4]. On the top of the shoulder, the channel divides into two branches [5]. One enters the body and passes through the Lung [6] diaphragm and the Large Intestine [7] which is its pertaining organ. Another one ascends externally along the neck [8] passes through the cheeks [9] and enters, internally, into the lower teeth and gum [10]. On the Exterior, it continues, it continues, curving around the upper lip and crossing to the opposite side of the nose (SeeFig. 3).

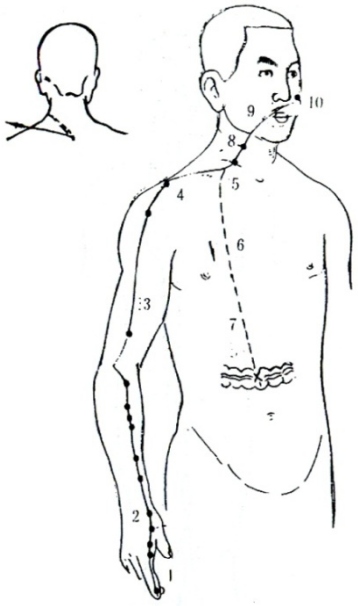

Fig. 3. The Large Intestine Channel of Hand Yangming

3.1.3 The Stomach Channel of Foot Yangming

The Stomach Channel begins, internally, where the Large Intestine Channel terminates, next to the nose [1]. It then ascends to the bridge of the nose, meeting the Bladder Channel at the inner corner of the nose. It enters the upper gum [2] and curves around the lips before passing along the side of the lower jaw bone [3] and through the angle of the jaw. It then runs upwards, running in front of the ear [4] to the corner of the forehead. A branch descends from the lower jaw [5] enters the body, and descends through the diaphragm. It then enters its pertaining organ, the Stomach, and connects with the Spleen [6]. Another branch leaves the lower jaw, but remains on the surface of the body as it crosses over the neck, chest [7] and abdomen [8] and terminates in the groin. Internally, the channel reconstitutes itself at the lower end of the stomach and descends inside the abdomen [9] to

reconnect with the external branch in the groin. From this point, the channel runs downwards over the front of the thigh [10] to the outer side of the knee [11] and continues along the center of the front of the lower leg to reach the top of the foot. It terminates at the lateral side of the tip of the second toe. A branch deviates from the Stomach Channel just below the knee [12] and ends at the lateral side of the middle toe. A short branch also leaves the top of the foot [13] and terminates at the medial side of the big toe to connect with the Spleen Channel (see Fig. 4).

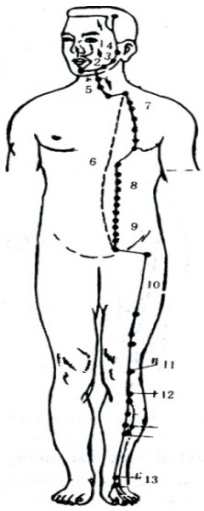

Fig. 4. The Stomach Channel of Foot Yangming

3.1.4 The spleen Channel

The spleen Channel originates at the medial side of the big toe. It then runs along the inside of the foot [1] turning in front of the inner ankle bone. From there, it ascends along the posterior surface of the lower leg [2] and the medial aspect of the knee and thigh [3] to enter the abdominal cavity [4]; it runs internally to its pertaining organ, the Spleen [5] and connects with the Stomach [6]. The main trunk of the channel continues on the surface of the abdomen. running upwards to the chest [7] where it again penetrates internally to follow the throat [8] up to the root of the tongue [9] under which it spreads its Qi and Blood. An internal branch leaves the Stomach, passes upwards through the diaphragm, and enters into the Heart [10] where it connects with the Heart Channel (see Fig. 5).

3.1.5 The Heart Channel of Hand Shaoyin

The Heart Channel has three branches, each of which begins in the heart [1]. One branch runs downwards through the diaphragm [2] to connect to the Small Intestine. The second branch runs upwards from the heart along the side of the throat [3] to meet the eye. The third branch runs across the chest from the Heart to the Lung [4] and then descends and emerges in the armpit. It passes along the midline of the inside of the upper arm [5] runs downwards across [6] crosses the wrist and palm [7] and terminates at the inside tip of the little finger, where it connects with the Small Intestine Channel (see Fig. 6).

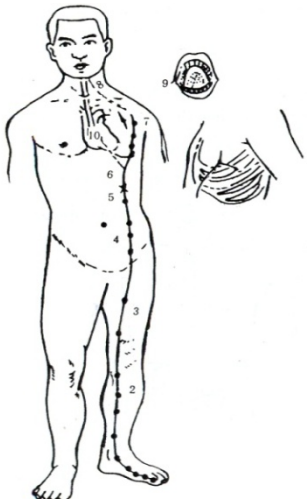

Fig. 5. The spleen Channel

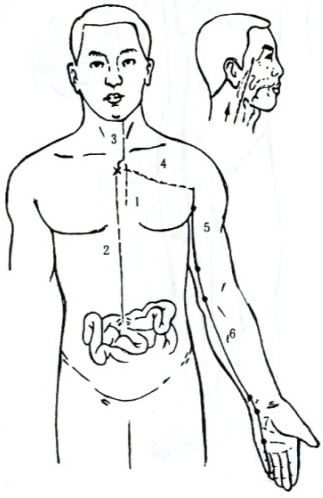

Fig. 6. The Heart Channel of Hand Shaoyin

3.1.6 The Small Intestine Channel of Hand Taiyang

The small Intestine Channel begins on the outside of the little finger, crosses the palm and wrist [1] and passes upwards along the posterior aspect of the forearm [2]. The channel continues upwards along the posterior border of the lateral aspect of the upper arm [3]. Circles behind the shoulder [4], and runs to the center of the uppermost part of the back (where it meets the Du Channel). Here, the channel divides into two branches, one entering internally [5] to connect with the Heart [6], diaphragm, and Stomach [7] before entering its pertaining organ, the Small Intestine [8]; the second branch ascends along the side of the neck [9] to the cheek [10] and the outer corner of the eye [11] before entering the ear. A short

branch leaves the channel on the cheek [12] and runs to the inner corner of the eye, where it connect with the Bladder Channel (see Fig. 7).

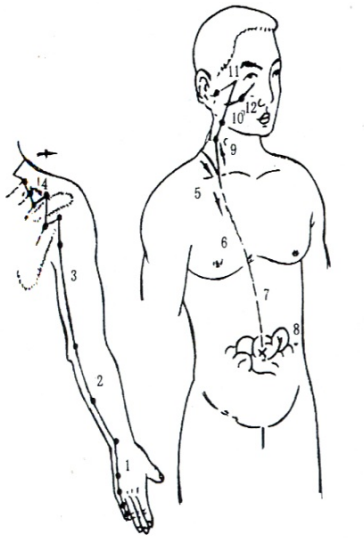

Fig. 7. The Small Intestine Channel of Hand Taiyang

3.1.7 The Bladder Channel of Foot Taiyang

The Bladder Channel starts at the inner side of the eye and ascends across the forehead [1] to the vertex of the head. From this point, a small branch splits off and enters into the brain [2] while the main trunk of te channel continues to descend along the back of the head [3] and bifurcates at the back of the neck [4]. The inner of these two branches descends a short distance to the center of the neck [5] and then descends parallel to the spine [6]. A branch splits off entering the body in the lumbar area and connecting to the Kidney [7] and its pertaining organ, the Bladder [8]. The outer branch traverses the back of the shoulder [9] descends adjacent to the inner branch and the spinal cord, and crosses the buttocks [10]. The two branches continue downwards along the posterior aspect of the thigh [11] and join behind the knee. The single channel now continues down the back of the lower limb [12] circles behind the outer ankle, runs along the outside of the foot [13] and terminates on the lateral side of the tip of the small toe, where it connects with the Kidney Channel (See Fig.8).

3.1.8 The Kidney Channel of Foot Shaoyin

The Kidney Channel starts from the inferior aspect of the small toe, runs across the sole of the foot [1] and emerges along the arch of the foot [2] to circle behind the inner ankle and pass through the heel. It then ascends along the medial side of the lower leg [3] to the medial side of the knee crease, climbs upwards along the innermost aspect of the thigh [4] and penetrates the body near the base of the spine [5]. This branch connects internally with the Kidney [6] its pertaining organ, and with the Bladder [7] before returning to the surface of the abdomen above the pubic bone and running upwards over the abdomen and chest [8]. Another branch begins inside at the Kidney [6] passes upward through the Liver [9] and

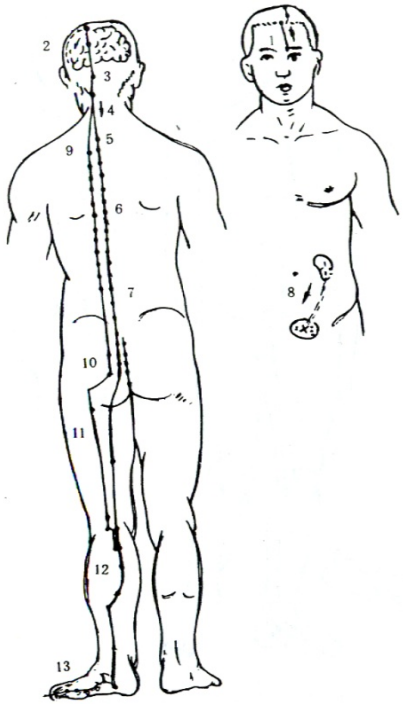

Fig. 8. The Bladder Channel of Foot Taiyang

diaphragm, and enters the Lung [11]. This branch continues along the throat [10] and terminates at the root of the tongue. A smaller branch leaves the Lung [11] joins the Heart, and flows into the chest to connect with the Pericardium Channel (see Fig.9).

3.1.9 The Pericardium Channel of Hand Jueyin

Beginning in the chest and in its pertaining organ, the Pericardium [1], this channel descends through the diaphragm [2] to link the upper middle and lower portions of the San Jiao. A second internal branch of the channel crosses the chest [3] emerging to the surface at the area of the ribs. The channel then ascends around the armpit [4] and continues down along the medial aspect of the upper arm [5] to the elbow crease. It runs further down the forefinger. A short branch splits off from the palm [8] to connect with the San Jiao Channel at the end of ring finger (see Fig. 10).

3.1.10 The san Jiao Channel of Hand Shaoyang

Beginning at the outside tip of the ring finger, the San Jiao Channel proceeds over the back of the hand [1] and wrist to the forearm [2]. It runs upwards, around the outer elbow, along the lateral aspect of the upper arm [3] to reach the posterior shoulder area [4]. From here, the channel travels over the shoulder [5] and enters into the chest underneath the breast bone. An internal branch passes from this point through the Pericardium, penetrates the diaphragm [6] and then proceeds downwards [7] to unite the Upper, Middle, and Lower Jioa. An external branch ascends toward the shoulder and runs internally up the neck [8]. It

reaches the posterior border of the ear [9] and then interiorly circles the face [10]. A short branch originates behind the ear, penetrates the ear, and emerges in front of the ear [11] to reach the outer end of the eyebrow and connect with the Gallbladder Channel (see Fig. 11).

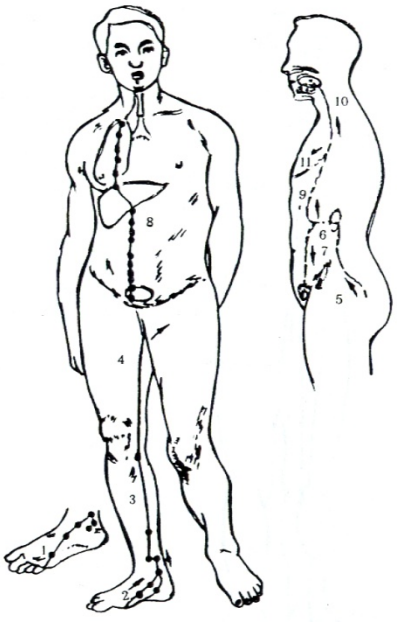

Fig. 9. The Kidney Channel of Foot Shaoyin

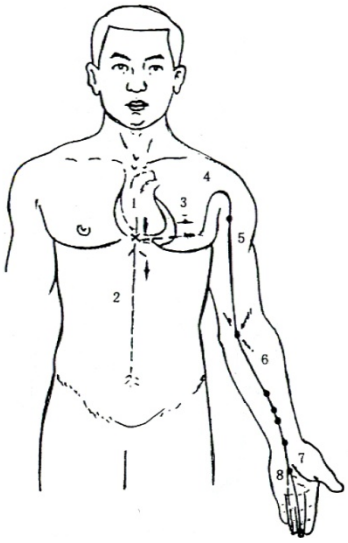

Fig. 10. The Pericardium Channel of Hand Jueyin

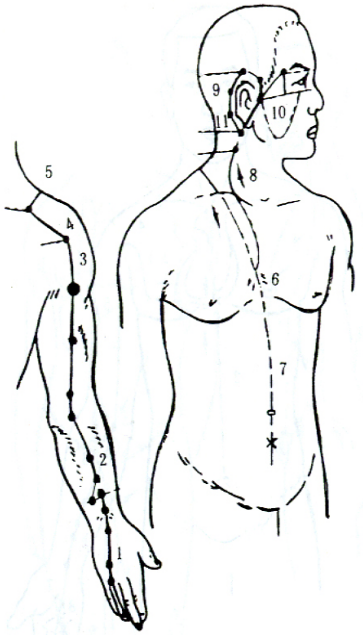

Fig. 11. The san Jiao Channel of Hand Shaoyang

3.1.11 The Gallbladder Channel of Foot Shaoyang

The Gallobladder Channel begins at the outer corner of the eye [1] where two branches arise. One branch, remaining on the surface, weaves back and forth on the head before curving behind the ear [2] to reach the top of the shoulder. It then continues downwards, passing in front of the armpit [3] and along the lateral aspect of the rib cage [4] to reach the lip area. The second branch traverses the cheek [5] internally and proceeds through the neck [6] and chest [7] to reach the Liver and its pertaining Organ, the Gallbladder [8], Continuing downwards, this branch emerges on the side of the lower abdomen, where it connects with the other branch in the hip area [9], The channel then descends along the lateral aspect of the thigh [10] and knee to the side of the lower leg [11] and further downwards in front of the outer ankle. It crosses the top of the foot [12] and terminates at the lateral side of the tip of the fourth toe. A branch leaves the channel just below the ankle to cross over the foot [13] to the big toe, where it connects with the Liver Channel (see Fig.12).

3.1.12 The Liver Channel of Foot Jueyin

Beginning at the top of the big toe, the Liver Channel traverses the top of the foot [1], ascending in front of the inner ankle and along the medial aspect of the lower leg [2] and knee. It runs continuously along the medial aspect of the thigh [3] to the pubic area, where it encircles the external genitalia [4] before entering the lower abdomen. It ascends internally [5], connects with its pertaining underneath the ribs [7] before pouring into the Lung [8] where it connects with the Lung Channel. The entire cycle of the channel system begins anew here. Reconstituting itself, the channel follows the trachea upwards to the throat [9] and connects with the eyes [10]. Two branches leaves the eye area. One descends across the

cheek to encircle the inner surface of the lips [11], another branch ascends across the forehead [12] to reach the vertex of the head (see Fig.13).

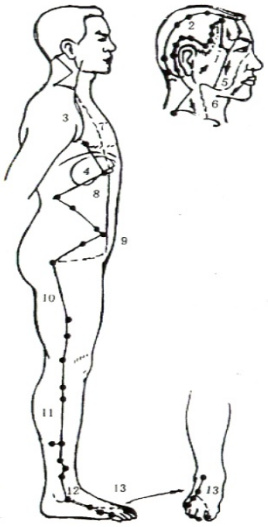

Fig. 12. The Gallbladder Channel of Foot Shaoyang

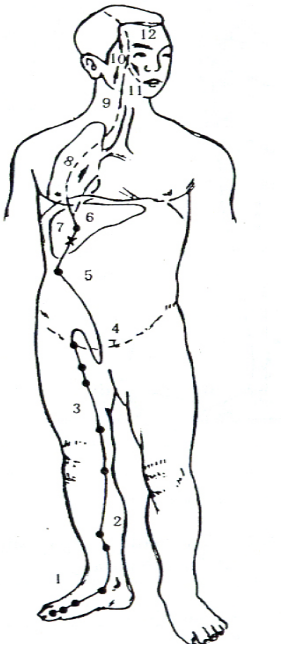

Fig. 13. The Liver Channel of Foot Jueyi

3.1.13 The Du Channel

The Du Channel begins in the pelvic cavity [1]. An internal branch ascends from here to the Kidney [2]. Another internal branch descends to emerge at the perineum [3] and passes through the tip of the coccyx. Ascending along the middle of the spine [4], it reaches the head [5] to penetrate into the brain [6]. The main branch continues over the top of the head, descends across the forehead [7] and nose to end inside the upper gum [8] (see Fig. 14).

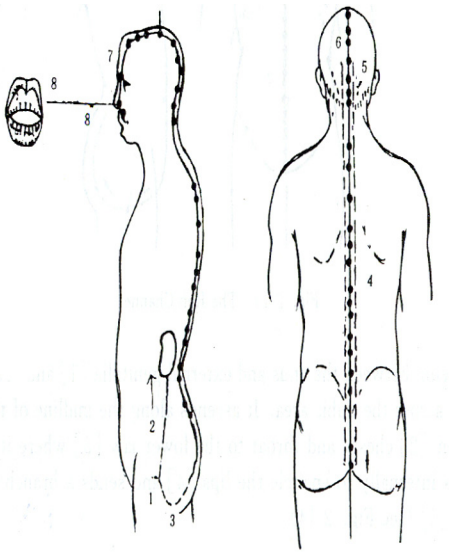

Fig. 14. The Du Channel

3.1.14 The Ren Channel

The Run Channel begins in the pelvic cavity, emerges at the perineum between the anus and external genitalia [1] and runs forward across the pubic area. It ascends along the midline of the abdomen [2] chest, and throat to the lower jaw [3] where it penetrates internally to encircle the lips [4] and sends a branch to the eyes [5] (see Fig.15).

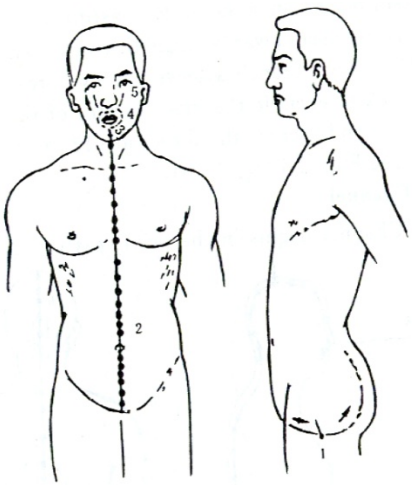

Fig. 15. The Ren Channel

4. Etiology and pathogenesis

In traditional Chinese medicine (TCM), every disease must be the result of some pathogenic factors. In the study of etiology, clinical manifestations are of basic importance, by analyzing symptoms and other indications, TCM is able to identify the causative factors of disease. Numerous factors can cause diseases, but in general the factors include the six climatic factors.

4.1 The six exogenous factors

Wind, cold, summer heat, damp, dryness and fire (mild heat and heat) are the six climatic changes found in nature. Diseases due to the six exogenous pathogenic factors are closely related to the seasonal changes in the weather and to the living environment. For example, heat syndromes mostly occur in summer, cold syndromes in winter, and damp syndromes are usually caused by prolonged exposure to damp.

Wind is the predominant Qi of the spring, although pathogenic wind and the diseases it causes are not confined only in the spring. They can occur in any season. TCM holds that wind is the most important pathogenic factor for exogenous diseases. Wind is a Yang pathogen and is characterized by "upward and outward dispersion." Thus, it attacks the Yang parts of the body easily, i.e. the body surface and the upper body; this leads to the loosening of the pores and the looseness of the body surface and the symptoms such as headache, sweating or aversion to wind, etc. usually appear.
Pathogenic wind, moreover, occurs in gusts and is characterized by rapid changes. Thus, a second characteristic of diseases caused by pathogenic wind is that they begin abruptly and symptoms spread to different parts of the body.

4.1.2 Cold

Cold is the predominant Qi in winter. In winter, being thinly clothed, or being exposed to the cold after sweating, caught in the rain, and wading in water, may all predisposes an individual to an attack of pathogenic cold.
Diseases caused by pathogenic cold can be divided into two types: an attack by cold on the body surface and an interior attack. The former is due to a decrease in the body's defensive Yang which reduces Yang dispersion on the body surface; the latter is due to a sudden injury of the Zang-Fu organs' Yang Qi.Cold has three specific properties and related pathological influences.
If the pathogenic cold attacks the body surface, the pores become obstructed as does the interstitial space, and the defensive Yang fails to disperse with the result of the symptoms such as aversion to cold, fever and absence of sweat. If the pathogenic cold attacks the blood vessels, the vessel contraction and the retarded flow of Qi and blood to the joints will occur, leading to cold, numbness and restricted movement of the limbs.

4.1.3 Summer-heat

Summer-heat is the predominant Qi of summer. Diseases caused by summer-heat are only seen in this season. Summer-heat is a Yang pathogenic factor. When a person is attacked by summer-heat, an excess of Yang occurs, leading to high fever, fidgeting, flushed face and a surging (Hong) pulse, massive sweating consumes body fluids, resulting in thirst and scanty dark urine .Sometimes shortness of breath, lassitude, or sudden loss of consciousness may occur; all are due to the loss of Qi following the exhaustion of body fluids.

4.1.4 Damp

Damp is the predominant aspect of the climate at the end of summer. The damp is a Yin pathogenic factor and is therefore apt to disturb the normal flow of Qi, leading to a stuffy chest, scanty urine and difficulty in bowel movement. The characteristic of the damp is that it tends to go downward and to impair the Yin parts of the body. So, pathogenic damp more often attacks the lower parts of the body and can cause edemas in the lower limbs. If the meridians and joints retain the damp, the dispersion of Yang Qi is hindered and the symptoms of soreness, pain and heavy sensations in the joints will occur. This is known as "Bi-syndrome (e.g. rheumatism) due to the damp." Pathogenic damp also tends to produce turbid excretions from the body: stickiness in the eyes, sticky stools, turbid urine, massive foul-smelling vaginal discharge, or oozing eczema, etc.

4.1.5 Dryness

Dryness is characteristic of autumn. During this season, the temperature and humidity gradually decrease. Lack of moisture causes many things in nature to dry out. Pathogenic dryness usually attacks the lung via the mouth and nose. Dryness depletes body fluids. Exhausting the body fluids, it causes such symptoms as dryness in the mouth and throat, thirst, dry and cracked skin, lusterless hair, scanty urine and constipation. Pathogenic dryness often impairs the functions of lung, the "delicate organ" which favors moisture over dryness, though the lung does not tolerate the pathogenic dampness, it reacts more adversely to the pathogenic dryness.

4.1.6 Fire (heat)

Fire and heat are caused by excessive Yang characteristic is its capability of "flaring up". Consequently, diseases due to pathogenic fire tend to display symptoms such as high fever, aversion to heat, fidgeting, thirst, perspiration, and a surging and rapid pulse .pathogenic fire often consumes the body fluids and Qi. Pathogenic fire predisposes the individuals to carbuncles, furuncles, boils and ulcers when it attacks the blood. In addition, ulcers and painful local red swellings are also clinically diagnosed as Yang and fire syndromes.

4.2 Phlegm and fluid retention, and blood stagnation

Phlegm, retained fluids, and stagnant blood are all pathological results of the dysfunction of the Zang-Fu organs. Since they can further affect the Zang-Fu organs and other tissues, either directly or indirectly, and can cause numerous diseases, they are also regarded as the pathogenic factors.

4.2.1 Phlegm and fluid retention

Phlegm and retained fluids are caused by the influence of the six exogenous pathogenic factors, irregular diet or abnormal emotional activities, all impair the functions of lung, spleen, kidney for the water metabolism, and the Qi of triple energizer. The main pathological manifestations of Phlegm and retained fluids are dizziness, nausea, vomiting, shortness of breath, palpitations or mania and semi-consciousness and sometimes visible phlegm or retained fluids.

4.2.2 Blood stagnation

Blood stagnation is a pathological product that appears in the course of certain diseases, yet, it becomes, in turn, the pathogenic factor of other diseases. By blood stagnation, it is meant that local blood stasis is in the meridians, Zang-Fu organs or other tissues, as well as the accumulation of extravasated blood somewhere. Deficient and stagnant Qi fails to propel blood circulation, because Qi is the commander of blood. The clinical manifestations of blood stagnation depend on the site and the cause of the disease. Stagnation of blood in the heart would cause palpitation, a suffocating sensation in the chest, cardiac pain and the purplish-colored lips and nails.

Blood stagnation in the lung produces pain in the chest and expectoration of blood. Stagnation in the stomach and intestines causes hematemesis and constipation.

4.3 Disharmony of Yin and Yang

Disharmony of Yin and Yang refers to the pathogenic changes involving either excess or deficiency of Yin or Yang, occurring when the body is invaded by the pathogenic Qi. Diseases will not occur unless the body is invaded by pathogenic factors which cause derangement of Yin and Yang in the interior. Yin-Yang disharmony, i.e. excess or deficiency of either Yin or Yang, is mainly manifested in the form of the syndromes of cold and heat and excess and deficiency. In general, heat syndromes of the excess type will occur in cases of the excess of Yang; cold syndromes of the excess type will occur in case of the predominance of Yin; cold syndromes of the deficiency type will occur in cases of the insufficiency of Yang, and heat syndromes of the deficiency type in cases of the deficiency of Yin.

4.4 Conflict between anti-pathogenic Qi and pathogenic Qi

The imbalance between the anti-pathogenic Qi and the pathogenic Qi refers to the struggle between the resistance powers of the body and any of the pathogenic factors. Invasion of pathogenic Qi destroys the Yin-Yang harmony of the body and causes functional disturbance of the Zang-Fu organs and meridians, derangement of Qi and blood. These are mainly manifested as the excess or deficiency syndromes.

Syndromes of the excess type are likely to occur if there is both the excessiveness of the pathogenic Qi and the anti-pathogenic Qi. It is commonly seen in the early and middle stages of diseases due to invasion by the exogenous pathogenic factors, and diseases caused by retention of phlegm fluid, stagnant blood. Deficiency mainly refers to the insufficiency of the anti-pathogenic Qi which is the pathological reaction dominated by the decline of the anti-pathogenic Qi. It is commonly seen in disease resulting from prolonged weakness of body constitution, hypo-function of the Zang-Fu organs, and deficiency of Qi.

5. Conclusion

Traditional Chinese medicine (TCM) studies human physiology and pathology according to basic theory, such as concepts of yin and yang, five elements, zang-fu organs, and the meridian system. Also TCM explain the etiology, pathogenesis, prevention and treatment of diseases. Basically in traditional Chinese medicine (TCM) that there is no disease without pathogenic factors. We must seeking the causative factors through the differentiation of symptoms and signs. So in TCM in order to provide a better guide for diagnosis and treatment, we studies not only the nature of causative factors and their special characteristics, but also their clinical manifestations.

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Part 2

Research About Physiologic Effects

2

Vascular Effects of Auricular Acupuncture Evaluated via Two Digital Volume Pulse Measurements

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1. Introduction

Stimulation of some auricular acupoints produces changes in the radial pulse (Nogier, 1989), and electric stimulation of the auricular acupoint stomach can modify the radial artery pulse wave (Ikezono et al., 2003). Measurement of light transmission through the finger pulp during the cardiac cycle (photoplethysmography) provides a digital volume pulse (DVP). Changes in the contour of the DVP associated with aging, vascular disease, and drugs have been described (Dillon & Hertzman, 1941; Morikawa, 1967). The contour of the DVP is mainly influenced by the systemic vasculature (Nichols, 2005).
A previous study has shown that the acute effects of manual acupuncture at the acupoint 6 of pericardium channel (PC6) on vascular pathophysiology in healthy and hypertensive subjects (Rivas-Vilchis et al, 2007), and non-smokers *versus* smokers (Rivas-Vilchis et al., 2008) can be detected by changes in the indices obtained from the second derivative of the DVP. Two indices derived from the DVP have been proposed. RI_{DVP} as an index of pressure wave reflection and SI_{DVP} as an estimate of pulse wave velocity (PWV) in the aorta and large arteries, i.e., an index of large artery stiffness (Chowienczyk et al., 1999; Millasseau et al., 2002; Millasseau et al., 2003). The purpose of the present study was to assess the acute differential vascular effects of auricular acupoints located in the right and left ear using two DVP indices.

2. Materials and methods

2.1 Subjects

Forty healthy volunteers, 23.4±1.44 years of age (mean±SD), were recruited by advertisement from our university. Studies were performed with the approval of the Research Ethics Committee of the División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana at Iztapalapa, conducted in accordance with the Declaration of Helsinki (2008) of the World Medical Association, and with the informed and written consent of the subjects. Subjects were randomly assigned to group A (n=20) or B (n=20), corresponding to recordings obtained for the left or right ear, respectively. The experimental design is depicted in Table 1. Each treatment was followed by a 1-week washout period in order not to carry over vascular effect according previous experiments not depicted.

Group A	Left auricular acupoint Shenmen	One week washout period	Left auricular acupoint Knee
Group B	Right auricular acupoint Shenmen		Right ear auricular acupoint Knee

Table 1. Experimental groups and treatments.

2.2 Methods

Measurements were obtained while subjects were in the supine position after an overnight fast and at least 20 min rest in a quiet temperature-controlled clinical laboratory (25±1°C) in order to achieve standard conditionsin all participants.

Acquisition of DVP. A photoplethysmograph (MP100, BIOPAC Systems, Goleta, CA, USA), which transmitted infrared light at 860±90 nm, was placed on the index finger of either the right or left hand to obtain the DVP. The frequency response of the photoplethysmograph was flat at 10 Hz. Digital output from the photoplethysmograph was recorded through a 12-bit analog-to-digital converter with a sampling frequency of 200 points/s using the analysis platform provided by AcqKnowledge v. 3.9 software (BIOPAC Systems).

In each subject, a 7-min-long registration DVP was obtained. The acupuncture needle was inserted unilaterally and guided within a plastic tube into the designated auricular acupoint at a depth of 2 mm. The needle was manually unilaterally stimulated for 5 s (60-65 s of registration period), contralateral to the side of the DVP recording, and was then immediately withdrawn.

The DVP indices obtained for each subject, corresponding to basal (25–35 s of the 1 min period before acupuncture) and post-acupuncture (365–385 s) periods, i.e, 5-min after acupuncture stimulation, were calculated using the average of 10 waves.

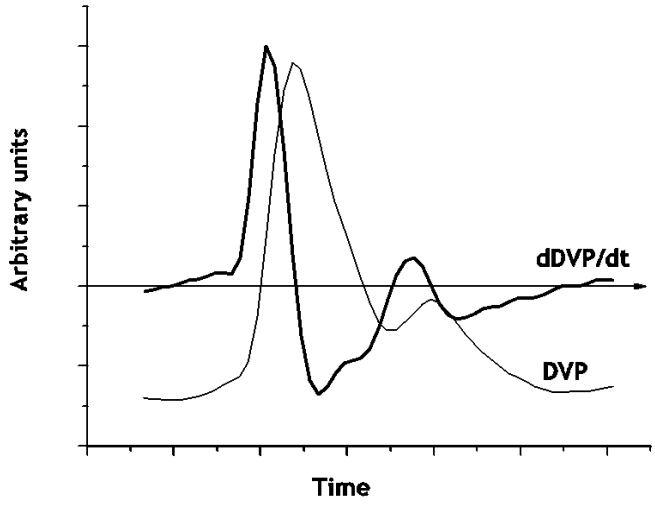

Fig. 1. DVP recording obtained by measuring the transmission of infrared light through the finger pulp, and its first derivative (dDVP/dt).

The first derivative of the DVP wave contour was obtained using the Origin graphics analyzer v. 7.5 (Microcal Software, Inc., Northampton, MA, USA) to specify inflection points (Figure 1). RI_{DVP} was determined as the height of the diastolic peak (or inflection point if no peak was present) and expressed as a percentage of the amplitude of the systolic peak. SI_{DVP} was calculated based on the subject's height (h) and the time between the systolic peak and diastolic peak/inflection points in the waveform (ΔT_{DVP}): $SI_{DVP} = h/\Delta T_{DVP}$.
RI_{DVP} was thus determined based on the relative amplitudes of the systolic and diastolic components of the DVP and SI_{DVP} according to the relative timing of these components (Figure 2). The diastolic peak/inflection point was defined as the point at which the first derivative of the waveform was closest to zero (Millaseau et al., 2002). A distinct peak occurred when the first derivative was zero (positive-to-negative zero crossing). In the absence of a distinct peak, the point of inflection was defined as the point at which the first derivative was closest to zero.

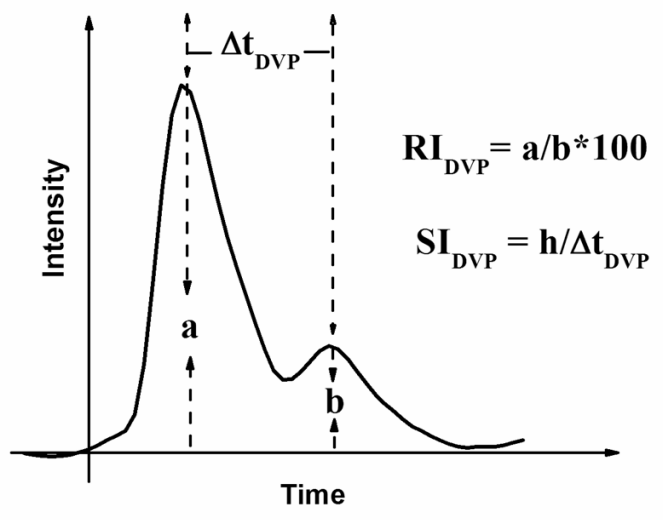

Fig. 2. RI_{DVP} and SI_{DVP} indices.

Acupuncture treatment. Acupuncture therapy was administered by a physician trained in both Western and traditional Chinese medicine. We used 1-cm long, 0.22-mm wide disposable stainless steel needles (Hwato, Suzhou, China), with no additional electrical or laser stimulation. The acupuncture needle was inserted unilaterally into the auricular acupoints Shenmen or Knee in the right or left auricle (Figure 3), gently stimulated for 5 s, and then removed. With respect to the primary objective of the study, the clinical investigators responsible for calculating the DVP indices were blinded and did not know whether the data belonged to the Shenmen or Knee auricular acupoints in either the left or right ear.

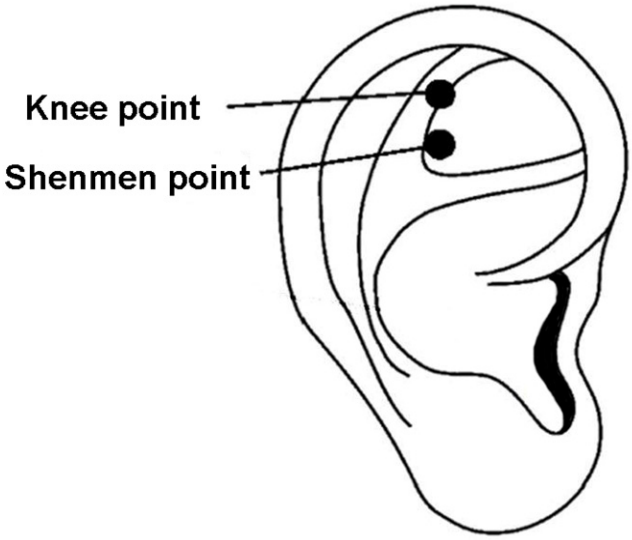

Fig. 3. Shenmen and Knee auricular acupoints.

Statistical analyses. Data are presented as means ± SD (subject characteristics) or as means ± SE (results). The Student's *t* test was used to compare normally distributed continuous variables. $p < 0.05$ was considered as significant, and all tests was two-sided. All statistical analyses were performed using SPSS software version 11.5 (SPSS Inc., Chicago, IL, USA).

3. Results

The study population consisted of 40 healthy male subjects having a global mean age of 23.4 ± 1.44 (21–26) years. The experimental groups did not significantly differ with regard to age, body mass index, or other relevant cardiovascular characteristics. Figures 4 and 5 show the changes observed in the RI_{DVP} and SI_{DVP} indices between the pre- and post-acupuncture periods.

In healthy subjects, manual stimulation of the right Knee auricular acupoint significantly reduced the RI_{DVP} (Figure 4) and manual stimulation of the left Knee auricular acupoint significantly increased the SI_{DVP} index (Figure 5), when comparing the pre- vs. post-acupuncture periods. Comparison of the post-acupuncture values revealed that the left Shenmen and Knee auricular acupoints elicited higher RI_{DVP} index values than their counterpoints on the right ($p < 0.05$ and $p < 0.001$, respectively).

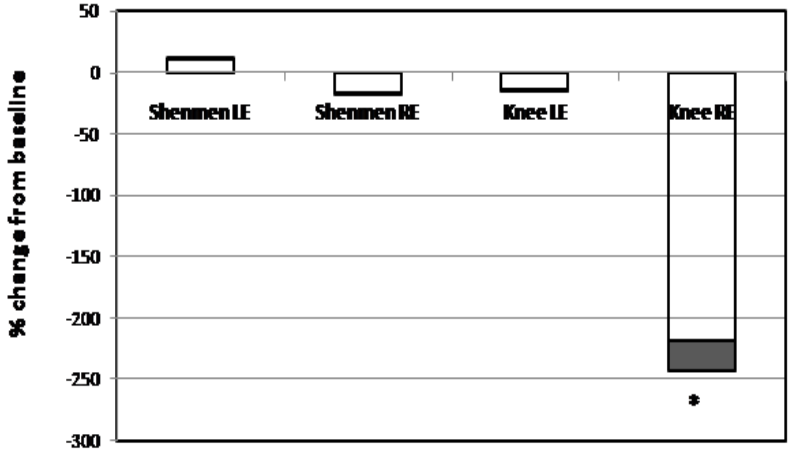

Fig. 4. Changes in percent units from baseline in RI_{DVP} index in healthy men (n=20) after 5 min of the five seconds acupuncture stimulation at the left or right auricular acupoints. LE = left ear; RE = right ear. *$p < 0.05$.

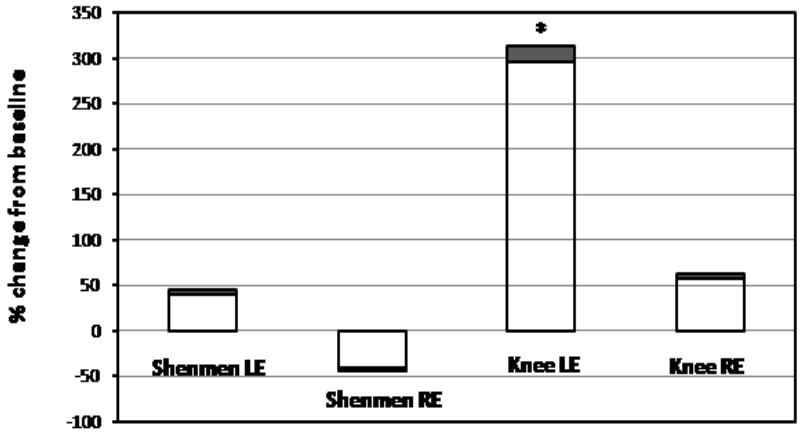

Fig. 5. Changes in percent units from baseline in SI_{DVP} index in healthy men (n=20) after 5 min of the five seconds acupuncture stimulation at the left or right auricular acupoints. LE = left ear; RE = right ear. *$p < 0.05$.

4. Discussion

The principal findings of the present study were that manual stimulation of the right and left Knee auricular acupoints in healthy subjects were associated with a significant decrease and increase in the RI_{DVP} and SI_{DVP} index, respectively. Auricular acupuncture elicited significant differences in RI_{DVP} indices between the left and right ears for both the Shenmen and Knee acupoints, with lower values detected for the right auricular acupoints in both cases.

The principal findings of the present study were that manual stimulation of the right Knee acupoint was associated with significant decrease in the RI_{DVP} and the left Knee acupoint was associated with significant increase in SI_{DVP} index. Auricular acupuncture elicited significant differences in RI_{DVP} indices between the left and right ears for both the Shenmen and Knee acupoints, with lower values detected for the right auricular acupoints in both cases.

Photoplethysmography provides a simple means of deriving the DVP. Several studies have revised the usefulness of the reflection and stiffness indices to assess and quantify effects of vasoactive drugs and nitrates in systemic haemodynamics, such as changes in heart rate and blood pressure (Wagner et al., 1990; Millaseau et al., 2006). To the best of our knowledge, RI_{DVP} and SI_{DVP} have not been previously used to study the vascular mechanisms of auricular acupoints. It has been suggested that DVP consists of a direct component arising from pressure waves propagating from the heart to the finger, as well as a delayed component arising from pressure waves reflected backward from the peripheral arteries, mainly in the lower body, which then propagate to the finger and possess a complex but predictable relationship with the aortic pulse pressure (Chowienczyk et al, 1999).

The RI_{DVP} index provides a measure of the amount of wave reflection in systemic circulation (Millasseau et al, 2006). The RI_{DVP} index is also sensitive to vasodilator inputs (Kelly et al., 2001; Stewart et al, 2003), it has been shown that nitrovasodilators such as glyceryl trinitrate produce reduction in RI index (Chowienczyk et al., 1999). SI_{DVP} is derived from the subject's height and the timing of the diastolic peak/inflection point of the waveform (Figure 2). It provides an estimate of the pulse wave velocity (PWV) in the aorta and large arteries, and therefore the stiffness of the large artery (Chowienczyk et al., 1999; Millasseau et al., 2003). Therefore, it has been shown that the powerful vasoconstrictor angiotensin II increase SI_{DVP} index (Millasseau et al., 2003).

Auricular acupoints have been shown to produce therapeutic effects on cardiovascular pathophysiology (Huang & Liang, 1992), as well as vascular peripheral modifications (Ikezono et al., 2003). The present findings demonstrating a correlation between needling the right Knee auricular acupoint and a decreased RI_{DVP} index probably indicates that this procedure evoked a vagal response. On the other hand, the correlation between needling the left Knee auricular acupoint and an increased SI_{DVP} index demonstrated herein indicates an increase in the PWV, most likely related to a sympathetic response. The RI_{DVP} and SI_{DVP} indices were selected because of their usefulness in assessing cardiovascular changes elicited by a variety of chemical and pharmacological stimuli. Our results clearly demonstrate the potential for auricular acupoints to modify these DVP indices.

5. Conclusions

The present study has one potential limitation, in that only two acupoints were analyzed. The mechanisms of the differences observed between right and left stimulation remain to be

elucidated. Contour analysis of the DVP provides a rapid means of evaluating vascular tone and arterial stiffness, and therefore can be used to assess the cardiovascular effects and the related mechanisms associated with auricular acupoints.

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3

The Effect of Acupuncture on Muscle Blood Volume and Oxygenation

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1. Introduction

A considerable number of patients (61.5/1000) who complain of shoulder stiffness visit oriental therapeutic clinics (Journal of Health and Welfare Statistics, 2007). Several studies have shown that acupuncture can be a useful modality for treating pain due to muscle spasms (Lundeberg et al., 1988: Sandberg et al., 2005: Inoue et al., 2006). It is believed that shoulder stiffness is caused primarily by restriction of blood flow to the working muscles where accumulated metabolites appear to activate sympathetic vasoconstrictors. Acupuncture stimulation (AS) is reported to increase local tissue blood flow in animals (Noguchi et al., 1999; Sato et al., 2000; Uchida et al., 2007) and humans (Sakai 2005; Sandberg et al., 2003; Sandberg et al., 2005) and are applicable in therapeutic interventions of shoulder stiffness.

The effects of acupuncture include neural, endocrinological, cardiovascular, and immunological functions. Among cardiovascular effects, in particular, acupuncture stimulation elicits enhanced muscle blood flow via peripheral vasodilatation. The enhanced blood flow response induced by acupuncture may be attributable to C-fibre mediated axon reflex (Nishijo et al., 1997) resulting from noxious mechanical stimulation. Conventional invasive techniques for evaluating muscle blood flow have limitations such as a relatively great burden on subjects and tissue destruction that may influence blood flow itself. On the other hands, noninvasive methodologies such as laser Doppler flowmetry and thermography could primarily measure and evaluate superficial skin blood flow response. In vivo near-infrared spectroscopy (NIRS) is a noninvasive technology for measuring muscle blood volume and oxygenation response with a depth of ~2cm from the skin surface (Hamaoka et al., 2007), which is suitable for assessing the effect of acupuncture on deep tissue blood flow and metabolic response.

First, we documented in the section 2.1 whether AS would provoke vasodilatation in the trapezius muscle and whether the influence of AS would propagate to a region distant from the stimulation point (spatial distribution of the stimulation). Further, the temporal response at the stimulation onset was also examined.

In the section 2.2, we reported the trapezius muscle blood volume and oxygenation in the stimulation region during four different acupuncture techniques, which are popular intervention techniques in Japan.

In the section 2.3, we described the trapezius muscle blood volume and oxygenation in the stimulation region for acupuncture-experienced and non-experienced individuals.
In the section 2.4, we documented the trapezius muscle blood volume and oxygenation in the stimulation region for individuals with and without shoulder stiffness, which originally motivated us to begin a series of experiment.
In the section 2.5, we examined the effects of acupuncture on the autonomic system and the trapezius muscle blood volume and oxygenation.
Finally, we examined in the section 2.6 that moxibustion, a potential analogous stimulus with acupuncture, would influence the trapezius muscle blood volume and oxygenation.

2. The effects of acupuncture and moxibustion

2.1 The effect of acupuncture on temporal and spatial blood volume and oxygenation response in muscle

In this section, we mentioned the trapezius muscle blood volume and oxygenation in the stimulation region and in a distant region in the same muscle during AS based on the reported literature (ohkubo et al. 2009).
There is no evidence whether the influence of AS would propagate to a region distant from the stimulation point across a single muscle. No temporal data either has ever been investigated regarding blood flow response at the onset of AS in humans. Thus, we compare the trapezius muscle blood volume and oxygenation in the stimulation region and in a distant region in the same muscle during AS and to estimate a latency at the onset of AS. We hypothesized that AS provokes a localized increase in muscle blood volume and oxygenation in the stimulation region.
Nine healthy acupuncture-experienced subjects who volunteered from a group of qualified acupuncture therapists were recruited for the experiment. Muscle oxygenation and blood volume were measured by a near-infrared spectrometer (Model HEO-200, OMRON Ltd. Inc., Japan). The equipment has a flexible probe consisting of 2 LEDs that emit light at 760 nm or 840 nm. The light can penetrate soft tissue up to approximately 2.0 cm when the detector is at a distance of 4 cm from the radiation source. Relative changes in oxygenated haemoglobin (oxy-Hb), deoxygenated Hb (deoxy-Hb), and total Hb (t-Hb) were calculated by the equation reported in a previous study (Shiga et al., 1997). However, continuous wave NIRS could not provide absolute values because of unknown physical properties such as the optical path length in tissue (Hamaoka et al., 2007; McCully & Hamaoka, 2000). Usually, arterial occlusion to the limbs makes it possible to produce zero physiological oxygenation and to compare NIRS values among varied individuals and different regions (Hamaoka et al., 1996). However, since the arterial occlusion method could not be applied to the shoulder muscle, oxygenation could not be quantified in the muscle of shoulder stiffness origination. To compare muscle oxygenation in the trapezius, isometric maximum voluntary contraction (MVC) was used in an attempt to induce maximal deoxygenation in the muscle (Matsumoto et al., 2005; Okubo et al., 2000). However, studies on this topic do not entirely address whether the MVC method can create maximal deoxygenation during the contraction and maximal post-contraction hyperemic response consistently in all subjects. Corrected concentrations (mM) considering the subcutaneous adipose tissue thickness (ATT) for oxy-Hb, deoxy-Hb, and t-Hb were obtained by dividing the concentrations with normalized measurement sensitivity S as shown in the literature (Niwayama et al., 2002). Two sets of

NIRS probes, with 40-mm light-source detector spacing, were placed on the right trapezius muscle, with a 50-mm distance between the probes (Fig.1).

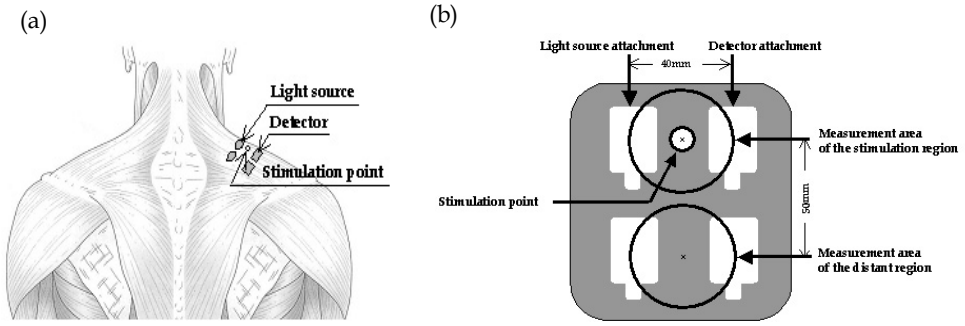

Fig. 1. Measurement site and stimulation point (a) and magnified measurement area and stimulation point (b)

NIRS probes were placed on the belly of the right trapezius muscle of the shoulder, with a 50-mm separation between probes (Fig. 1a & 1b). The measurement range is in the hemisphere of 1/2 of the distance between the light source and detector (20 mm) (Chance et al., 1992). It is necessary to separate the measurement areas by 40 mm or more to prevent the near-infrared rays interfering when two NIRS are measured simultaneously. Therefore, we assumed a distance of 50 mm in consideration of this issue (Fig. 1 b). Changes in muscle oxygenation (oxy-Hb) and blood volume (t-Hb) in stimulation and distant regions (50 mm away from the stimulation point) were measured.

In this experiment, we determined the stimulation point anatomically in the center of the line which tied up the acromion edge to the seventh cervical spine spinous process. This position is the same as GB21 (Jianjing) unified by WHO/WPRO (*WPRO*, 2008). The other probe located on the same muscle, 50 mm away from the stimulation region, was provided as the reference point (distant region). Measurements began with a 3-min rest period, followed by "Jakutaku" (AS) for 2 min, and recovery after stimulation.

There was no change in any parameters for the control group. There was a significant increase in oxy-Hb and t-Hb in the stimulation region compared to the distant region (Fig.2). Although the distribution of C polymodal nociceptors has been examined in the skin of human limbs but not in the muscle (Ochoa & Torebjork, 1989), the results of this study indicated that the effect of the AS might be, at most, localized ~3 cm away from the AS point or an area ~57 cm^3. One might argue that the increases in oxy-Hb and t-Hb found in this study could be due to vasodilatation of the skin. However, since the path of the photons follows the so-called banana-shaped characteristics, the contribution of the skin to the signal should be far less than that of the muscle when an appropriate source-detector spacing of 4 or 5 cm is used (Chance et al., 1992; Ferrari et al., 2004). In the stimulation region, a significant increase in oxy-Hb and t-Hb compared with the pre-stimulation level was first noted at approximately 58 s and 14 s, respectively, after the onset of stimulation. A slower response for oxy-Hb suggests that refilling of oxygenated blood into the small vessels, especially to the capillaries and venules, required a certain length of time after vasodilatation of the arterioles and venules where AS-induced vasodilators might directly

have an influence. The delayed vasodilatation response (14-s latency for the increase in t-Hb after stimulation) might be due to the nature of the response time for nociceptors and C-fibres. It has been reported that vasodilatation induced by the stimulation of peripheral terminals of nociceptors develops with a latency of 15–20 s and outlasts the time of stimulation (Häbler et al., 1997). It has also been suggested that C-fibre activation evokes an ~5-s delay from the onset of AS at a very low (1 Hz) frequency of stimulation (Ochoa & Torebjork, 1989). The long-lasting effects, even after the termination of stimulation where the needle remains stationary, on hyperoxygenation indicate that vasoactive substances such as calcitonin gene-related peptide (CGRP) from the sensory nerve terminals are involved in the mechanisms (Sandberg et al., 2003).

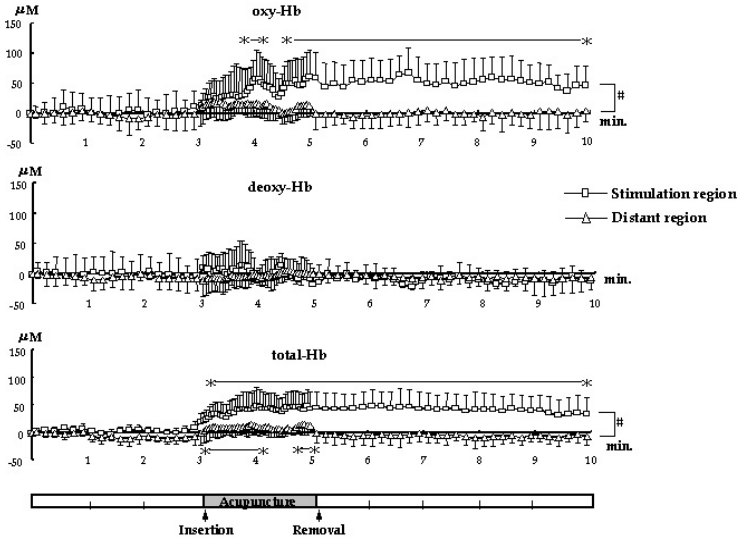

Fig. 2. Changes in oxy-Hb, deoxy-Hb, and t-Hb at pre-stimulation rest and during acupuncture stimulation (AS) and recovery from AS in the stimulation and distant regions in the trapezius muscle

In this study, it is suggested that vasodilative substances induced by AS, mediated by the axon reflex via polymodal receptors in the skeletal muscle and the skin, played a role in the local muscle oxygenation response to the stimulation. The oxygenation and blood volume response was localized to the region where AS was applied.

2.2 The effect of varying acupuncture techniques on muscle blood volume and oxygenation response

In this section, we compared the trapezius muscle blood volume and oxygenation in the stimulation region during four different acupuncture techniques that we have selected as typical interventions in Japan.

Nine healthy acupuncture-experienced subjects who volunteered from a group of qualified acupuncture therapists were recruited for the experiment. Muscle oxygenation and blood volume were measured by a near-infrared spectrometer (Model HEO-200, OMRON Ltd.

Inc., Japan). The equipment has a flexible probe consisting of 2 LEDs that emit light at 760 nm or 840 nm. The light can penetrate soft tissue up to approximately 2.0 cm when the detector is at a distance of 4 cm from the radiation source. Relative changes in oxygenated haemoglobin (oxy-Hb), deoxygenated Hb (deoxy-Hb), and total Hb (t-Hb) were calculated by the equation reported in a previous study (Shiga et al., 1997). Corrected concentrations (mM) considering the subcutaneous adipose tissue thickness (ATT) for oxy-Hb, deoxy-Hb, and t-Hb were obtained by dividing the concentrations with normalized measurement sensitivity *S* as shown in the literature (Niwayama et al., 2002). Two sets of NIRS probes, with 40-mm light-source detector spacing, were placed on the right trapezius muscle, with a 50-mm distance between the probes.

Measurements began with a 3-min rest period, followed by one out of four different acupuncture techniques in random order each for 2 min, and recovery after stimulation. Four different acupuncture techniques are static "Chishin method" (retaining needle) and three dynamic methodes such as "Jakutaku method" (small vertical motion), "Sennen method" (half spin returning) and "Shinsen method" (delicate vibration).

We found the increase in blood volume and oxygenation during acupuncture stimulation for all techniques. The kinetics for static stimulation is illustrated in Fig. 3a. However, techniques for static stimulation produced smaller response than the dynamic ones (Fig. 3b), indicating dynamic stimulation is one of the important factors for creating greater vasodilation response by acupuncture. The increase in oxy-Hb and t-Hb during and after stimulation found in this study indicates enhanced blood flow (oxygen supply) to the small vessels, including arterioles, capillaries, and venules (McCully & Hamaoka, 2000). The increase in blood volume is faster than that of oxygenation, which indicates that arteriolar vasodilatation primarily occurs and then oxygenated blood enters into the capillary networks resulting in the increase in tissue oxygenation.

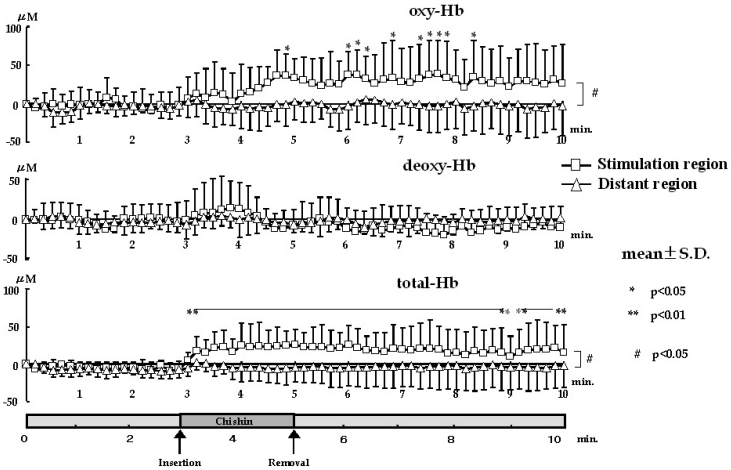

Fig. 3a. Changes in oxy-Hb, deoxy-Hb, and t-Hb at pre-stimulation rest and during static acupuncture stimulation (AS) and recovery from AS in the stimulation and distant regions in the trapezius muscle

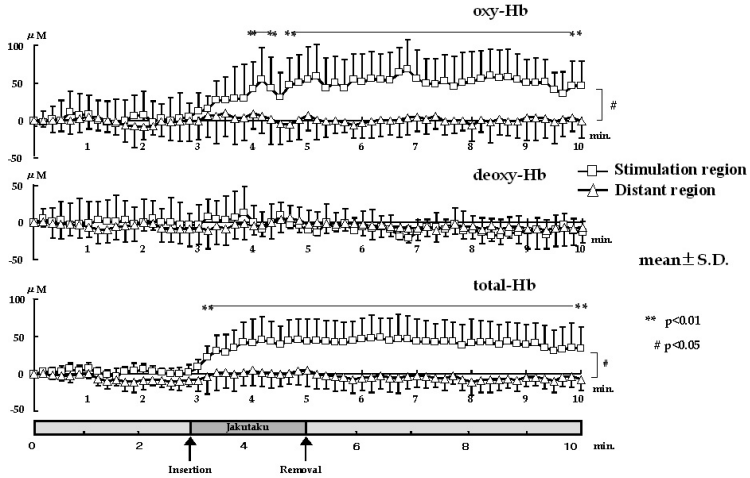

Fig. 3b. Changes in oxy-Hb, deoxy-Hb, and t-Hb at pre-stimulation rest and during dynamic (Jakutaku) acupuncture stimulation (AS) and recovery from AS in the stimulation and distant regions in the trapezius muscle

2.3 The difference in muscle blood volume and oxygenation response between acupuncture-experienced and non-experienced individuals

In this section, we compared the trapezius muscle blood volume and oxygenation in the stimulation region for acupuncture-experienced and non-experienced individuals.

The subjects in the study consisted of 7 healthy acupuncture-experienced adults and 5 healthy non-experienced adults. Muscle oxygenation and blood volume were measured by a near-infrared spectrometer (Model HEO-200, OMRON Ltd. Inc., Japan). The muscle blood volume was measured by placing the light source and the detector 40 mm apart and the middle point between the light source and the detector of the right shoulder was used as the stimulation point. Measurements began with a 3-min rest period, followed by "Jakutaku" acupuncture stimulation without provoking the de-qi sensation for 2 min, and recovery after stimulation. After 15 minutes, isometric contraction of the trapezius was carried out for 20 seconds, and the change was measured during the following 5 minutes.

To compare muscle oxygenation in the trapezius, isometric maximum voluntary contraction (MVC) was used in an attempt to induce maximal deoxygenation in the muscle (Matsumoto et al., 2005; Okubo et al., 2000). The blood volume of the trapezius was calibrated as the ratio of the peak value after isometric contraction of the muscle to the blood volume change during acupuncture stimulation (Fig. 4).

We found the increase in blood volume and oxygenation during acupuncture stimulation for both groups (Fig. 5).

However, the vasodilatation response was greater for acupuncture-experienced individuals, indicating some psychological factors may play a role for blunted response for non-experienced individuals. For examples, the increase in sympathetic activity to the vessels in muscle provoked by anxiety for the intervention may one of the causes for the blunted response. Regarding the effects of blood flow, eliciting the de-qi sensation is superior to merely inserting the needle into the muscle (Sandberg et al., 2003). However, insertion into

the muscle without de-qi sensation resulted in a greater increase in blood flow than that into the skin (Sandberg et al., 2003). Painful sensation is not evoked by microstimulation of C-fibre afferents at frequencies less than 2 Hz and even low-discharge frequency stimuli might induce some physiological effects in subjects without any de-qi sensation (Kawakita et al., 2006). Taken together, the stimulation, regarded as low- to moderate-intensity even without the de-qi sensation used in this study, is effective for creating a vasodilatation response, presumably with the presence of C-fibre stimuli without any de-qi sensation and pain.

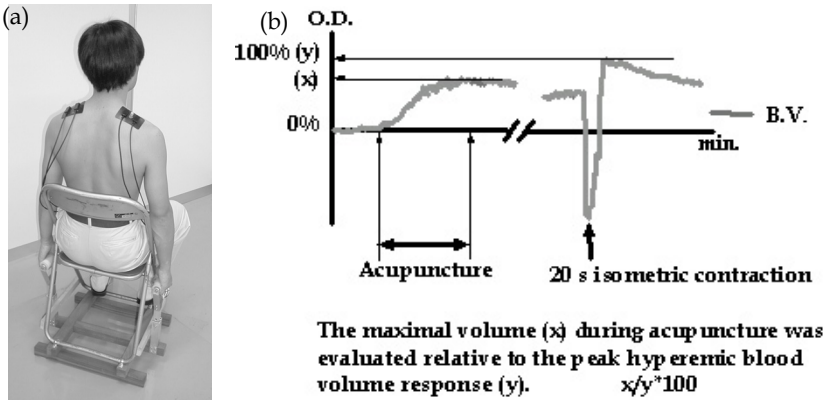

Fig. 4. Experimental setup for NIRS calibration using isometric contraction of the trapezius (a) and representative change in blood volume during acupuncture stimulation, 20 s isometric contraction, and recovery after contraction (b)

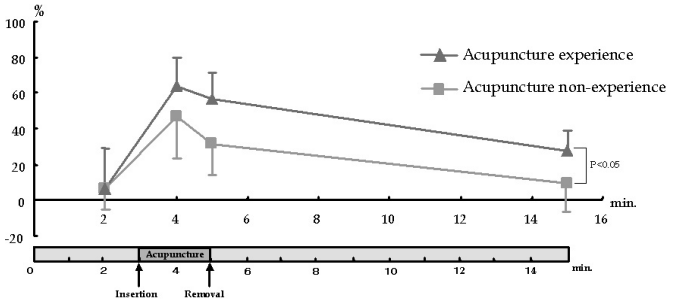

Fig. 5. Changes in blood volume for acupuncture experienced and non-experienced individuals

2.4 The difference in muscle blood volume and oxygenation response between individuals with and without shoulder stiffness

In this section, we attempted to elucidate the difference in vasodilatation response between individuals with and without shoulder stiffness. We compared the trapezius muscle blood volume and oxygenation in the stimulation side where stiffness is prominent for individuals with symptoms and in the non-stimulation contra-lateral shoulder for individuals with and without shoulder stiffness.

The subjects in the study consisted of 6 adults suffering from a shoulder stiffness and 7 healthy adults. Muscle oxygenation and blood volume were measured by a near-infrared spectrometer (Model HEO-200, OMRON Ltd. Inc., Japan). The muscle blood volume was measured by placing the light source and the detector 40 mm apart and the middle point between the light source and the detector of the right shoulder was used as the stimulation point. Measurements began with a 3-min rest period, followed by "Jakutaku" acupuncture stimulation without provoking the de-qi sensation for 2 min, and recovery after stimulation. After 15 minutes, isometric contraction of the trapezius was carried out for 20 seconds, and the change was measured during the following 5 minutes. To compare muscle oxygenation in the trapezius, isometric maximum voluntary contraction (MVC) was used in an attempt to induce maximal deoxygenation in the muscle (Matsumoto et al., 2005; Okubo et al., 2000). The blood volume of the trapezius was calibrated as the ratio of the peak value after isometric contraction of the muscle to the blood volume change during acupuncture stimulation.

Measurements began with a 3-min rest period, followed by the acupuncture into the trapezius muscle for 2 min, and recovery after stimulation. After 15 minutes, isometric contraction of the trapezius was carried out for 20 seconds, and the change was measured during the following 5 minutes. Muscle oxygenation and blood volume were measured using near-infrared spectrometer (Model HEO-200, OMRON Ltd. Inc., Japan).

We found the increase in blood volume and oxygenation during acupuncture stimulation for both groups in the stimulation shoulder with the response being smaller in the symptom (+) group (Fig. 6).

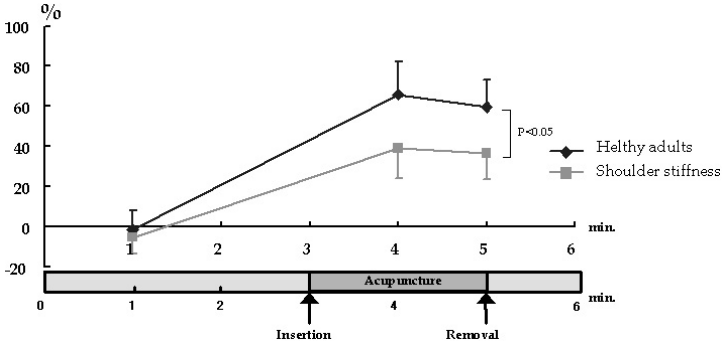

Fig. 6. Changes in blood volume for individuals with and without shoulder stiffness

The response was similar for the two groups in the non-stimulation shoulder, but subjective pain relief was only found in stimulation region for shoulder stiffness individuals. The result of this study indicates that the pain relief response by acupuncture could be brought about only in the stimulation side, presumably due to some local responses such as C-fibre mediated axon reflex.

It is believed that shoulder discomfort occurs due to blood flow restriction resulted from the increase in muscle stiffness and vasoconstriction. The results of this study may be explained by the greater sympathetic nerve activity (SNA) against local metabolic vasodilatation activity for individuals with shoulder stiffness.

2.5 The difference of acupuncture on autonomic system and localized trapezius blood volume and oxygenation responses

In this section, we examined the effects of acupuncture on the autonomic system and the trapezius muscle blood volume and oxygenation.

The subjects in the study consisted of 9 healthy adults. Muscle oxygenation and blood volume were measured by a near-infrared spectrometer (Model HEO-200, OMRON Ltd. Inc., Japan). The equipment has a flexible probe consisting of 2 LEDs that emit light at 760 nm or 840 nm. The light can penetrate soft tissue up to approximately 2.0 cm when the detector is at a distance of 4 cm from the radiation source. Relative changes in oxygenated haemoglobin (oxy-Hb), deoxygenated Hb (deoxy-Hb), and total Hb (t-Hb) were calculated by the equation reported in a previous study (Shiga et al., 1997). However, continuous wave NIRS could not provide absolute values because of unknown physical properties such as the optical path length in tissue (Hamaoka et al., 2007; McCully & Hamaoka, 2000). Usually, arterial occlusion to the limbs makes it possible to produce zero physiological oxygenation and to compare NIRS values among varied individuals and different regions (Hamaoka et al., 1996). However, since the arterial occlusion method could not be applied to the shoulder muscle, oxygenation could not be quantified in the muscle of shoulder stiffness origination. To compare muscle oxygenation in the trapezius, isometric maximum voluntary contraction (MVC) was used in an attempt to induce maximal deoxygenation in the muscle (Matsumoto et al., 2005; Okubo et al., 2000). However, studies on this topic do not entirely address whether the MVC method can create maximal deoxygenation during the contraction and maximal post-contraction hyperemic response consistently in all subjects. Corrected concentrations (mM) considering the subcutaneous adipose tissue thickness (ATT) for oxy-Hb, deoxy-Hb, and t-Hb were obtained by dividing the concentrations with normalized measurement sensitivity S as shown in the literature (Niwayama et al., 2002). Two sets of NIRS probes, with 40-mm light-source detector spacing, were placed on the right trapezius muscle, with a 50-mm distance between the probes.

The sensor of the tonometry device was placed over the left radial artery and the manchette on the left upper arm to monitor the peripheral volumetric pulse waveform and blood pressure (BP). Electrocardiogram (ECG) electrodes were placed on the chest to monitor the heart rate (HR) during the experiment. The subjects were instructed to report the perceived sensation at the end of AS to evaluate a subjective perception of the stimulation. The BP and R-R interval were continuously measured using applanation tonometry and HR monitors, respectively (BP-508, Colin Medical Instruments). The HR and BP recordings were sampled at 2 Hz each during 128-s intervals. All data were acquired via ANS-508 (Colin Medical) and digitized for beat-to-beat R–R interval variability analysis. The frequency domain variables high- (HF, 0.15–0.40 Hz) and low-frequency power (LF, 0.04–0.15 Hz) and the LF/HF-ratio (LF/HF) were analyzed using fast Fourier transform (FFT).

There was a significant increase in oxy-Hb and t-Hb in the stimulation region as shown in Fig. 2. The data for HR, BP, LF, and LF/HF were averaged from 1 to 3 min at rest, from 3 to 5 min during stimulation, and from 8 to 10 min after stimulation; they were denoted as PRE, STM, and PST, respectively. The HR during STM (69.1 ± 7.7 bpm) was significantly lower than that at PRE (74.2 ± 7.8 bpm) and PST (73.3 ± 8.2 bpm) (Fig. 7). There were no significant changes in systolic BP (SBP), diastolic BP, or mean BP. The HF and LF/HF values obtained from HR variability did not show any significant changes throughout the AS experiment. The LF values obtained from SBP variability did not show any significant changes throughout the AS experiment. We found a decreased HR without any modification of sympathetic and parasympathetic activities and the increase in blood volume and

oxygenation during acupuncture stimulation. The peripheral blood pressure and sympathetic responses did not change by acupuncture.

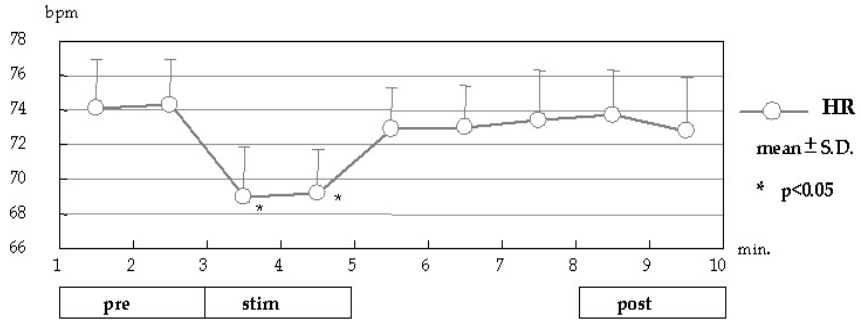

Fig. 7. Change in heart rate (HR) by acupuncture stimulation

We found no changes in LF power for BP variability, an indicator for peripheral SNA, evaluated by the tonometry of the radial artery both during and after AS and MS. There are several reports regarding the influence of AS on SNA in animals and humans. In the line of the results obtained from hypertensive animal experiments (Yao et al., 1982), Knardahl et al. (1998) hypothesized that SNA would decrease during AS by reducing stress. However, they found an increase in SNA during AS in humans. They speculated that the discrepancy between the finding in hypertensive animals (Yao et al., 1982) and the lack of acupuncture-induced sympathoinhibition (Knardahl et al., 1998) could be attributable to a difference in responsiveness related to normo- versus hypertension. However, there is also some evidence for decreasing blood pressure via acupuncture-induced suppressed response of SNA even in normotensive animal experiments (Ohsawa et al., 1995). The discrepancy in SNA response reported in the study of Knardahl et al. (1998) and ours might be due to the degree of central command or mental stress from the AS that is known to bring about an increase in SNA (Anderson et al., 1987).

Central circulation is one of the determinant factors for peripheral circulation. In this study, we did not find any changes in the parameters related to cardiac output (CO) except for HR (an average decrease of 5 rpm during AS, but a return to the baseline levels after AS). Since BP and SNA, indicators of peripheral resistance, and the cardiac sympathetic/parasympathetic activity did not change during AS, it is speculated that the CO remained relatively constant during and after AS. Further, muscle oxygenation levels remained elevated after AS, whereas the HR returned to the pre-AS resting levels following the decrease during AS. Therefore, there is no influence of the central circulation on the response of local muscle oxygenation changes during and after AS. Usually, a parallel decrease in HR is observed with acupuncture-induced BP increase as a result of a baroreflex response (Nishijo et al., 1997; Lin et al., 2000). After the end of the stimulation, a parasympathetic tone or a decreased sympathetic tone appears dominant (Jänig, 1995).

In contrast, weaker stimuli may induce a decrease in both BP and HR (Damen and Brunia, 1987). However, we found only a decrease in HR without any changes in BP or sympathetic activity during and after AS. The study (Nishijo et al., 1997) examined HR response following AS with the administration of atropine and propranolol in conscious humans. Vagal nerves as well as sympathetic nerves can respond well to AS, being moved up and down about 5 mm at a frequency of 1 Hz with a 20 mm insertion depth. The mechanism

attributable to reducing the HR in this study might be the dominant activation of the parasympathetic cholinergic system or cardiac vagal activation.

2.6 The effect of moxibustion on localized trapezius blood volume and oxygenation response

We examined the effects of moxibustion, a potential analogous stimulus with acupuncture, on the trapezius muscle blood volume and oxygenation. The subjects in the study consisted of 9 healthy adults. Muscle oxygenation and blood volume were measured by a near-infrared spectrometer (Model HEO-200, OMRON Ltd. Inc., Japan). The equipment has a flexible probe consisting of 2 LEDs that emit light at 760 nm or 840 nm. The light can penetrate soft tissue up to approximately 2.0 cm when the detector is at a distance of 4 cm from the radiation source. Relative changes in oxygenated haemoglobin (oxy-Hb), deoxygenated Hb (deoxy-Hb), and total Hb (t-Hb) were calculated by the equation reported in a previous study (Shiga et al., 1997). Corrected concentrations (mM) considering the subcutaneous adipose tissue thickness (ATT) for oxy-Hb, deoxy-Hb, and t-Hb were obtained by dividing the concentrations with normalized measurement sensitivity *S* as shown in the literature (Niwayama et al., 2002). Two sets of NIRS probes, with 40-mm light-source detector spacing, were placed on the right trapezius muscle, with a 50-mm distance between the probes.

The warming moxa (Kamaya moxa Co. Ltd.) was placed on the stimulation point, and the timing for the ignition was adjusted such that the subjects experienced a thermal perception 3 min after the start of the measurement. The measurement was continued for 7 min thereafter (total: 10 min).

We found a robust increase in localized blood volume and oxygenation during and after moxibustion stimulation periods and the increase was larger than that of acupuncture. The magnitude of stimulation could be greater in moxibustion than that of acupuncture, but the response is still localized and is not propagated to the contralateral trapezius (Fig. 8).

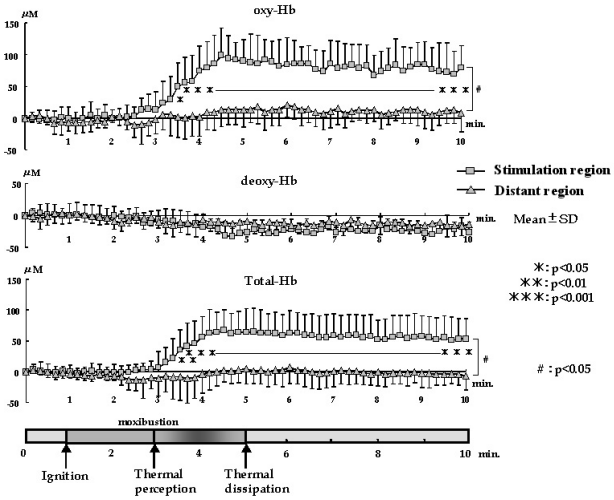

Fig. 8. Changes in oxy-Hb, deoxy-Hb, and t-Hb at pre-stimulation rest and during moxibustion stimulation (MS) and recovery from MS in the stimulation and distant regions in the trapezius muscle

It is suggested that vasodilative metabolites induced by MS, presumably mediated by the axon reflex via polymodal receptors in the skeletal muscle and the skin, attributed to the local muscle oxygenation response to the stimulation. The oxygenation and blood volume response was localized to the region where MS was applied similar to the AS.

3. Summary

In this study, vasodilative substances induced by AS and MS, mediated by the axon reflex via polymodal receptors in the skeletal muscle and the skin, played a role in the local muscle oxygenation response to the stimulation. The oxygenation and blood volume response was localized to the region where AS and MS were applied. However, techniques for static AS produced smaller response than the dynamic ones, indicating dynamic stimulation is one of the important factors for creating greater vasodilation response by acupuncture.

The vasodilatation response was greater for acupuncture-experienced individuals, indicating some psychological factors may play a role for blunted response for non-experienced individuals. We found the increase in blood volume and oxygenation during acupuncture stimulation for both groups in the stimulation shoulder with the response being smaller in the symptom (+) group. The HR during AS was lowered, but BP and cardiac and peripheral SNA did not change. We found a robust increase in localized blood volume and oxygenation during and after moxibustion stimulation periods and the increase was larger than that of acupuncture.

NIRS is able to provide an objective indication for examining the degree of vasodilatation (hyperoxygenation) response. Monitoring NIRS indications would be useful, in particular, for treating patients with shoulder stiffness or muscle spasms to determine the optimal intensity and frequency of AS and MS.

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4

An fMRI Investigation on Brain Activity in Response to Unilateral Acupuncture, Electroacupuncture and Electromyostimulation on ST36 and ST39

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1. Introduction

It is known that unilateral resistance exercise training may result in an increased muscular strength not only in the exercised muscle, but also in the unexercised homologous muscle in the contralateral limb. This phenomenon is termed cross education. Cross-education effect has been shown in hand, arm and leg muscles after various types of resistance training (Farthing, 2009; Hortobagyi, 2005; Lee & Carroll, 2007; Lee et al., 2010; Munn et al., 2004; Zhou, 2000). Furthermore, it has been reported that repeated unilateral transcutaneous electrical stimulation on a limb muscle or a nerve trunk may also cause cross-education effect (Bezerra et al., 2009; Cabric & Appell, 1987; Hortobagyi et al., 1999; Singer, 1986; Tachino et al., 1989; Zhou et al., 2002). Although the magnitude of strength gain in the contralateral limb is generally less than that in the exercised limb, this cross-over effect might have clinical values in neuromuscular rehabilitation (Farthing et al., 2009; Singer, 1986; Woo et al., 2006).

More interestingly, unilateral therapy for treatment of conditions on the contralateral side of the body has been used in traditional Chinese medicine for centuries (Kim et al., 2010; Woo et al., 2006). One particular type of treatment, *juci*, involves acupuncture on one side of the body to affect the function of the other side (Lin & Pan, 2004). This appears to be similar to the concept of cross education. A recent investigation in our laboratory has demonstrated that four weeks of electroacupuncture on tibialis anterior muscle (TA) of one limb can significantly increase dorsiflexion muscle strength in both the stimulated limb and the contralateral limb (Huang et al., 2007).

The exact mechanism of cross education is not clear. In principle, muscle strength can improve in adaptation to voluntary exercise training due to either or both an improved neural control and/or muscle hypertrophy. Because there has been little evidence of a significant muscle hypertrophy associated with improved strength in the contralateral limb,

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it is believed that cross education is primarily caused by adaptations in the central nervous system (Bezerra et al., 2009; Dragert & Zehr, 2011; Everaert et al., 2010). Several candidate mechanisms have been proposed in the literature, including ipsilateral innervation to the muscle due to a small proportion of uncrossed nerve fibres in the corticospinal pathway; bilateral activation of muscles for maintenance of posture during a unilateral exercise; and bilateral interaction between the two hemispheres of the brain (Carroll et al., 2006; Farthing, 2009; Hortobágyi et al., 2011; Zhou, 2000). However, the neural mechanisms for the cross education induced by electric stimulation or electroacupuncture may not be the same as that induced by voluntary exercise because the stimulation is applied to a peripheral nerve or muscle that by-passes the corticospinal pathway. It has been speculated that the sensory afferents may play an essential role in mediating cross-education effect induced by electric stimulation and electroacupuncture (Hortobagyi, 2005; Huang et al., 2007). Furthermore, whether the cross-education effect caused by electrical stimulation on surface or via needling at the acupoint involves similar or different neural mechanisms has not been examined.

The research presented in this Chapter utilised the functional magnetic resonance imaging (fMRI) technique to examine the brain activities during unilateral electric stimulation and acupuncture. The technique of fMRI is based on detection of blood oxygenation level dependent (BOLD) signals that provides a direct and precise indication of the regions in the brain involved in a given sensory-motor task.

The aim of the present study was to compare the areas of the brain that were activated, as indicated by fMRI, during unilateral transcutaneous electrostmulation, manual acupuncture and electroacupuncture on the acupoints of ST36 (*Zusanli*) and ST39 (*Xiajuxu*) in healthy young adults, in order to obtain a better understanding of the differences in the areas activated during these tasks and how these might contribute to the mechanisms of cross education or unilateral therapy. It was hypothesised that electroacupuncture on acupoints and transcutaneous electric stimulation on the same areas of the tibialis anterior muscle would induce similar level of activities in certain regions of the contralateral and ipsilateral side of the brain; and manual acupuncture at the same acupoints may also cause activation of the same regions in the brain, but to a less magnitude, as indicated by the BOLD signals.

2. Methods

Six healthy young men with a mean age of 23 years (range 21-26 years) volunteered for the study. All participants were right-foot dominant as identified using an established questionnaire (Li, 1983) and without current neuromuscular, orthopedic, diabetes mellitus and cardiovascular diseases or neuromuscular injuries. Participants were physically active, without a history of specific sport training, especially muscle strength training, during the six months prior to the study, and had no previous experience with acupuncture or electric stimulation. They did not show a fear to acupuncture and electric stimulation. All participants gave their consent to participation prior to the experiment. The experiment was carried out in accord with the Declaration of Helsinki, and the procedure obtained approval by the Human Research Ethics Committee of Southern Cross University, Australia.

One week prior to the formal experiment, each participant was given two familiarisation trials, one in the University's laboratory and the other in the MRI room at the hospital. During the familiarisation trials the detailed experimental procedure was explained to the participants. The participants were given transcutaneous electric stimulation, manual acupuncture and

electroacupuncture at the acupoints ST36 and ST39 of the right leg, respectively. The stimulation intensity was increased gradually till the level that the participant could maximally tolerate. A post-hoc investigation (n=5) found that the electromyogram (EMG) activity of the left anterior tibialis muscle showed no significant changes ($P>0.05$ by ANOVA with repeated measures) between resting and when the right leg received electric stimulation (contraction intensity was up to 40% maximal voluntary contraction, MVC, at the maximum tolerance), electroacupuncture (up to 30%MVC) or manual acupuncture (up to 10%MVC). The EMG activity of the left anterior tibialis during the stimulation or acupuncture on the right leg was generally below 1.5% of that during a maximal voluntary contraction.

During the experimental trial for fMRI scanning, participant was in a supine position, relaxed and with eyes closed. The head position in the head coil was stabilised by foam padding. The right leg of the subject was strapped into a custom-built device at the thigh and foot with the knee joint angle at 0 degrees and the ankle joint at 15 degrees in plantar flexion. The left leg was in full extension and the participant was told to relax the muscles.

The experiment included three tasks with block-design to detect BOLD signals. The tasks were performed in the order of transcutaneous electric stimulation, manual acupuncture and electroacupuncture on the ST36 and ST39 of the right leg. In each task, there was three sets of 1 min rest (reference period) followed by 1 min stimulation or acupuncture. A minimum of 10 minutes rest was given between the tasks.

The location of the acupoints ST36 and ST39 were determined according to the description of traditional Chinese medicine (Beijing College of Traditional Chinese Medicine et al., 1980) by an accredited acupuncturist who had been practicing in hospital for 10 years. The ST36 is located at 3 *cun* distance (*cun* is a unit of length relative to patient's body size in traditional Chinese medicine. Three *cun* is the breadth of the patient's index, middle, ring, and little fingers at the level of proximal interphalangeal joint at the dorsum of the middle finger) from the depression below the patella and lateral to the patellar ligament, and one finger breadth lateral to the anterior crest of the tibia. The ST39 is located at 9 *cun* distance from the depression below the patella and lateral to the patellar ligament and one finger breadth lateral to the anterior crest of the tibia (Figure 1).

During the surface electric stimulation task, the stimulation was applied to the tibialis anterior muscle via a Trio300 stimulator (ITO, Japan). Two 5 cm x 5 cm self-adhesive MRI-compatible electrodes (ITO, Japan) were placed over the muscle with the cathode on ST36 and the anode on ST39. The stimulator was located outside of the MRI room and linked to the electrodes via MRI-compatible wires. The stimulation was delivered at a duty cycle of 5 s rest followed by 5 s stimulation, 6 cycles per set (a total of 1 minute). The electrical pulses were square waves at 50 Hz, with the pulse width of 200 μs and intensity of 48-55 mA according to the level that the participant could maximally tolerate in the familiarisation trials.

During the manual acupuncture task, a pure silver acupuncture needle with diameter of 0.3 mm and length of 50 mm (GB2024-94, Suzhou Medical Appliance Company, Ltd., China) was inserted vertically into the muscle at each acupoint to a depth of 20 to 30 mm by the accredited acupuncturist. The needles were manually twirled at 1 Hz to induce a feeling of *de qi*. The *de qi* sensation is a combination of aching, pressure, soreness, heaviness, fullness, warmth, cooling, numbness, tingling, and dull pain, but not a sharp pain (Hui et al., 2007). The acupuncture was applied for 60 s after 60 s rest in each set. Three sets were performed.

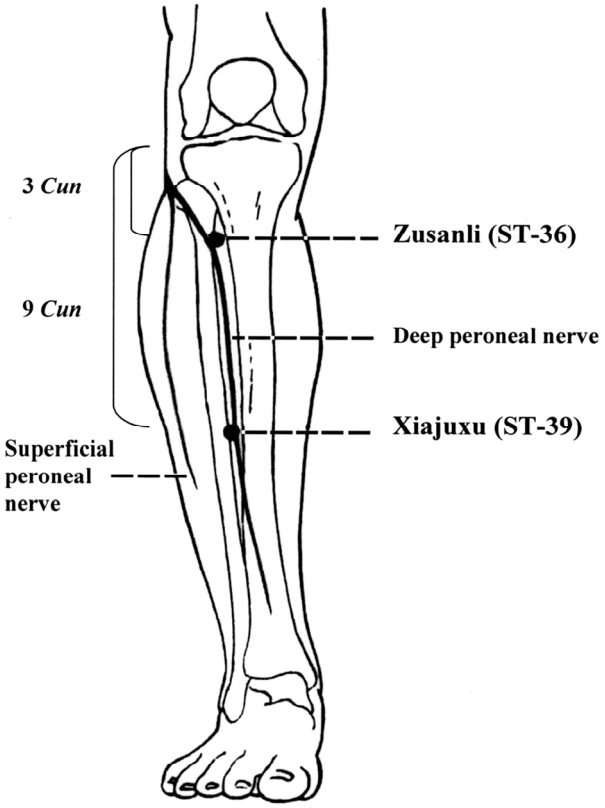


Fig. 1. Location of the acupoints ST36 and ST39 [Figure from Huang et al. (2007). Journal of Alternative and Complementary Medicine, 13(5), 539-546, with permission].

The protocol of electroacupuncture task was similar to that of electric stimulation, with the surface electrodes replaced by acupuncture needles inserted into the muscle at the acupoints. The electrical pulses were delivered to the needles by using an electroacupuncture apparatus (SDZ-II, Suzhou Medical Appliance Company, Ltd., China), with square waves at 50 Hz, pulse width of 200 μs and intensity of 8 to 11 mA which was the maximal level that the participant could tolerate in the familiarisation trials.

A 1.5 T whole body MRI scanner (GE, 1.5 T twin speed infinity with Excite II, USA) was used for fluid attenuation inverse recovery T1-weighted imaging (FLAIR T1WI) and gradient echo-echo planar imaging (GRE-EPI) fMRI scanning. Anatomical images were acquired with a repetition time (TR) of 2250 ms, a time for echo (TE) of 11.6 ms, a time for inversion of 760 ms, bandwidth (BH) 19.32 KHz, field of vision (FOV) 24 cm x 18 cm, data matrix 320 x 224, slice thickness 6 mm with 1 mm gap, for 20 slices that covered the distance from the apex of the scull to the lower edge of the cerebellum. The fMRI used the GRE-EPI technique with parameters of TR 3000 ms, TE 40 ms, flip angle 90 degrees, BH 62.50 KHz, FOV 24 cm x 24 cm, data matrix 128 x 128 for an in-plane resolution of 1.875 mm x 1.875

mm. Slice thickness 6 mm, gap 1 mm, with 20 slices that corresponded to the T1WI. Three scans with a total scanning time of 7 minutes were performed for each task.

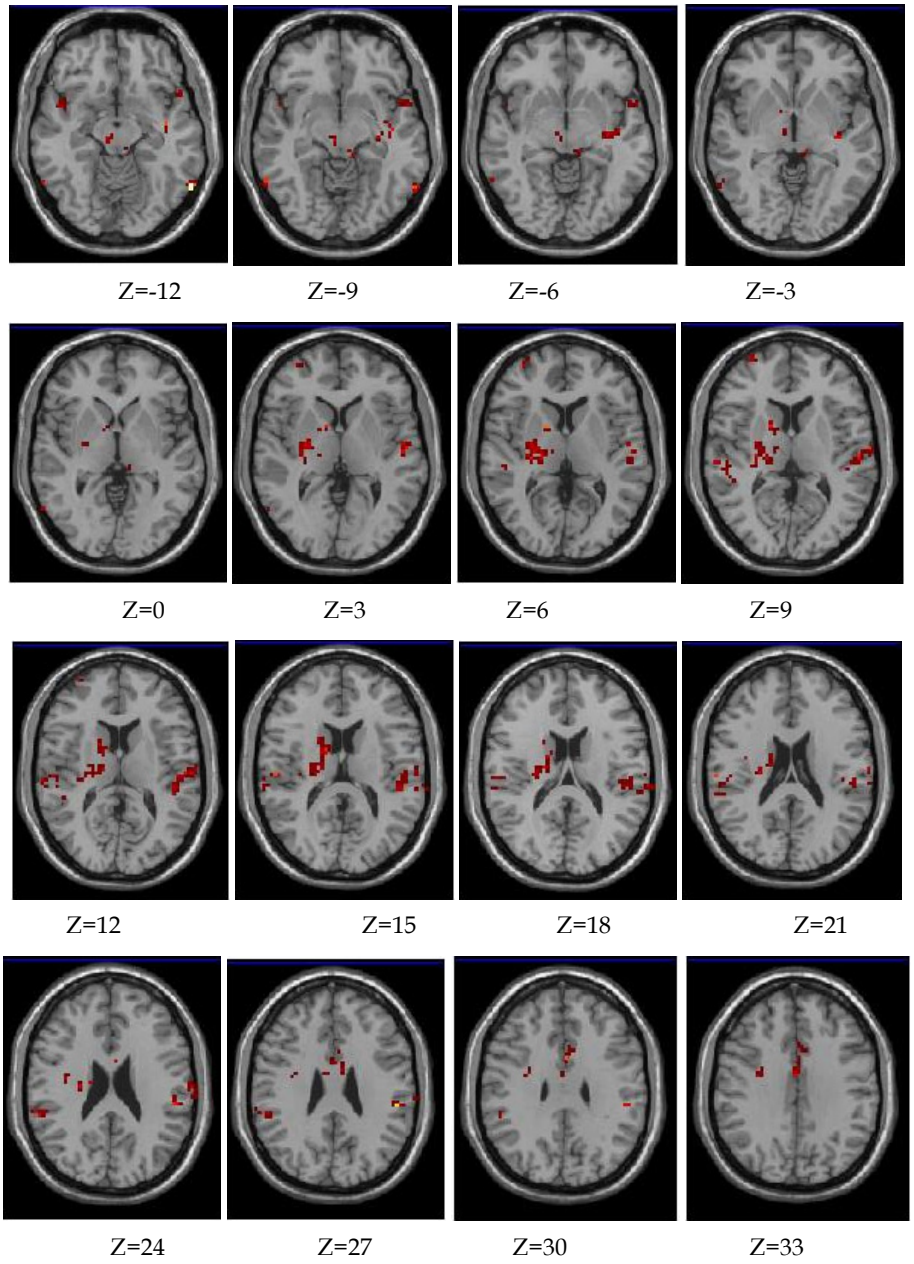
The fMRI signals were monitored by a real time imaging processor. If participant's head position moved for more than 5 mm, the scan would stop automatically. The participants in this study cooperated well and there was no case of significant head movement that required repositioning. The original fMRI data in DICOM format was transferred to a computer where the statistics parameter mapping software was used for analysis (SPM99, Wellcome Department of Imaging Neuroscience, University College London, UK). Conjunction analysis was performed to compare the fMRI signals among the three tasks. The activation of the areas in the brain was estimated by correlative analysis for temporal-signal intensity curve with stimulation. The threshold for identifying an active area was set as 10 voxels. The fMRI image analysis was performed by a medical imaging specialist. Paired *t*-test was used to identify task-related activities as compared to the resting period, with the Alpha level of 0.005 was used for significant differences (uncorrected) (Bai et al., 2009).

3. Results

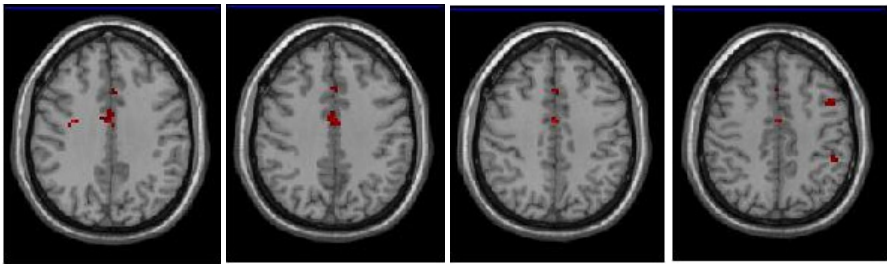
All fMRI trials were completed successfully. During the transcutaneous electric stimulation task, significant activation ($p<0.005$) was detected in the areas of bilateral gyrus postcentralis (GOPC, Brodmann area [BA]43), lobulus parietalis inferior (LPi, BA40), gyrus frontalis medius (GFm, BA8,10), inferior temporal gyrus (GTi, BA37), gyrus temporalis superior (GTs, BA38,42) and brain stem; ipsilateral gyrus frontalis superior (GFs, BA6), insula (INS) and hippocampus (HI); and contralateral gyrus frontalis medialis (GFd, BA6), gyrus cinguli (GC, BA24), nucleus lentiformis (NL), lobulus paracentralis (LPC, BA5,7), gyrus precentralis (GPRC, BA4), cerebellum (Cb) and internal capsule (IC) (Table 1, Figure 2).

Activated Area	Brodmann	Contralateral (mm)			T value	Ipsilateral (mm)			T value
		x	y	z		x	y	z	
GPOC	43	-57	-21	15	14.63	45	-39	63	22.38
LPi	40	-60	-39	24	9.4	51	-30	27	21.70
GTi	37	-63	-54	-9	13.17	60	-60	-12	31.78
GTs	38, 42	-66	-24	12	8.12	63	-12	9	14.07
GFm	8, 10	-36	60	12	9.00	39	12	48	14.14
Brainstem	/	-6	-21	-9	9.61	15	-30	-39	11.59
INS	/	/	/	/	/	39	-9	-12	15.09
HI	/	/	/	/	/	30	-18	-9	8.13
GFS	6	/	/	/	/	9	9	63	10.82
GFd	6	-3	-21	63	12.47	/	/	/	/
GC	24	-3	-6	33	10.10	/	/	/	/
NL	/	-18	6	6	15.70	/	/	/	/
LPC	5, 7	-6	-33	63	16.86	/	/	/	/
GPRC	4	-33	-6	36	9.73	/	/	/	/
Cb	/	-15	-66	-33	9.58	/	/	/	/
IC	/	-15	0	15	12.54	/	/	/	/

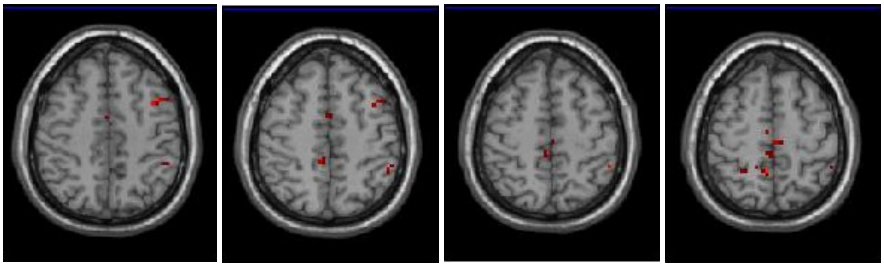
Table 1. Montreal Neurology Institute (MNI) coordinates of local maxima of cortical clusters showing significant ($P<0.005$) activity associated with transcutaneous electric stimulation at the acupoints of ST36 and ST39 in the right leg.



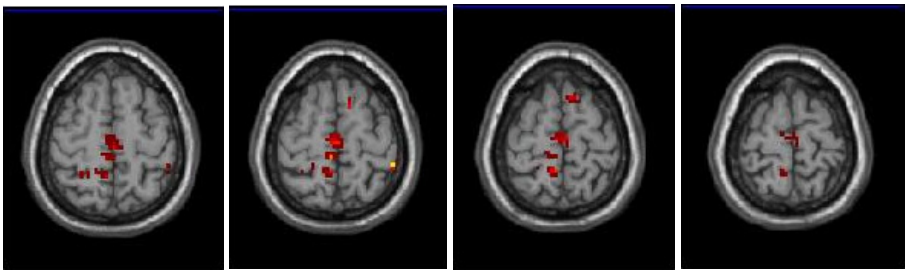
(Figure 2)



Z=36 Z=39 Z=42 Z=45



Z=48 Z=51 Z=54 Z=57



Z=60 Z=63 Z=66 Z=69

(Figure 2 continuing)

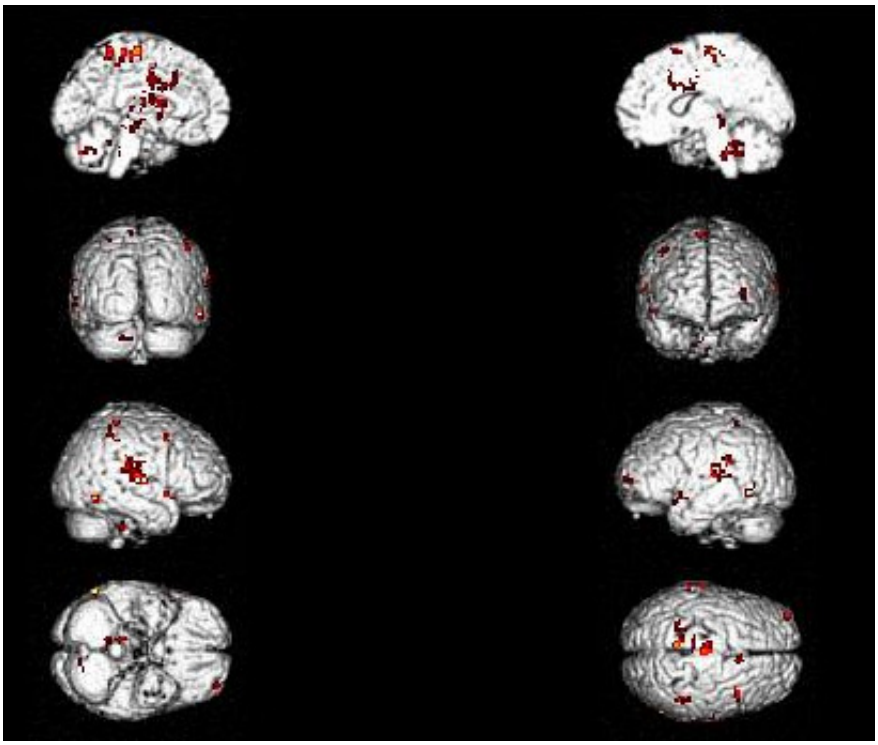
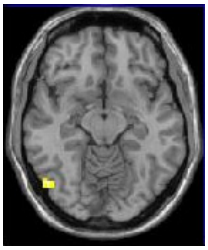


Fig. 2. Brain areas activated (coloured areas) during transcutaneous electric stimulation on the acupoints of ST36 and ST39 in the right leg (P<0.005).

During the manual acupuncture task, significant activation was detected only in the area of the contralateral gyrus occipitalis medius (Gom, BA19) (Table 2 and Figure 3).

Activated	Brodmann	Contralateral (mm)			T value	Ipsilateral (mm)			T value
Area		x	y	z		x	y	z	
Gom	19	-51	-69	-12	5.55	/	/	/	/

Table 2. Montreal Neurology Institute (MNI) coordinates of local maxima of cortical clusters showing significant (P<0.005) activity associated with manual acupuncture at the acupoints of ST36 and ST39 in the right leg.



Z=-12

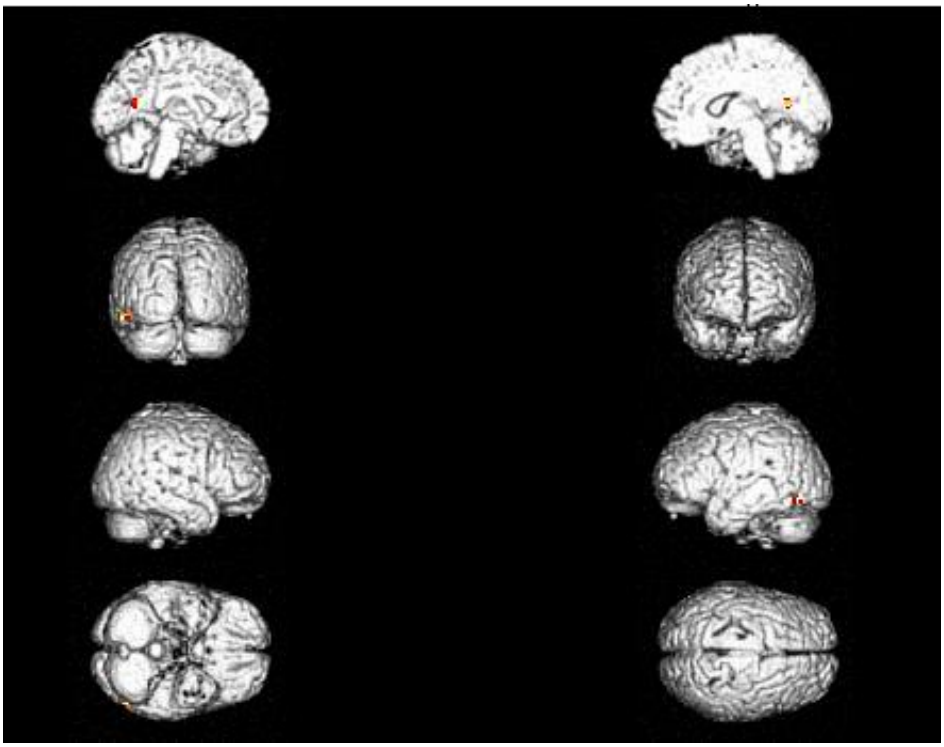
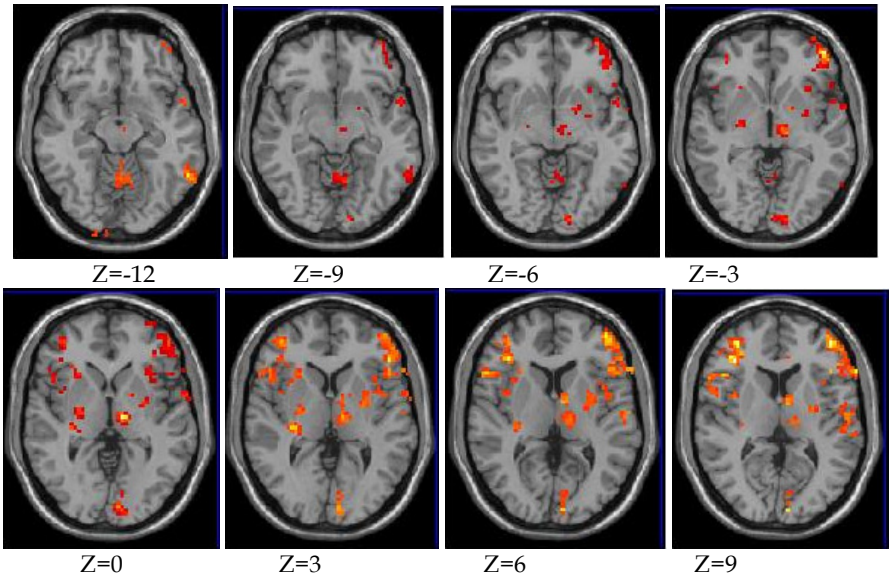


Fig. 3. Brain areas activated (coloured areas) during manual acupuncture on the acupoints ST36 and ST39 in the right leg ($P<0.005$).

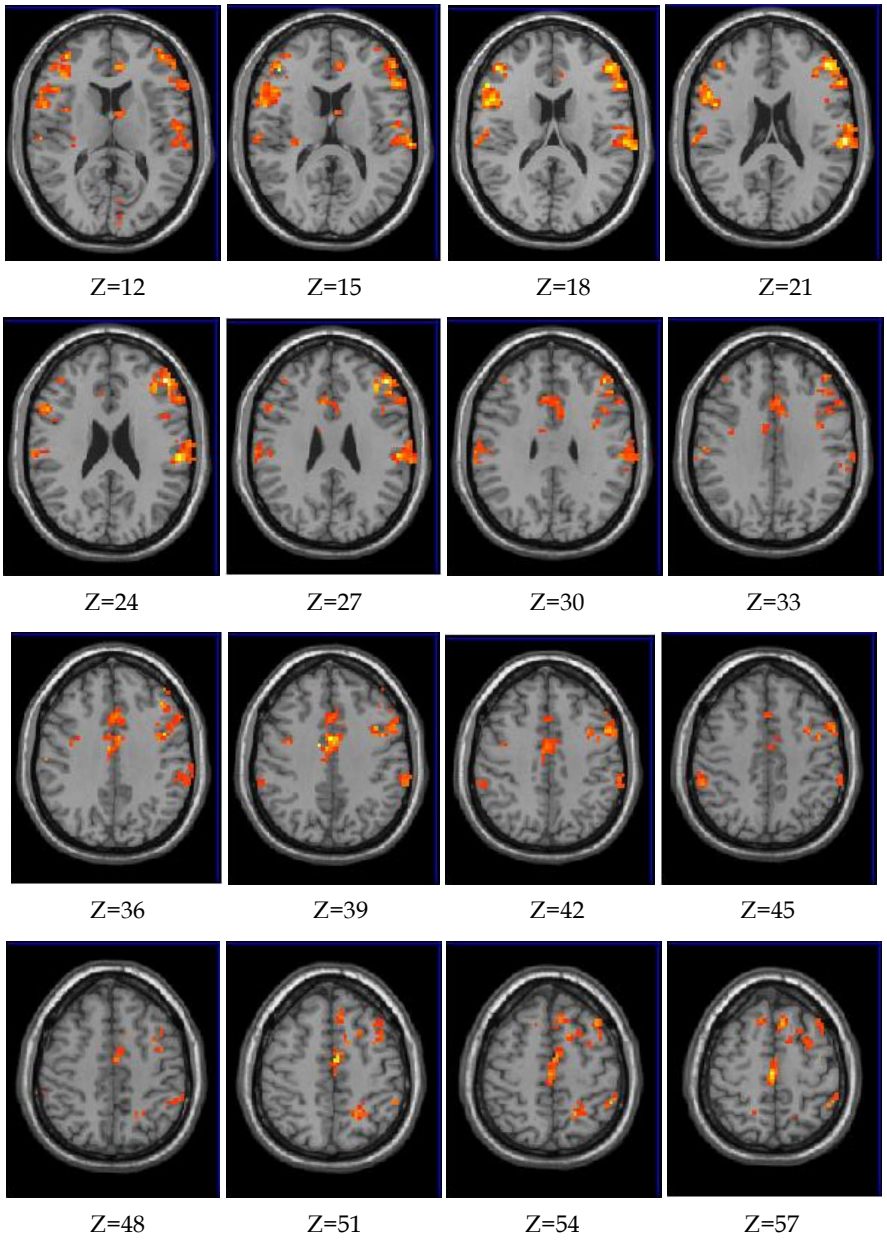
During the electroacupuncture task, significant activation was identified in the areas of bilateral gyrus frontalis superior (GFs, BA6), gyrus frontalis medius (GFm, BA6,10,46), gyrus frontalis inferior (GFi, BA44-47), gyrus frontalis medialis (GFd, BA6,8), gyrus postcentralis (GPOC, BA40), lobulus parietalis inferior (LPi, BA40), precuneus (PCU, BA7), nucleus lentiformis (NL), insula (INS, BA13), gyrus cinguli (GC, BA 24,32), cerebellum (Cb) and brain stem; ipsilateral thalamus (TH) and cuneus (CU, BA18); and contralateral gyrus parahippocampalis (GH, BA35) and gyrus precentralis (GPRC, BA4) (Table 3, Figure 4).

Activated Area	Brodmann	Contralateral(mm)			T value	Ipsilateral(mm)			T value
		x	y	z		x	y	z	
GPOC	40	-66	-24	21	6.69	60	-24	21	11.52
GFd	6, 8	-3	-21	57	9.17	6	27	51	5.80
GFm	6, 10, 46	-36	48	9	10.87	45	39	24	13.27
GFi	44,45,46,47	-39	33	9	12.40	33	24	0	4.69
GC	24, 32	-3	0	39	9.67	6	39	12	6.54
Brainstem	/	-3	-24	-30	5.52	3	-18	-27	6.56
GTS	22, 42	-60	-21	12	6.12	66	-27	15	9.69
GL	18	-18	-102	-15	6.00	9	-93	-9	6.58
PCU	7	-18	-54	57	3.91	18	-54	54	6.85
LPi	40	-57	-39	45	5.85	48	-45	54	7.79
NL	/	-24	-12	0	5.96	15	3	-3	7.09
INS	13	-39	9	0	3.83	39	9	6	4.56
U	28, 36	-27	-3	-39	4.91	33	6	-24	5.64
GFs	6, 8	-12	24	57	5.38	6	21	57	7.20
Cb	/	-9	-72	-33	7.52	6	-60	-9	5.85
TH	/	/	/	/	/	9	-15	0	11.83
GTm	21	/	/	/	/	57	-54	-12	9.14
CU	18	/	/	/	/	6	-93	6	8.54
GPRC	4	-39	-3	39	5.87	/	/	/	/
GH	35	-27	0	-30	7.11	/	/	/	/
IC	/	-33	-24	3	7.97	/	/	/	/

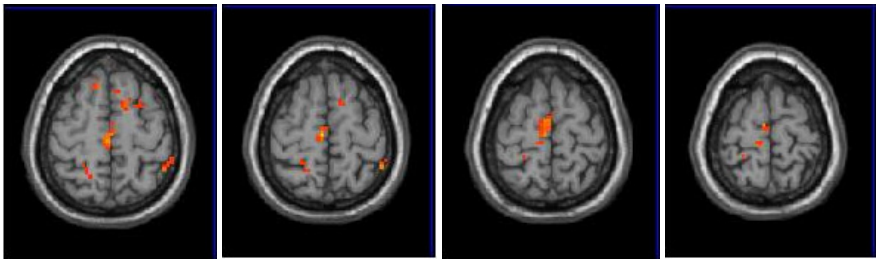
Table 3. Montreal Neurology Institute (MNI) coordinates of local maxima of cortical clusters showing significant (P<0.005) activity associated with electroacupuncture at the acupoints of ST36 and ST39 in the right leg.



(Figure 4)



(Figure 4 continuing)



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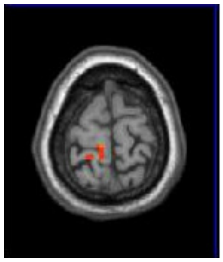

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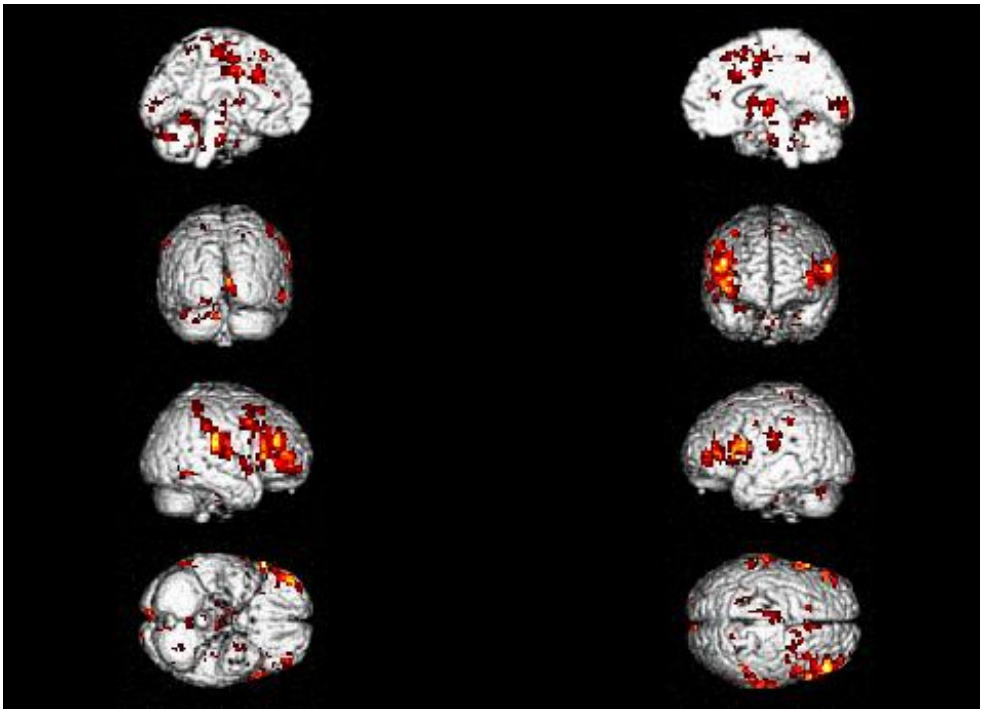

Fig. 4. Brain areas activated (coloured areas) during electroacupuncture on the acupoints of ST36 and ST39 in the right leg ($P<0.005$).

4. Discussion

The results of present study demonstrated significant changes of BOLD signals in multiple brain areas during the tasks of unilateral transcutaneous electric stimulation and electroacupuncture on the acupoints of ST36 and ST39 (Table 1 and 3, Figure 2 and 4). In contrast, significantly activity was observed only in one area during the manual acupuncture task (Table 2 and Figure 3).

The present findings on the fMRI changes in response to electric stimulation appear to be in line with the information obtained from other studies. There have been reports that unilateral electric stimulation to upper limb muscles (wrist extensors and flexors) caused increased BOLD signals in the bilateral secondary somatosensory cortex, the supplementary motor area and anterior cingulate cortex; contralateral primary motor cortex, primary somatosensory cortex and premotor cortex; and the ipsilateral cerebellum (Blickenstorfer et al., 2009; Han et al., 2003). In respect of lower limb muscles, Francis and associates compared BOLD signals during unilateral active and passive dorsiflexion movement, and electric stimulation-induced dorsiflexion, and reported that the electric stimulation induced greater brain activities than passive movement, but lower activities than that induced by active movement (Francis et al., 2009). The major brain areas that showed significant activities during the electric stimulation task included bilateral dorsal and ventral premotor areas and cerebellum; and contralateral primary motor, primary sensory, secondary somatosensory areas, as well as in supplementary motor area and cingulate motor areas (Francis et al., 2009). The present results showed a similar pattern with significant activities found in the contralateral (BA4, 6, 8, 10) and bilateral (BA8, 10) motor-related areas and somatosensory areas (BA40) (Table 4).

Brain area	Electric stimulation		Electroacupuncture	
(Abbreviation, Brodmann area)	Contralat.	Ipsilat.	Contralat.	Ipsilat.
Gyrus presentralis (GPRC, BA4)	+		+	
Gyrus frontalis medius (GFm, BA8,10)	+	+	+	+
Gyrus frontalis medialis (GFd, BA6)	+		+	+
Gyrus frontalis superior (GFs, BA6,8)		+	+	+
Gyrus frontalis inferior (GFi, BA44-47)			+	+
Brain stem	+	+	+	+
Cerebellum (Cb)	+		+	+
Gyrus postcentralis (GOPC, BA40)	+	+	+	+
Lobulus parietalis inferior (LPi, BA40)	+	+	+	+
Precuneus (PCU, BA7)			+	+
Nucleus lentiformis (NL)	+		+	+
Gyrus cinguli (GC, BA24)	+		+	+
Uncus (U, BA28,36)			+	+
Insula (INS, BA13)		+	+	+

Table 4. Comparison of brain areas that showed significant changes in fMRI signals during transcutaneous electric stimulation and electroacupuncture on acupoints of ST36 and ST39. "+" indicates a significant change in BOLD signals at $P<0.005$ level.

A number of studies have also reported the effects of acupuncture and electroacupuncture on brain activities as indicated by BOLD signal changes. For example, Bai and colleagues

reported fMRI signal changes in response to manual acupuncture at ST36 in sensorimotor cortices, bilaterally in BA9, 10, 11, 40, 44 and 45, brainstem and cerebellum; ipsilaterally in BA6; and contralaterally in BA3, etc. (Bai et al., 2009). Zhang and associates investigated the effects of electroacupuncture on acupoints of *Yanglingquan* (GB34) and *Xuanzhong* (GB39) on the left leg, and reported significantly increased brain activities bilaterally in BA4, 6, 10, 11, 18, 23 and 24, insula and cerebellum; and contralaterally in BA44 and 45, caudate, putamen and midbrain (Zhang et al., 2007). The present study also found significantly increased activities in bilateral BA6, 7, 8, 10, 13, 28, 26, 40 and 44-47, brainstem and cerebellum; and contralaterally in BA4 (Table 3 and 4). However, the manual acupuncture induced significant activity only in BA19 of the contralateral side (Table 2). The lack of significant changes in BOLD signals compared with other reports might be related to the higher threshold (10 voxels vs 3 voxels used in Bai et al., 2009) and alpha level (0.005 vs 0.05 or 0.01 used in Zhang et al., 2007) used in this study. Another possible reason could be that only the twirling manipulation of the needles was used during the experiment without the use of lifting and thrusting techniques. Therefore, the stimulation might not be strong enough to produce wider activations in the brain.

Electric stimulation has been widely used as a supplementary training method for improving muscular strength and fitness as well as a means of rehabilitation (Maffiuletti, 2010; Paillard, 2008). Electroacupuncture has recently been shown to be able to improve muscle strength as well (Huang et al., 2007). Although the electric stimulation and electroacupuncture can directly activate peripheral nerve and muscle fibres and by-pass the central nervous system, recent evidence has suggested that the training/therapeutic effects of electric stimulation may also include neural plasticity (Chae et al., 2008; Maffiuletti, 2010; Wolpaw, 2007). The current results provide new evidence about the cortical responses by comparison of the BOLD signal changes during electroacupuncture and electric stimulation.

It was interesting to find that there were common bilateral activations during electric stimulation and electroacupuncture tasks in the somatosensory areas (GPOC and LPi) and secondary motor areas (GFm) that are known to be associated with somatosensory inputs processing and motor preparation, motor program encoding and sensorimotor integration (Table 4). Furthermore, electroacupuncture induced much wider bilateral brain activation than surface electric stimulation and manual acupuncture.

In the present study, the stimulation intensity utilised in the electric stimulation and electroacupuncture task was limited by participants' tolerance to the discomfort and pain. Obviously, a common factor in these two treatments was the nociceptive afferents. Pain (from nociceptors) has been shown to elicit activation of the sensorimotor cortices, rostral anterior cingulate cortex, insula, cerebellum, hippocampus and brain stem (Hui et al., 2005; Hui et al., 2009; Kong et al., 2007; Lewith et al., 2005; Seidler et al., 2004) through spinothalamus bundle. A number of cortical areas have been shown to be involved in pain processing, including the primary somatosensory cortex, the secondary somatosensory cortex, the insula, the anterior cingulate, the prefrontal cortex, the hypothalamus and periaqueductal gray (PAG) (Kong et al., 2009; Zhang et al., 2004). The primary and secondary somatosensory cortices are involved in the sensory discriminative aspect of pain processing (Liu et al., 2010), such as intensity determination and location of nociceptive stimulation (Tracey, 2005), as components of the sensory (pain) network (Wager, 2005). Afferent inputs have shown to alter the excitability of motoneurons and interneurons that affects the contralateral limb at the spinal (Robinson et al., 1979) or higher levels in the central nervous system (Hortobagyi et al., 2003; Kaelin-Lang et al., 2002). Therefore, it is

speculated that the nociceptive inputs, together with other somatosensory afferents, may play an important role in mediating neural plasticity in adaptation to unilateral electric stimulation training and electroacupuncture. However, changes in fMRI signals observed in this study can only provide neuroanatomical evidence for the brain areas involved in the prescribed tasks and cannot provide direct evidence for the functional links between these cortical areas. How the sensory afferents affect motor function that results in improved expression of muscular strength requires further investigations.

An interesting finding of the present study is that the electroacupuncture induced a much wider cortical activation than the surface electric stimulation at the two acupoints. One possible explanation is that acupuncture required insertion of a needle into the muscle and manipulation of the needle to induce the sensation of *de qi*. The *de qi* sensation reported by the participants included aching, soreness and pressure, tingling, numbness, dull pain, heaviness, warmth, fullness and coolness without sharp pain. The complex sensations in *de qi* suggest involvement of a wide spectrum of myelinated and unmyelinated nerve fibers, particularly the slower conducting fibers in the tendinomuscular layers (Hui et al., 2007). In addition of *de qi*, electric pulses were delivered to the needle.

Whether the wider cortical responses observed in the electroacupuncture task compared with the surface electric stimulation task indicates the unique treatment effect of inserting needle at the acupoint would be an interesting question. There has been a study on rats that compared the effects of acupuncture or electroacupunture at different depth (sham acupuncture), and with (true electroacupuncture) or without electric current (sham electroacupuncture) (Chiu et al., 2003). The results of the study showed that there was no neural activation caused by the sham acupuncture. The sham electroacupuncture only induced a slight increase in brain activity in the hypothalamus; however, true electroacupuncture elicited enhanced hypothalamus response (Chiu et al., 2003). The results suggested that electroacupuncture at the acupoint produce greater activation of the central nervous system. However, one limitation of using animal models to investigate the effects of acupuncture is that no *de qi* sensation can be substantiated as that observed in humans. It has been suggested that transcutaneous electric stimulation should make no difference as compared with electroacupuncture on the identified points in respect of pain control (Han, 1997). However, the present results indicate there are differences between the cortical responses to the surface and needle stimulation at the given acupoints.

It has been reported that the increases of BOLD signal in secondary somatosensory area and insula were the most consistently observed regardless of acupoints or acupuncture modes (Kong et al., 2007). The insula is purported to process sensory/discriminative, rather than affective information (Craig et al., 2000; Napadow et al., 2005). Recent studies showed that insula is widely connected with cortex, subcortex and brainstem structures and is involved in decision making and subsequent behavior emotional experience and pain-related modulation. The fMRI imaging data also indicated that insula played an important role in a dynamic switching between the central-executive network and the default mode network (Liu et al., 2010; Sridharan et al., 2008). The posterior middle cingulate cortex, receiving information from insula, appeared to be important in coding acupuncture sensation intensity, then interacting with the posterolateral parietal cortex in orienting the body in response to somatosensory stimuli (Vogt, 2005). Recently, a functional imaging study also suggested a central role for the precuneus in a wide spectrum of highly integrated tasks (Hsieh et al., 2010). Based on these reports and present findings, we speculate that these

structures may be responsible for integration of the sensory inputs associated with pain and proprioceptors, and the motor cortices for manifestation of cross education.
In summary, the present results showed that the electroacupuncture induced a much wider activation of brain areas bilaterally than that induced by the transcutaneous electric stimulation over the same acupoints, as indicated by the BOLD signal changes. Therefore the outcomes of the study did not fully support the hypotheses that electroacupuncture and surface electric stimulation at the same areas would induce similar level of activities in the brain; and that manual acupuncture at the same acupoints may also cause activation of the same regions in the brain.

5. Conclusion

The results of this study indicated that the unilateral electroacupunture task induced a wider bilateral activation in the brain than that induced by the unilateral transcutaneous electric stimulation at the acupoints of ST36 and ST39, although the electrical current utilised in the electroacupuncture was lower. The manual acupuncture did not induce a sufficient level of activation in the brain, as indicated by the BOLD signals, possibly due to the high threshold for identifying activation and the needle handling technique used in the study. Although the bilateral brain activities as indicated by the BOLD signals cannot directly explain the functional changes in adaptation to unilateral interventions, this study has provided new evidence for the differences between the effects of the three treatments.

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5

Electroacupuncture and Stimulatory Frequencies in Analgesia

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1. Introduction

The electroacupuncture was first used in France in 1970 by Roger de La Fuy with analgesics objective. Long before, however, the use of electric currents for therapeutic purposes, it was getting the usual, especially in the area of physical rehabilitation (Amestoy, 1998). The therapeutic effects depend on the type of waveform, intensity, duration and direction of current flow on the type of tissue in which it is applied, involving electrochemical, electrophysical and electrothermal phenomena (Cameron, 2003).
Electrical stimulation of a tissue triggers an increase in the movement, in special potassium and sodion ions along the axon of the nerve cell. This fact accelerates the familiar process of neuronal depolarization, responsible for nerve conduction (Gyuton, 2002).
The fisiological responses by electrophysical stimulation can be perceived by contraction of skeletal or smooth muscle, indirect vascular responses and activation of endogenous mechanisms of analgesia (Alon, 2003)
There is a superior analgesic with eletroacupuncture as compared totraditional acupuncture systemic, especially in musculoskeletal pain (Silvério-Lopes & Nohama, 2007). The increase promoted by electroacupunture analgesic is justified by the acceleration of cell membrane depolarization and consequently more rapid conduction of the stimulus to the central nervous system. There is also specificity in the release of neurotransmitters, depending on the frequencies used in the electrical stimulation system (Silvério-Lopes & Nohama, 2008).
One of the most relevant and studied physical parameters in electroacupuncture is stimulation frequency, especially its relationship with endogenous opioid release in analgesic and anti-inflammatory processes (Han, 2003).
In the first generation of electroacupuncture research, studies were conducted on rats with induced pain in rats to relate stimulation frequencies to biochemically released substances such as: dinorphin at 100Hz (Han, 2003); endorphin at 2Hz (Han, 2004); encephalin and dynorphin at 2 and 100Hz (Zhang et al., 2005a); endomorphin at 2Hz (Han, 2004), and substance P at 10Hz (Zhang et al., 2005b).
The studies on humans, as well as those involving higher frequencies, are scarce and use different methodologies, such as analgesia in back pain with the application of 2500 Hz (Mehret, 2010) postoperative analgesia at 100Hz (Amestoy, 1998; Lin, 2002) neck pain at 120Hz and 250Hz (Qing et al., 2000) or with 1000Hz and 2500Hz (Silvério-Lopes & Nohama, 2009). The scarcity of scientific studies on humans in this area can be explained by the difficulties which surround the assessment of human pain, as well as methodological errors,

which have already been criticized by other authors (Ezzo et al., 2000; Pomeranz 2005). Therefore, it is important to evaluate the analgesic effects of therapeutic procedures to determine whether they should continue to be used.

The largest number of patients in acupuncture treatments are cases of musculo-skeletal pain (Filshe, 2002), such as; low back pain and neck pain. The symptom of neck pain due to muscular tension was chosen because it is part of the population profile since it affects a great number of individuals. Neck pain affects 30% of men and 43% of women at some point in their lives, and it is a complaint that keeps a large number of workers away from their professional activities (Côté et al., 2004). Neck pain can have several sources, such as postural changes, mechanical traumas, spine rectifications, and others. It is known that neck pain due to muscular tension is not a pathology in itself, but a symptom or a manifestation of muscle pain syndromes. Another relevant aspect in choosing this symptomatology was the fact that acupuncture has already shown good therapeutic results in neck pain (Qing et al., 2000; Vas et al., 2006).

To the extent that there is an unquestionable clinical applicability of electroacupuncture in pain, there is no standardization in the physical parameters that should contain stimulators (Silvério-Lopes et al., 2006). It noted the growing interest of health professionals by the use of electrical stimulation for therapeutic, as the technological resource. Even in places with in Chine, surrender in the news electroacupuncture as an additional resource in classic systemic acupuncture was for thousand years the basis of Tradicional Chinese Medicine (TCM).

However, the extent to which the interest of traders grows in using electroacupuncture, grows along the arsenal of new equipments by electrical stimulation. It is necessary to have electronic stability, safety for the operator and the user, and technical specifications to be used, ensuring adequate therapeutic effects. We also recall that in the case of electroacupuncture for the care that the electrode is invasive represented by the needle inserted into the skin. This is different from surface electrodes used in physiotherapy, like the Transcutaneos Electrical Nerve Stimulation (TENS).

Of all the physical parameters of electroacupuncture, it is believed that the frequency has a stimulatory relevance and need for clinical studies. Currently there is still disagreement on what is the best stimulatory frequencies to be used in analgesia by electroacupuncture.

This chapter of this book brings a paper with a study of different stimulatory frequencies involved in the analgesia of neck pain, induced by electroacupuncture. The objective of this paper is to evaluate what the best stimulatory frequencies with electroacupuncture, and which promotes better analgesic effects in a population of individuals with cronic neck pain.

2. Methods

For the experimental protocol, we used stainless steel disposable acupuncture needles (0.25 diameter x 40mm length); 70% alcohol solution; absorbent cotton; a chronometer; a Wagner digital algometer; and a sharps disposal box. We also used a class I, BF type electrostimulator (NKL, model EL608, ANVISA 80191680002) with microprocessed stimulus generation and control and 8 isolated outputs through pulse transformers. The output current can reach a maximum value of 10mA per pulse or mean intensity of 6mA.

The pulsed shape generated by the stimulator was configured as monophasic, rectangular, asymmetrical, with secondary phase in decreasing exponential obeying, a pulsed pattern with 4-second stimulation periods and 3-second resting periods, according to Knihs (2003).

The equipment was calibrated at the Rehabilitation Engineering Laboratory of PUC / PR, following the technical norms NBR IEC 60601-1 and NBR IEC 60601-2 (Associação Brasileira de Normas Técnicas-ABNT 1977; ABNT 1997)

The subjects were recruited at the outpatient clinics of Instituto Brasileiro de Therapias e Ensino (IBRATE) at Curitiba -Brazil. Initially, following the inclusion criteria, a population sample of 88 subjects was selected. However, at the time of intervention, a few subjects showed inadequacies such as drop in blood pressure, fear, intolerance to the electrical stimulation, use of analgesic drugs, among others. These subjects received treatment but were not considered as part of the sample. The sample consisted of 66 individuals, aged 18 to 53 years with a mean age of 33.67±9.97 years, 89.5% female and 10.5% male.

A subject screening instrument was prepared and validated using the technical reports of 10 orthopedics specialists. The objective of this instrument was to characterize the volunteers as neck pain sufferers due to muscular tension to outline the sample profile to guarantee group homogeneity. Based on the defined inclusion criteria, we selected: normotensive individuals, with neck pain due to muscular tension in the trapezius and neck muscle region, at least in the last 4 weeks before the selection. The exclusion criteria were: smokers, because tobacco was pointed out by Piovesan et al. (2001) as a factor in the decrease in nociceptive sensibility in algometry evaluation; pacemaker carriers and pregnant women, because the use of electroacupuncture is contraindicated for those individuals (Filshe & White, 2002); individuals who had received physical therapy treatment, massage or acupuncture in the last two weeks before the intervention, or who had taken anesthetic drugs, painkillers, muscle relaxants, psychotropic drugs or anti-inflammatories in the last two days before the intervention.

This project was approved by the Research Ethics Committee of PUC-PR, protocol CEP 1035/2006 and registered in the Australian New Zealand Clinical Trials Registry (ANSCTR) under the number 083456. All the volunteers signed a consent form. With the intention of partially blinding the study, a physical therapist was invited to evaluate the subjects, who were systematically distributed between the groups. The measurement instruments were also evaluated before and after the therapeutic intervention.

Initially, the subjects were asked to score the pain on the visual analog scale (VAS) where zero was defined as "no pain", and ten as "the worst pain". The subject's heart rate was then measured. The evaluation through pressure algometry began with an explanation about the test and how the subject should verbalize the tolerance to the pressure. An example was given before the real test for clarity. The example consisted of a mechanical stimulus applied to the right elbow crease until the subject expressed discomfort to the pressure by immediately saying "stop". At that moment, the compression was instantly blocked, and the reading was checked on the algometer. For the pressure measurement, the algometer (with calibration certificate) was set at the C function (self-calibration in kgf/cm^2). The tolerance was standardized as the expression of the onset of discomfort caused by the pressure of the algometer's rubber tip on the skin**,** according is ilustred in the **figure 1.**

For the pressure readings, we selected three bilateral and symmetrical combinations of points on the neck and trapezius muscle with a total of six reading areas: 1 and 2 (occipital insertion of the right and left trapezius, respectively); 3 and 4 (midpoint of the upper border of the right and left trapezius, respectively); 5 and 6 (supraspinatus muscle above the medial border of the right and left spine of the scapula, respectively), as demonstrated in Figure 2. These points were chosen based on the literature because they are painful points in myofascial pain syndromes (Melzack et al, 1977).

Fig. 1. Digital Pressure Algometer used at work.

The VAS, heart rate and pressure algometry procedures were performed at least 10 minutes before the intervention, taking advantage of the interview time when the subject remained seated and at rest. The procedures were repeated 10 minutes after the acupuncture needles were removed.

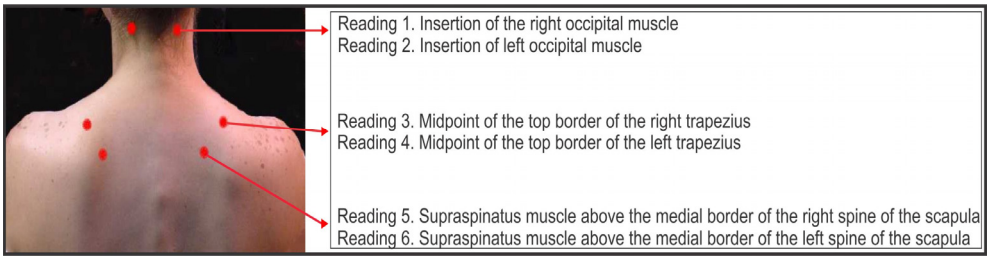

Fig. 2. Algometry points and anatomical/topographical references.

The subject remained seated during all of the procedures. A sequence of algometry readings was standardized in such a way that, when the first reading of the six points was completed, a new "round" of readings in the same sequence began. Overall, three readings were performed on each point, before and after the intervention. The values were grouped for mean calculation, considering measure 1 with measure 2, 3 with 5, and 4 with 6. After the pre-intervention evaluations were completed, the acupuncture needles were applied bilaterally. The acupuncture points were selected based on bibliographical indications for neck pain as follows: B10 (*tianzhu*), VB21 (*jianjing*), TA15 (*tianliao*), IG4 (*hegu*) and ID3 (*houxi*) (Stux & Pomeranz, 2004; Lian et al., 2007). The needles used on points TA15 and VB21 (trapezius muscle, bilaterally) were selected to receive electrical stimulus, acting as needle-electrodes. These points were chosen due to the anatomical proximity to the painful region,

to the muscle relaxation function attributed to these points, and the fact that the needles can be easily and more comfortably applied to them. The needle's depth of insertion was approximately 1.27cm (0.8 in), except in ID3 (on the hand), where the depth was about 0. 4cm (0.3 in). The needles were inserted and removed in the same sequence for all the subjects.

The groups were coded by draw with letters A (2500Hz), B (2Hz), C (1000Hz), D (100Hz) and E (without electrical stimulation). The subject and the researcher had no knowledge of the frequencies that corresponded to each letter. The stimulation frequency was the variable modified during the experiments because it was the physical parameter under evaluation. The adjustment of the current intensity respected the stimulus tolerance of each subject, therefore individualized, based on the electroacupuncture technique (Knihs, 2003; Filshe, 2002).

The subjects were divided into groups A, B, C, D and E by systematic distribution conducted by the invited examiner. The amount of time the needles were left in place, including the time of electrostimulation, was 20 minutes. At the end of this interval, the electrostimulator cables and the needles were removed. Care was taken to avoid pressure close to the reading locations. A rest period of 10 minutes was standardized until the VAS, heart rate and algometry evaluations were repeated, which constituted the post-intervention data collection. The present study included 66 volunteers divided into five groups: A (2500Hz, n=13), B (2Hz, n=13), C (1000Hz, n=13), D (100Hz, n=13), E (without electrical stimulation, n=14).

In order to accomplish the statistical treatment, the analysis of covariance (ANCOVA) was applied to the algometry data. The non-parametric Kruskal-Wallis test was used for the VAS, and the Wilcoxon and *t* tests were used for within-group evaluations.

3. Results

3.1 Tolerance to pressure

An evaluation per anatomical region (**Figure 2)** found statistical significance between pre- and post-intervention pressure tolerance. This form of evaluation, from a statistical point of view, reduces individual variability among subjects because it compares each individual to himself (paired sample). **Table 1** shows that there was statistical significance for groups A (2500Hz) and D (100Hz) in all evaluated anatomical regions, which demonstrates the effectiveness of the therapeutic intervention. The other groups did not show.

Group A	Time	n	Mean	Median	Low	High	Standard deviation	P value
Region 1-2	Before	13	3,03	2,51	1,54	5,45	1,27	**0,006**
	After	13	3,62	3,53	1,51	5,68	1,33	
Region 3-5	Before	13	3,24	2,62	1,02	7,35	1,99	**0,003**
	After	13	4,11	3,88	1,14	7,04	1,83	
Region 4-6	Before	13	3,09	2,39	0,91	8,17	2,14	**0,013**
	After	13	3,93	4,04	1,05	6,63	1,71	

Group B	Time	n	Mean	Median	Low	High	Standard deviation	P value
Region 1-2	Before	13	2,53	2,24	1,07	4,68	1,09	0,254
	After	13	2,76	2,58	1,41	5,77	1,21	
Region 3-5	Before	13	2,53	2,63	0,58	4,84	1,24	0,100
	After	13	2,93	2,55	0,91	6,45	1,56	
Region 4-6	Before	13	2,77	2,39	0,68	5,46	1,58	0,821
	After	13	2,81	2,45	0,95	6,34	1,42	

Group C	Time	n	Mean	Median	Low	High	Standard deviation	P value
Region 1-2	Before	13	2,53	2,31	1,07	4,62	0,89	0,906
	After	13	2,51	2,44	1,20	3,91	0,77	
Region 3-5	Before	13	2,28	2,13	0,78	4,14	0,87	0,257
	After	13	2,52	2,44	1,19	3,65	0,80	
Region 4-6	Before	13	2,45	2,32	0,80	4,16	0,92	0,249
	After	13	2,71	2,61	0,99	3,91	0,90	

Group D	Time	n	Mean	Median	Low	High	Standard deviation	P value
Region 1-2	Before	13	2,36	2,43	1,10	4,66	1,19	**0,035**
	After	13	2,85	2,66	1,33	5,52	1,19	
Region 3-5	Before	13	2,53	2,39	0,90	4,92	1,39	**0,016**
	After	13	3,12	2,74	1,29	6,64	1,65	
Region 4-6	Before	13	2,58	2,48	1,03	5,31	1,45	**0,038**
	After	13	3,09	2,45	1,34	7,06	1,79	

Group E	Time	n	Mean	Median	Low	High	Standard deviation	P value
Region 1-2	Before	14	2,70	2,72	0,81	6,02	1,16	0,634
	After	14	2,81	2,45	1,30	5,78	1,30	
Region 3-5	Before	14	2,73	2,29	0,49	9,14	2,06	0,457
	After	14	2,92	2,46	1,25	9,10	1,92	
Region 4-6	Before	14	2,78	2,30	0,44	7,28	1,62	0,614
	After	14	2,91	2,38	1,08	7,81	1,78	

Table 1. Variations in the pressure tolerance measurements and statistical significance (kgf/cm^2) before and after the intervention within groups A (2500Hz), B (2Hz), C (1000Hz), D (100Hz) and E (without electrical stimulation).

3.2 Pain score (VAS)

There was statistical significance to the reduction in the percentage variation of the mean pain scores, which shows improvements in the analgesic effect noticed by the subjects in all groups. The values were: A (2500Hz) reduction of 52.12% and p=0.003; B (2Hz) reduction of

32.93% and p=0.028; C (1000Hz) reduction of 52.41% and p=0.002; D (100Hz) reduction of 41.92% with p=0.013; and E (without electrical stimulation) reduction of 65.95% and p=0.002.

3.3 Heart rate

The percentage variation between pre- and post-intervention heart rate had no significant difference between groups (p=0.716). In all groups, there were subjects with no change in heart rate; however, some had an increase and others had a reduction, as demonstrated in **Figure 3.** Group E (without electrical stimulation) had the highest number of cases of increased heart rate (43%).

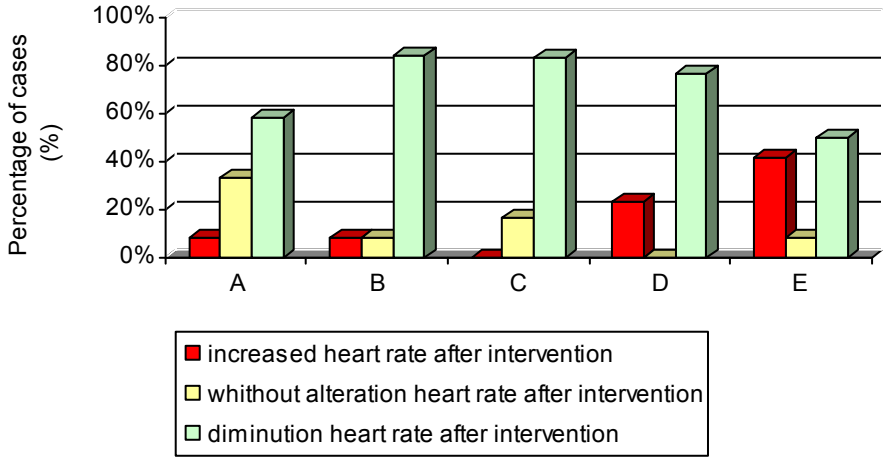

Fig. 3. Comparison of groups regarding the incidence of cases where the heart rate had increased, not changed and decreased after the intervention. Groups A (2500Hz), B (2Hz), C (1000Hz), D (100Hz) and E (without electrical stimulation).

4. Discussion

The results and statistical analyses show that there was statistical significance in all groups between the pre- and post-intervention pain score (VAS) and heart rate, which indicates therapeutic improvement, but without prominence of a specific group. However, the evaluation of within-group therapeutic performance for pressure tolerance showed better results for 2500Hz, followed by the 100Hz frequency. This result was confirmed in all the regions evaluated by pressure algometry.

These results disagree with some authors such as Han (2003) and Filshe & White (2002) who point out the advantages of using low-stimulation frequencies (2Hz) for analgesic effects based on biochemical and immunohistological studies on rats and mice. Research in animals are important because it is based on the analgesic effects of neurotransmitter release. In contrast, it does not take into account emotional, cultural and biomechanical variables experienced in human pain.

Filshe & White (2002) conducted a survey of controlled experiments on humans which had very few findings, but verified that lower electroacupuncture frequencies had better analgesic results than the higher frequencies. The authors also reported that the therapeutic effects last longer in chronic painful conditions. Unfortunately, electroacupuncture studies in humans are still scarce, particularly the ones which intend to compare parameters.

Yin (2000), Cui et al. (2004) and Tienyou (2000), defend that electroacupuncture has analgesic advantages over acupuncture. The results of the present study partially confirm this statement by showing that there was statistical significance for pressure algometry in all evaluated regions in two out of four groups treated with electroacupuncture (2500Hz and 100Hz), and that there was no difference in the group treated only with acupuncture. However, the results of the VAS evaluation show that group E, which received only acupuncture, demonstrated the highest mean reduction in the pain score (65.95%), although there was no statistical difference in comparison to the other groups. A possible explanation for this result is based on the fact that the mere possibility of an electrical current passing through the body causes anxiety in the subject and consequent negative psychological effect. It is worth noting that the VAS score has a subjective and emotional component, according to Ferreira (2001).

In pressure algometry, however, the reference is more quantitative and it is associated with nociceptive sensibility based on a concrete mechanical stimulus, which is the rubber tip of the algometer. In addition, the algometry reading points chosen for the present study were close to the insertion location, and the stimulus caused by the electrical current in the groups with electroacupuncture also had an enhanced local effect, unlike the stimulus of acupuncture needles alone. With regard to heart rate variations, before and after the therapeutic intervention, there were no differences between the researched groups.

There are no studies in the literature that associate heart rate with analgesic effects of acupuncture or electroacupuncture. Although there was no statistical difference between the evaluated groups, one result is worth noting: most of the subjects in the groups submitted to electroacupuncture demonstrated a reduction in heart rate after the intervention **(Figure 3).** The same fact did not occur in the group which received only acupuncture (without electrical stimulation), in which 43% of the subjects had an increase in heart rate after the intervention, 50% had reduction and 7% showed no change.

Wall & Melzack (1999), and Fox (2007) discussed the influence of stress and external stimuli on heart rate modulation, as well as the anatomical and physiological pathways of that influence. Pomeranz (2005) found a relationship between low-frequency electroacupuncture and analgesic and sedative effects, which suggests possible indirect effects on heart rate. The studies by Yang et al., (2002) confirm that electroacupuncture reduces heart rate, blood pressure and catecholamine release, reducing stress. Based on these references, the results of the present study indicate that electroacupuncture has a greater effect on the autonomous and hypothalamic tonic regulation than acupuncture, which explains the higher proportion of subjects with heart rate reduction in the groups with electrostimulation.

5. Concluding remarks

In the electroacupuncture, there is a predominance of basic research in biochemistry and immunohistological investigation, with most being in cobaias. It was found a dearth of research controlled clinical trials with humans, and even more rare that proposes to comparative studies of physical parameters used in electroacupuncture.

The methodology of this paper proved to be adequate from the standpoint of protecting the uniformity of the sample, assessment tools, and statistical analisis. There was convergence of results between the different features of evaluation used (algometry pressure, heart rate and VAS.

It is recommended that future studies with algometry (tolerance to pressure) suitable for regions seeking to standardize the evaluation of analgesic effects of acupuncture and electroacupuncture, thus assisting the appropriate methodological support to encourage clinical research in humans.

Although no significant statistical differences were found between groups with regard to pain score and heart rate, the present study recommends electroacupuncture application at a frequency of 2500Hz and 100Hz for analgesia of neck pain due to muscular tension because these frequencies demonstrated the highest individual efficiency in the algometry evaluation.

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Part 3

New Proposed Concepts

6

Is Acupuncture Meridians a Novel System for Superoxide Disposition

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1. Introduction

According to Traditional Chinese Medicine (TCM) theory, acupuncture meridian is recognized as the most important network responsible for transporting *Qi*, the energy flow and therefore determining surviving status of human body. *Qi* (also *Chi or Ki*) in traditional Chinese culture is an active principle forming part of any living thing. *Qi* is frequently translated as "energy flow", but some researchers stand in different attitudes.

Energy flow is the most used phrase to explain *Qi* in traditional Chinese culture. Before Scientific Revolution, ancient Chinese philosophers believed that *Qi* was the invisible and basic unit of all life and matter, endowed things with specific characteristics. In other words, *Qi* as basic unit was combined to create distinctive matters, providing matters various functions. The concept of *Qi* in Chinese philosophy divides into two opposite basic characteristic as *Yin* and *Yang*, which is used to describe how polar or seemingly contrary forces are interconnected and interdependent in the natural world, and how they give rise to each other in turn. Opposites thus only exist in relation to each other. *Yin* aspect of the *Qi* was described as cold, stationary, negative, objective; *Yang* aspect of the *Qi* was described as hot, active, positive and virtual. Taking blood circulation for example, the TCM physicians believe that blood consists of the tangible *Qi* of *Yin* characteristic and the circulation motion was powered by the other invisible *Qi* of *Yang* characteristic. To expand the notion of *Yin* or *Yang* aspect of the *Qi*, the negative portion of *Qi* composes the objects such as human body and the positive portion performs the particular function like breath and thinking.

What the ancient Chinese philosophers perceive as *Qi* is similar to the concept of energy in modern science. Energy is the capacity of a system to do work. In other words, energy like the *Yang* aspect of the *Qi* imparted the ability of function to matters. On the other hand, matter like the *Yin* aspect of the *Qi* has mass and occupies volume. According to Einstein's the theory of relativity, however, mass and energy are two names for the same thing, neither one appears without the other, just like *Qi* has two opposite and interconvertible characteristic.

But in Traditional Chinese Medicine, energy concept of *Qi* is difficult to be understood how human body functions in different conditions, such as in health or in disease. For example, human inner organs in TCM called *Zang-fu* possess respective *Qi*. The particular *Qi* flows out of respective inner organ along an acupuncture meridian intitled as the organ name circulate in the body, and somehow absorb or release *Qi* from or to environment via acupoints. Meridians are channels along with *Qi* circulates, balances energy throughout the

body. Once *Qi* was blocked, stagnated or weakened, it could result in physical, mental or emotional ill health. Rather, when *Qi* flowed freely through the meridians, the body was balanced and healthy. Maintaining meridian's proper functions constitutes the key principles for traditional Chinese medical modalities. Energy have been defined several forms as thermal energy, chemical energy, electrical energy, radiant energy, nuclear energy, magnetic energy, elastic energy, sound energy, mechanical energy, luminous energy, etc. What the *Qi* concept belong to is indistinct yet.

Wu & Jong (C. C. Wu & Jong, 1990) used radionuclide to inject into acupoints on a subject, in order to find the acupuncture meridian's relationship with the veins and to understand the physiological function of acupoint. They took a photo of those radionuclide flew along a meridian in subject body. Yang et al. (H. Q. Yang, et al., 2007) investigated the meridians and acupoints of human bodies at natural condition are among 30 healthy volunteers by infrared thermal imaging technique, which give clear evidence of the existence of infrared radiant tracks along human meridian courses. They believed that the infrared radiant track along human meridian courses is a normal vital and physiological phenomenon appearing in human beings, which indicated that *Qi* has strong connection with the thermal energy. van Wijk et al. (van Wijk, et al., 2010) discussed the relationship between connective tissue and meridian function in terms of energy transmission. The network of hydrogen-bonded water molecules interspersed within the collagen fibrillar matrix in connective tissue has properties of ultraweak photon emission and human photon was considered as *Qi* in Traditional Chinese Medicine. Brătilă & Moldovan (Brătilă & Moldovan, 2007) used harmonic sounds to stimulate acupoints, testing the theory that the body rhythms synchronize to an outer rhythm applied for therapeutic purpose, can restores the energy balance in acupuncture meridians and organs and the condition of well-being. Lee et al. (Lee, et al., 2010) measured the electrical potential along the stomach meridian to investigate the bioenergy consensus between the operator and subject during acupuncture stimulation, which might indicate the transfer of bioenergy between operator and subject by the meridian electrical potentials. They believed that *Qi* was some kind of electrical potential.

Other researchers tried to found the certain molecule to define what the *Qi* is. Ma et al. (Ma, et al., 2007) quantified total nitrate and nitrite (NOx-) collected from the skin surface along acupoints in meridian and determined whether non-enzymatic reduction of nitrate by bacteria is involved in chemical generation of nitric oxide (NO) on acupoints. They conclude that NO is physiologically released from the skin surface with a higher level at acupoints, and the chemical generation of NO on skin acupoints has significantly relate to *Qi* in meridian. Zhang et al. (W. B. Zhang, et al., 2009) used a highly sensitive CO_2 instrument to measure the transcutaneous CO_2 emission at 13 points along the pericardium meridian line, found that the distribution of transcutaneous CO_2 emission is highly related to acupoints on the body. They concluded that there is a strong correlativity of energy metabolism activity between the body surfaces along the meridian, and an even stronger correlativity between the acupoints on the meridian. Zhai et al. (Zhai, et al., 1995) used nuclear magnetic resonance (NMR) spectrum analysis to measure the state of energetic metabolism of hepatic cells at the organic level. They found that moxibustion on acupoint Guanyuan (CV4) could remarkably increase the ATP molecules in the hepatic cells, significantly raise the thermodynamic reserve and phosphate potential of the hepatic cells, so as to enhance the functional activities of liver. Chris Kresser (Kresser, 2010) defined *Qi* as air or the essence of air as oxygen. He believed that *Qi* can also refer to the function of something (i.e. the *Qi* of

an organ would refer to the function of that organ) and the weather rather *Qi* does not mean energy. What's more, Chris Kresser further thought about how the ancient Chinese philosophers recognize the essence of life, the surrounding environment and the connection between both above. He believed that although those ancient Chinese philosophers have no appropriate technology to extract oxygen from air, they did understand the air we breathed was essential to human beings and this vital air called *Qi* was circulated around the body to support physiological processes. The *Qi* circulation tracts in body portray the meridian lines. So the literal translation of *Qi* is air, breath, or gas. To speak of oxygen, nitric oxide and carbon dioxide both are derivative of oxygen.

The failure, however, to demonstrate decisive evidences of the anatomical existence of such channels have rendered the whole TCM practice to suffer from the persisting suspicion and even despise from the science community. The greatest effort in meridian research remains mostly the identification of differences between tissues along the meridians and their adjacent ones in physical properties such as electric conductivity, light, heat or sound transmission. It hardly contributes anything to the understanding of the meridian system since they must be only the peripheral expressions of an unknown cause. It may be necessary to understand the biological function of the meridian before its anatomical properties can be revealed. In other word, you can find it only after you know what it is rather than the other way around.

2. Discovery of the linkage between acupuncture meridian and superoxide

Superoxide is another derivative of oxygen. It is important as the product of the one-electron reduction of oxygen, which occurs widely in nature. With one unpaired electron, the superoxide ion is a free radical, and, like oxygen, it is paramagnetic (Isenberg, 1964). Superoxide is biologically quite toxic and is deployed by the immune system to kill invading microorganisms (Bus & Gibson, 1982).

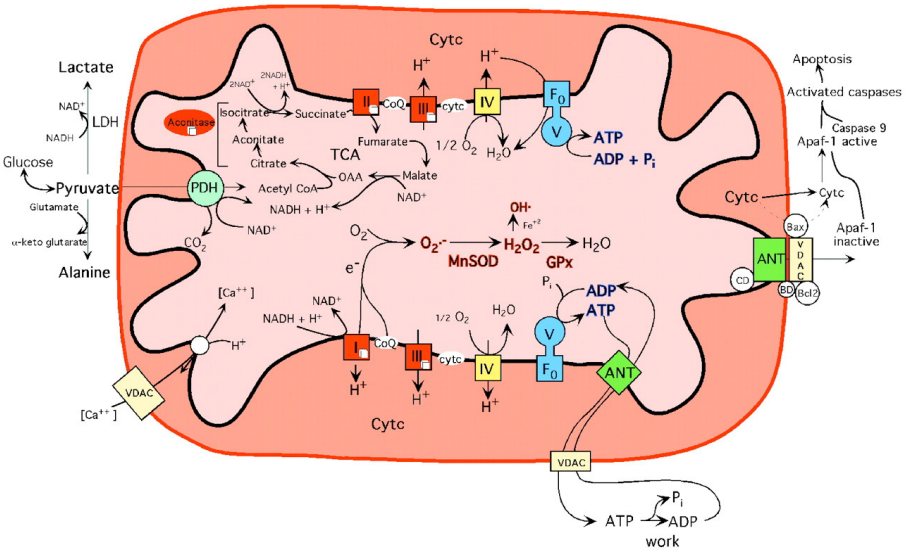

Fig. 1. Superoxide production in mitochondrial, cited from (Raha & Robinson, 2000)

Superoxide in biology is important because it underlies oxidative damage in much pathology and contributes to reduction-oxidation (redox) signalling from the organelle to the nucleus and cytosol (Murphy, 2009). Superoxide is generated by the mitochondrial respiratory chain (Raha & Robinson, 2000), which depends critically on the $NADH/NAD^+$ and $CoQH_2/CoQ$ ratios and the local O_2 concentration.

Superoxide in mammalians cell causes the propagation of lipid peroxidation reactions and initiation in biological membrane systems (Gutteridge & Halliwell, 1990). What's worse, superoxide also caused main protein degradation, such as main enzyme and channel protein (Webster & Nunn, 1988). Protein and lipid peroxidation can arise as a consequence of tissue injury in many disease states and may sometimes contribute significantly to worsening the tissue injury (Martínez-Cayuela, 1995).

Superoxide in signal transduction increases the expression of several pro-arteriosclerotic genes, such as monocyte chemoattractant protein-1, tissue factor, and vascular endothelial growth factor (Brandes, 2003). Superoxide also mediates the actions of angiotensin II in the central nervous system, including promotion of thirst, regulation of vasopressin secretion, and modulation of sympathetic outflow (Zimmerman, et al., 2002). In certain cells, superoxide as a signal-transduction messenger plays a role in ras-induced transformation, resulting in cellular unchecked proliferation and malignant transformation (J. Q. Yang, et al. 2002). Dolowschiak et al. (Dolowschiak, et al., 2010) reported to describe epithelial cell-cell communication in response to innate immune activation by NADPH oxidase (Nox) 4-dependent superoxide formation. This epithelial communication facilitates a coordinated infectious host defence at the very early stage of microbial infection. Superoxide generated in skeletal muscles could control excitability of muscle fibers through redox modulation of membrane ion channels (Luin, et al., 2011), on the contrary, myotube depolarization generates superoxide through NAD(P)H oxidase (Espinosa, et al., 2006).

Because superoxide is toxic, nearly all organisms living in the presence of oxygen contain isoforms of the superoxide scavenging enzyme, superoxide dismutase (SOD). SOD is an extremely efficient enzyme, which catalyzes the neutralization of superoxide nearly as fast as the two can diffuse together spontaneously in solution (McCord & Fridovich, 1988).

Although exogenous SOD is available to protect against oxidative stresses, the major problem in using exogenous SOD is that SOD can not be delivered into cells, thus resulting in their inability to detoxify intracellular superoxide (Eum, et al., 2004). As a promising solution to the problem, several protein segments have been identified as protein transduction domains (PTDs) which can transport exogenous protein into living cells (Eum, et al., 2004). One of these PTDs is TAT, an 11 peptide derived from HIV-1 trans-activator of transcription protein (Watson & Edwards, 1999). Once SOD fused with TAT by gene-recombinant expression, TAT-SOD can be delivered cross the cell membrane while maintaining SOD activities (Eum, et al., 2004).

We discovered the linkage between the meridian and superoxide by accident in our work with this fusion protein consisting of human liver Cu,Zn-superoxide dismutase covalently bonded with TAT peptide, which can ferry the enzyme across the cell membrane while retaining its activity. When TAT-SOD was applied topically on certain spots of pain around the neck, the pain was removed while the throat was instantly cleared. That fact that the instant and remote action of the molecule resembled the effect of acupuncture had inspired us to formulate an assumption that the meridian line was an alignment of cells full of superoxide.

Adult respiratory distress syndrome
Aging
Alcoholism
Allergic encephalomyelitis
Alzheimer's disease
Atherosclerosis
Autoimmune vasculitis
Bronchopulmonary dysplasia
Cancer
Cataract
Chronic autoimmune gastritis
Cirrhosis
Contact dermatitis
Dermatomyositis
Favism
Glomerulonephritis
Gout
Haemochromatosis
Ischemia-reperfusion injury
Lipofuscinosis
Malaria
Multiple sclerosis
Muscular dystrophy
Myasthenia gravis
Pancreatitis
Parkinson's disease
Porphyria
Pulmonary emphysema
Retrolental fibroplasia
Rheumatoid arthritis
Senile dementia
Sickle cell anemia
Stroke
Systemic lupus erythematosus
Thalassemia
Ulcerative colitis

Table 1. Diseases and clinical disturbances that may involve free radical reactions, cited from (Martínez-Cayuela, 1995)

When intracellular superoxide indicators of 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) and MitoSOX™ Red (Bass, et al., 1983; Robinson, et al., 2006) were intravenously at the tail vein and topically applied to SD rats, respectively. These SD rats were 6 weeks old and all of them were male. 1 ml of DCFH-DA solution (10 mg in 1 ml dimethyl sulfoxide) was injected through the SD rat tail vein. 30 minutes later, the rat frontal

abdominal wall was incised from over iliac crest then along the midaxillary line on both sides up to the rib bone so that the interior wall could be exposed for fluorescent imaging. On the other hand, the interior abdominal wall thus exposed was applied with MitoSOX Red reagent (100 μg in 1 ml dimethyl sulfoxide) by direct smearing with living rats without any other prior treatments. Fluorescent imaging for superoxide distribution in SD rat was carried out with the exciting lights, which were generated by a lamp through band-pass filters of 488 nm for DCFH-DA and 510 nm for MitoSOX Red reagent, respectively. The fluorescence images of the interior abdominal wall were collected by a camera equipped with band-pass filter of 525 nm for DCFH-DA and 588 nm for MitoSOX Red reagent, respectively. The photograph of the fluorescent image under exciting light was with an exposure time of 10 seconds.

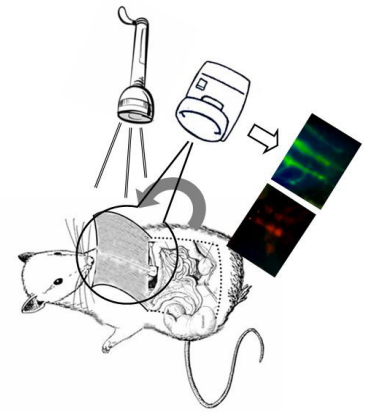

Fig. 2. Anatomical sketch of the interior abdominal wall of the rat for fluorescent imaging. The rat frontal abdominal wall was incised from over iliac crest then along the midaxillary line on both sides up to the rib bone, cited from (Guo, et al., 2000)

Under the exciting light, green and red fluorescent lines were beautifully revealed respectively on SD rat's interior frontal abdominal wall. The obtained fluorescent image of Fig. 3A is shown in Fig. 3B, in which five green fluorescent lines were revealed on the interior abdominal wall the rat. While line 1 represents the outburst of oxidative stress along the edge of the abdominal wall due to incision, lines 2-5 can be interpreted as nothing but the unusual distribution of intracellular superoxide in the form of lines in the abdominal wall or the existence of lines consisting of cells under high oxidative stress.

As is shown in Fig. 4B, line 6 and line 7, two broadened red fluorescent lines were revealed by direct smearing of MitoSOX on the abdominal wall of a rat in Fig. 4A, line 6 and line 7 correspond to line 4 and 5 in Fig. 3B, respectively, confirming the unusual pattern of superoxide distribution. As a result of the intravenous delivery of higher dosage of DCFH-DA than mitochondrial superoxide-specific MitoSOX, a stronger fluorescence was generated, which may be accounted for the better visualization in Fig. 3B than Fig. 4B.

Furthermore, while superoxide pattern in Fig. 3B and Fig. 4B resemble neither abdominal veins nor nerves, it is almost perfectly superimposable on the human acupuncture meridian

network (World Health Organization Regional Office for the Western Pacific, 2008) shown in Fig. 5, with Line 2 for the spleen meridian, 3 for the stomach meridian, 4 and 6 for the kidney meridian and conception vessel, and 5 and 7 for the symmetric stomach meridian .

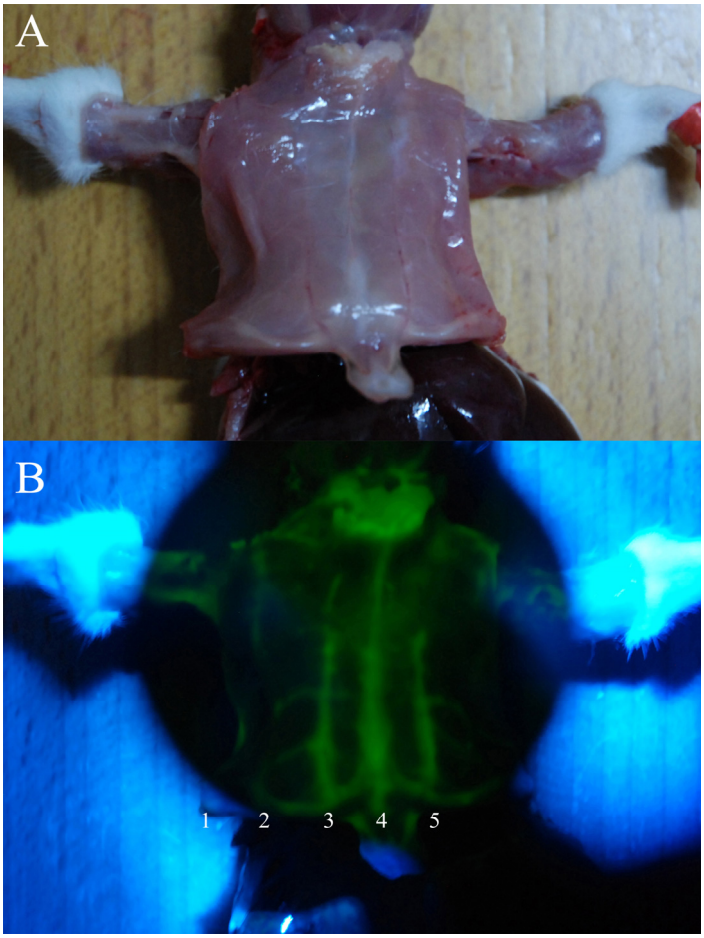

Fig. 3. A) Image of the frontal abdominal interior wall of a 6 weeks old male SD rat whose abdominal wall was incised 30 minutes after the injection of 1 ml of DCFH-DA solution (10 mg in 1 ml dimethyl sulfoxide) through the tail vein. B) Fluorescence image of the frontal abdominal interior wall of the SD rat shown in Fig. 3A. The exciting light was generated by an UltraFire MCU WF-1200L lamp through a band-pass filter of 488 nm, and the image was collected by a NIKON (model D-80) camera equipped with a band-pass filter of 525 nm., cited from (Guo, et al., 2000)

The lines could be almost superimposable with human meridian lines in the similar region, revealing meridian like images at the highest resolution in the largest area in animal. It indicated an exciting hitherto unknown connection of meridian with superoxide, a derivative of oxygen and one of the most important molecules to life. A hypothesis was

proposed thereupon that acupuncture efficacy was due to the mechanical removal of intracellular superoxide at acupoints by the puncture of a needle and that enzymatic removal of intracellular superoxide at acupoints could achieve similar clinical results.

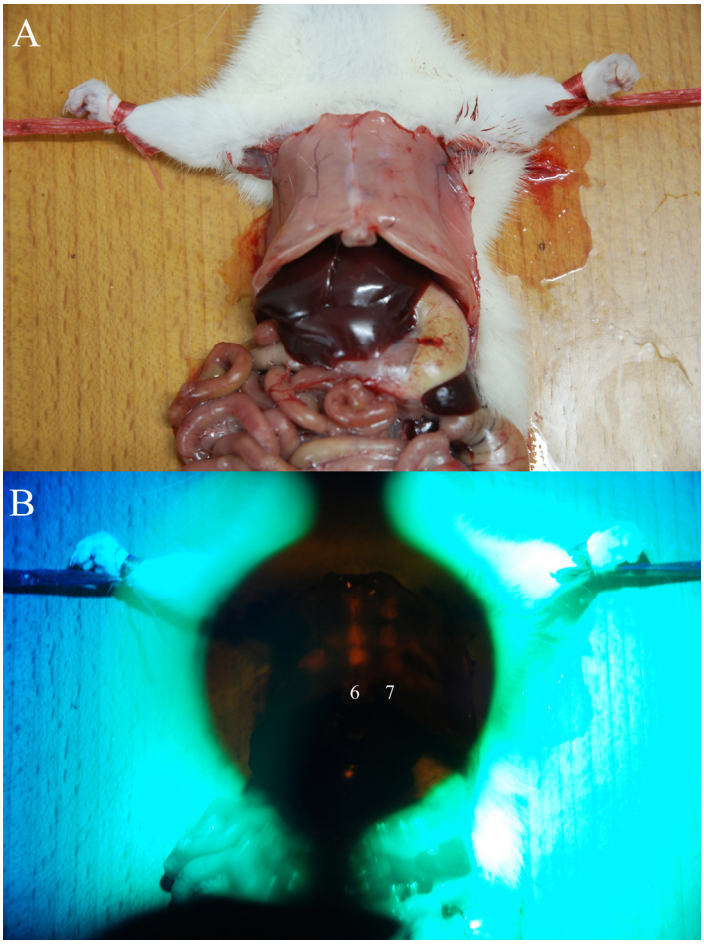

Fig. 4. A) Image of the frontal abdominal interior wall of a 6 weeks old male SD rat whose abdominal wall was applied with MitoSOX™ Red reagent (100 μg in 1 ml dimethyl sulfoxide) by direct smearing with living rats without any other prior treatments. B) Fluorescence image of the frontal abdominal interior wall of the SD rat shown in Fig. 4A. The images were collected by the camera equipped with a band-pass filter of 510 nm with exciting light generated by the lamp through a band-pass filter of 580 nm, cited from (Guo, et al., 2000)

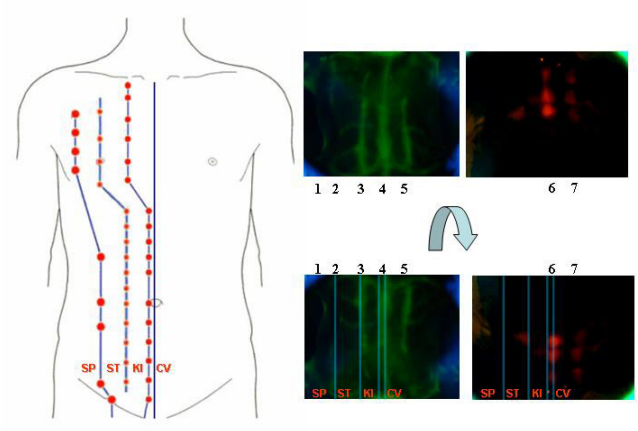

Fig. 5. Illustrations for location of human acupuncture meridian and the superimposition with ROS distribution patterns in Fig. 3B and Fig. 4B. SP, spleen meridian; ST, stomach meridian; KI, kidney meridian; CV, conception vessel, cited from (Guo, et al., 2000)

3. Clinical confirmation of the linkage between acupuncture meridians and intracellular superoxide

Acupuncture has been demonstrated to be effective in treating simple obesity, but other than scientifically intangible *Qi* concept and mysterious theory in Traditional Chinese Medicine, there is no any understanding about what exactly happens immediately after a needle is inserted into acupoints in the modern scientific terms, not to mention the whole mechanism of the treatment. A recent discovery about superoxide's involvement in meridians implies that the unavoidable leakage of intracellular superoxide caused by the puncture of a needle into cells at acupoints may be associated with the acupuncture efficacy. As a novel intracellular superoxide quencher, TAT-SOD can be used to test the hypothesis since TAT-SOD can do nothing but to be delivered into cells to remove superoxide there when it is applied topically to acupoints.

Simple obesity is a prevalent, refractory, and serious problem (Kenny, 2011). Individuals with overweight (BMI >25 kg/m^2) or obese (BMI >30 kg/m^2) are at greater risk for a variety of medical conditions including diabetes, dyslipidemia, hypertension, cardiovascular disease, and sleep apnea (National Heart, Lung and Blood Institute, 1998).The psychological consequences are also severe, which include impaired quality of life, among the severely obese, depression and body image disparagement (Pan, et al., 2011; Donini, et al., 2010). In 2005, the Chinese Center for Disease Control and Prevention reported that 17.6% of Chinese are overweight and 5.6% are obese (Y. F. Wu, et al., 2005). It is estimated that 7% of adults are obese worldwide, and two to three times as many are considered overweight (Seidell, 1999). The prevalence of simple obesity in developed countries (Europe, US, Canada, Japan, etc) is estimated to be 15-23% (Lau, et al., 2007; Seidell, 2000). In developing countries, the estimated prevalence of childhood overweight and simple obesity of Africa in 2010 is 8.5% and is expected to reach 12.7% in 2020. The prevalence is lower in Asia than in Africa (4.9% in 2010), but the total number of affected children (18 million) is higher in Asia (de Onis, et al., 2010).

The main treatment for simple obesity consists of dieting and physical exercise (Lau, et al., 2007). Weight loss may be produced by diet programs over a short term (Strychar, 2006), but maintaining this weight loss is frequently difficult and always requires exercises and a lower calorie diet for a long term as a person's lifestyle (Shick, et al., 1998; Tate, et al., 2007). As one medication, orlistat (Xenical) is currently widely available and approved for long term use with modest weight loss of a average 2.9 kg at 1 to 4 years, but accompanies high rates of gastrointestinal side effects (Rucker, et al., 2007).

Bariatric surgery is the most effective treatment for simple obesity. For severe obesity, it is associated with risks of long-term weight loss and a decreased overall mortality. Swedish Obese Subjects Study (Sjöström, et al., 2007) in 2007 found a weight loss of between 14% and 25% by bariatric surgery at 10 years, and a 29% reduction in all cause mortality when compared to conventional treatment. However, due to the high surgery cost and high risk of complications, other effective but less invasive treatments are sought after.

Acupuncture has been demonstrated to be effective in treating simple obesity (Z. H. Wu, 2009; Lin, et al., 2010; Hu, et al., 2010; Lacey, et al., 2003). In Traditional Chinese Medicine, simple obesity has been conceptualized in a variety of ways, such as a deficiency of *Qi* in the spleen and stomach (Li, 1999), Heat in the stomach and intestine (Shi, et al., 2006; Bai & Fu, 2007). Based on these beliefs about the causes of simple obesity, a variety of acupoints are targeted in the treatment of simple obesity, including (Bai & Fu, 2007): Stomach Meridian acupoints, bilateral Tianshu (ST 25), bilateral Huaroumen (ST 24), bilateral Wailing (ST 26); Spleen Meridian acupoints, bilateral Daheng (SP 15); Conception Vessel Meridian, Xiawan (CV 10), Shimen (CV 5).

Overweight is associated with alterations in lipid concentrations and an activation of inflammatory markers. Reactive oxygen species, particularly superoxide anions, evoke endothelial cell activation through many pathways (Kobayasi, et al., 2010). The discovery about superoxide's involvement in meridians implies that the unavoidable leakage of intracellular superoxide caused by the puncture of a needle into cells at acupoints may be associated with the acupuncture efficacy. One of the immediate consequences caused by the puncture of a needle into an acupoint is the mechanic damage of cells around the point, and it is logic to postulate that a leakage of intracellular superoxide might occur as a result of the cellular damage. If it is true, enzymatic removal of intracellular superoxide with TAT-SOD at acupoints should generate an efficacy on simple obesity comparable to acupuncture since as a novel intracellular superoxide quenching enzyme with high specificity TAT-SOD can do nothing but to be delivered into cells to remove superoxide there once it is applied topically to acupoints. The hypothesis was tested in this clinical study.

3.1 Materials and methods

3.1.1 Study design

To investigate the effect of the intracellular superoxide removal at acupoint with TAT-SOD on simple obesity and to elucidate the mechanism of acupuncture treatment of obesity, 60 test subjects suffering from simple obesity were divided evenly at random into 2 groups to receive 12 week's TAT-SOD cream and acupuncture treatments, respectively.

The curative effect was evaluated as no improvement (BMI decrease less than 2), moderate (BMI decrease 2~4) or marked (BMI decrease 4) improvement, clinical recovery (BMI is getting close to 25), Adverse events were recorded.

Written informed consent was obtained from all participants before enrollment. This study was conducted in Hospital of Fujian Traditional Chinese Medicine (TCM) University (research center), and approved by the Institutional Review Board at the Fuzhou University.

3.1.2 Subjects

Healthy adults between the ages of 16 and 55 years with simple obesity were recruited between November 3, 2008, and September 13, 2010. The following subjects were excluded from this study: patients with a history or physical examination suggestive of renal, hepatic, or cardiovascular disease; pregnant or lactating women; those with secondary obesity; undertaking weight reduction by medication or other measures during the past six months, or with severe organopathy. Ultimately, 52 female subjects and 8 male subjects were enrolled. The body weight, body height, BMI, waist circumference (WC), hip circumference (HC) and waist-hip ratio (WHR) of each subject before and after the treatment were measured.

3.1.3 TAT-SOD preparation

TAT-SOD was prepared by recombinant expression of a fusion protein of human Cu, Zn-SOD fused with TAT peptide in *E. coli* as follows: Constructs preparation: The nucleic acid sequence encoding TAT-SOD fusion protein was constructed by DNA recombinant technology and inserted into expression vector pGEX-2T; Cell culture and transfections: *E. coli* (BL21, DH5α) cells were transformed with the expression vector pGEX-2T containing the inserted *TAT-SOD*; TAT-SOD fusion protein preparation: TAT-SOD was expressed in the E. coli by the induction of IPTG and prepared by affinity chromatography to electrophoretically pure for use (C. Zhang, et al., 2007).

3.1.4 TAT-SOD treatment

Patients of TAT-SOD treatment group received the topical application of TAT-SOD 5000 U/ml, 0.2 ml at 10 mm^2 area around the acupoints, 3 times per day 30 min after food intake for 12 weeks.

3.1.5 Acupuncture location

The prescription consists of bilateral Tianshu (ST 25), bilateral Huaroumen (ST 24), bilateral Wailing (ST 26), bilateral Daheng (SP 15), Xiawan (CV 10), Shimen (CV 5). The location of points was performed according to Table 2 (World Health Organization Regional Office for the Western Pacific, 2008).

Points	location
ST 25: Tianshu	On the upper abdomen, 50 mm lateral to the centre of the umbilicus.
ST 24: Huaroumen	On the upper abdomen, 25 mm superior to the centre of the umbilicus, 50 mm lateral to the anterior median line.
ST 26: Wailing	On the lower abdomen, 25 mm inferior to the centre of the umbilicus, 50 mm lateral to the anterior median line.
SP 15: Daheng	On the upper abdomen, 100 mm lateral to the centre of the umbilicus.
CV 10: Xiawan	On the upper abdomen and on the anterior midline, 50 mm above the centre of the umbilicus.
CV 5: Shimen	On the lower abdomen and on the anterior midline, 50 mm below the centre of the umbilicus.

Table 2. Acupoints and their anatomical positions

3.1.6 Acupuncture needling procedures

"Hwato" disposable pre-sterilized needles were used in the trial, which selected was 0.30 mm in diameter. The length of the needle selected varied according to the point location. Subjects were advised to lie in a supine position for needling of the acupoints. In this treatment, pre-injection swabs with 70% v/v alcohol and dry sterile cotton wool were used when withdrawing the needles. In brief, for this research, the needle was inserted perpendicularly with 30 mm in depth. The needle was retained for 25 minutes with applying the needle techniques as even movement, reducing, tonification and scraping every 10 minutes before the needle was withdrawn.

3.1.7 Statistical analysis

The primary outcomes were the body weight, body height, WC, and HC. Other outcomes were BMI (body weight/body height^2, kg/m^2) and WHR (WC/HC).

Data are reported as means (SEM). All statistical analyses were carried out using the Microsoft Excel version 2003 software. Results in TAT-SOD group and acupuncture group were compared using two-sample *t*-test.

3.2 Results

Sixty subjects were screened and entered into the study. No subject dropped out and no adverse events occurred throughout the study. The groups were matched at entry for age, sex, body weight, body height, BMI, WC, HC and WHR (see Table 3).

Parameter/treatment	TAT-SOD	Acupuncture
No.	30	30
Age (y), mean (SEM)	31.0 (1.6)	30.6 (1.6)
Sex (M/F)	3/27	5/25
Body weight (kg) , mean (SEM)	73.85 (9.99)	72.83 (7.40)
Body height (m) , mean (SEM)	1.58 (0.07)	1.57 (0.06)
BMI (kg/m^2), mean (SEM)	29.48 (3.51)	29.43 (2.58)
WC (cm), mean (SEM)	86.71 (4.51)	87.86 (3.64)
HC (cm), mean (SEM)	93.88 (5.86)	94.75 (4.66)
WHR, mean (SEM)	0.92 (0.06)	0.93 (0.05)

Table 3. Baseline characteristics of simple obesity subjects, F, Female; M, male

The mean BMI scores before and after the treatment for TAT-SOD and acupuncture groups are shown in Table 4. Both TAT-SOD group and acupuncture groups demonstrated a significant decrease in BMI scores (1.79, 2.08 respectively), and there is no significant differences between the two groups.

Treatment		Score, mean (SEM)	BMI decrease	*P* value*
TAT-SOD	Before	29.48 (3.51)		
	After	27.69 (3.28)	1.79	
Acupuncture	Before	29.43 (2.58)		0.950
	After	27.35 (2.46)	2.08	0.651

Table 4. BMI scores of simple obesity subjects, *Two-sample t-test comparing treatment means of TAT-SOD versus acupuncture

Mean scores for the body weight, WC, HC and WHR before and after the treatment are presented in Table 5. Received 12 week's TAT-SOD cream and acupuncture treatments, the TAT-SOD group and acupuncture group also demonstrated a significant decrease body weight (4.54 kg, 5.13 kg respectively), WC (3.74 cm, 4.89 cm respectively) and HC (2.53 cm, 3.58 cm respectively), and a slight decrease in WHR (0.01, 0.02 respectively). No significant differences were observed between the groups on each measurement.

	Treatment	BW (kg)	WC (cm)	HC (cm)	WHR
Before	TAT-SOD	73.85 (9.99)	86.71 (4.51)	93.88 (5.86)	0.92 (0.06)
	Acupuncture	72.83 (7.40)	87.86 (3.64)	94.75 (4.66)	0.93 (0.05)
After	TAT-SOD	69.31 (8.98)	82.97 (4.79)	91.35 (5.40)	0.91 (0.05)
	Acupuncture	67.70 (6.66)	82.63 (2.98)	91.17 (4.14)	0.91 (0.03)

Table 5. Body weight (BW), WC, HC and WHR of simple obesity subjects, mean (SEM). *$p < 0.05$, TAT-SOD versus acupuncture

The percent of subjects in each category of relief with each treatment after treatment are presented in Table 6. The overall clinical effective rates were 60% and 77%, respectively with significant difference ($P<0.01$), when comparing all the measurements before and after the treatment for both groups, and no significant differences were observed between the two groups.

Treatment	None	Moderate	Marked	Clinical recovery
TAT-SOD	12 (40 %)	13 (43 %)	4 (13 %)	1 (3 %)
Acupuncture	7 (23 %)	12 (40 %)	8 (27 %)	3 (10 %)

Table 6. Therapeutic response of simple obesity subjects, No. (percentage)

3.3 Discussion

The total efficacy rate of 77% for simple obesity treatment by acupuncture in this work is within the range of the previously reported ones (Lacey, et al., 2003) although the acupoints selected may somewhat different among the reported treatments. The treatment significantly decreased body weight 4.54 kg in 12 week, more effective than the approved anti-obesity dugs (orlistat, sibutramine, rimonabant) as is reported by Rucker (Rucker, et al., 2007) of an average decrease in body weight by 2.9 kg in adults in 1 to 4 years. The major criticism about the reported works on the acupuncture treatment of simple obesity is that most trials are descriptive in nature, and therefore, its efficacy in a rigorous scientific sense is not conclusive. However, joining numerous reported works, the readily repeatable treatment efficacy as is demonstrated in our work suggests a valuable solid base for the treatment as an effective popular clinical practice before a rigorous scientific validation is available, which may be an impossible goal before the mechanism is elucidated.

The efficacy of the obesity treatment with the topical application of TAT-SOD cream instead of needle stimulation, is somewhat lower than that by acupuncture without significance though. It may be attributed to the less complete quenching of intracellular superoxide at acupoints due to the insufficient delivery of TAT-SOD by applying a limited amount of the cream with a content of 5000u SOD activity/ml. Further trial is necessary to determine an appropriate dosage for TAT-SOD application.

What is worth noting is that the subjects' WHR in TAT-SOD group merely decreased 0.01, which indicated that stimulation at acupoints lost little more weight around the waist than that around the hip. As is shown on acupuncture group or what Yang (J. J. Yang, et al., 2010) report, acupuncture decrease WHR of subject little more than what TAT-SOD application do at 0.02 in 9~12 weeks. The difference of 0.01 between TAT-SOD treatment and acupuncture without significance though may be also attributed to nonoptimal dosage for TAT-SOD application.

Subjects receiving both acupuncture and TAT-SOD treatment reported a feeling of loss in appetite (data not shown), indicating that TAT-SOD treatment may possibly work by the same mechanism as acupuncture on weight loss through appetite modulation. Han (Han, 2003) reported that acupuncture or electrical stimulation in specific frequencies applied to acupoints can facilitate the release of specific neuropeptides in the central nervous system (CNS), such as neuropeptide Y (NPY) in appetite modulation. Further work to monitor the change in neuropeptide Y after TAT-SOD application and to compare the change pattern with that of acupuncture is necessary to decisively conclude if the both treatments share the same mechanism.

The overall of efficacy of TAT-SOD treatment as a preliminary test is encouraging that such a simple and safe protocol and may possibly replace the high-skill required practice of acupuncture in order to achieve comparable treatment results. The patient acceptance of a topical application can be extremely different from the puncture of a needle into body.

The overall clinical effective rates were 60.00% and 76.67%, respectively with significant difference ($P<0.01$) when comparing all the measurements before and after the treatment for both groups but no significant differences between the groups. The results indicate that enzymatic removal of intracellular superoxide at acupoints could achieve comparable efficacy with acupuncture, implying a novel mechanism for acupuncture, and reconfirming superoxide's key role in meridians.

4. Acupuncture meridians as a novel system for superoxide disposition?

Enzymatic removal of intracellular superoxide has generated remarkably similar efficacy in treating simple obesity, suggesting that acupuncture may work on the same mechanism of intracellular removal at acupoints. What is different with acupuncture may be that it removes intracellular superoxide at acupoints by puncturing cells containing superoxide and cause a leakage rather than enzymatic scavenging inside the cells.

What is more interesting with the TAT-SOD treatment is

1. Mechanical removal of superoxide by acupuncture results in the same results as enzymatic removal of superoxide by TAT-SOD.
2. Confirmation of our discovery of superoxide's involvement in meridian.
3. A novel alternative solution to the dilemma of placebo in acupuncture efficacy validation.
4. Source of superoxide in meridian may be from the stressed cells constituting the organs connected to meridian.
5. Conduction of superoxide to acupoints may be due to electric effect. Medtronic's device behind the stomach to curb appetite may be related to this pathway.

Many researchers (Liu, et al., 2006; Kim, et al., 2009; Yu, et al., 2010) concluded that acupuncture treatment displayed antioxidative properties to human body, but still didn't know how the antioxidative properties work to meridian and what the connection between

acupuncture and antioxidative properties is in the path of acupuncture meridian. We used TAT-SOD as cream solute to smear on subject's acupoint achieved acupuncture-like efficacy, these results confirmed that TAT-SOD traversed into acupoint or puncture a needle into acupoint could modulate appetite by remotely increasing SOD activity in target tissues. It also confirmed the discovery of superoxide's involvement in meridian from our previous work (Guo, et al., 2000) on demonstrating the alignment of cells with a high content of superoxide almost perfectly superimposable on the meridian lines. Meridian line was unsearchable channel which was on the knees of the gods, but now possibly is a novel channel for superoxide transporting and disposal between acupoints and the action target of acupuncture, as is in simple obesity a channel between ST 25, ST 24, ST 26, SP 15, CV 10, CV 5 and target tissues.

Obviously, more works employing more sophisticated approaches are necessary before the proposed concept of the removal of intracellular superoxide at acupoints as the mechanism of acupuncture can be established. The current results clearly suggest a great possibility of replacing a mysterious and cumbersome treatment of acupuncture with an intracellular superoxide scavenging treatment at acupoints no more difficult than applying skin care products, and also provide a novel alternative solution to the dilemma of placebo in acupuncture efficacy validation.

On the other hand, it would be extremely interesting to trace where those superoxide molecules in the meridian line originate from, how they are transported from cell to cell along the meridian lines and how they end up in the acupoints, and what is the influence of each of the processes on human body. Base on the results of our previous work (Guo, et al., 2000), cells along the meridian lines were not damaged by the high concentration of intracellular superoxide as indicated by the strong fluorescence intensity. It implied that the superoxide could be in a transit status caused probably by the electric potential along the meridian lines and the potential magnitude may be proportional to superoxide generated by visceral organs or tissues linked by the meridian lines, thus can possibly indicate performing status of the organ or tissue. We tried to electrostimulate ST 24 (cathode) and ST 26 (anode) by 9-volt DC battery, resulting in a subject 2-week appetite loss and 2.5 kg weight loss (data not shown).

With further characterization of constituting cells along the meridians, elucidation of superoxide's conducting mechanism and disposal of superoxide at acupoints, a complete picture of meridians as a novel scientific system is on the horizon, which will not only provide a solid scientific basis for acupuncture but also reveal an unknown bioelectric system for reporting and controlling body function. Conduction of superoxide to acupoints may be due to electric effect. Medtronic's device (Arndt, 2005) behind the stomach to curb appetite may be related to this pathway.

If all those questions are answered, what we can obtain will not only be a convincing mechanism of acupuncture and many simpler, and safer and more effective treatment alternatives to needle puncture, but an unprecedented new understanding about physics, physiology and biology of a living body.

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7

Blood Flow Volume as an Indicator of the Effectiveness of Traditional Medicine

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1. Introduction

In pulse diagnosis, an important diagnostic technique in traditional medicine (TM), treatment plans for traditional therapies are adjusted according to a patient's observed pulse. Use of this approach suggests that blood flow can be an ideal indicator of the effectiveness of TM in humans.

To our knowledge, few studies have examined the relationship between peripheral artery haemodynamics and traditional therapies in humans.

We must overcome some difficulties to confirm this hypothesis. First, it is necessary to use a very simple intervention in the study to investigate its effect and add reproducibility. Second, we need to use a quantitative indicator to evaluate the effect of traditional interventions such as acupuncture, moxibustion, and herbal medicine. Acupuncture and moxibustion therapies are complex interventions, and this complexity causes a loss of objectivity for the study. In our recent studies, we employed a simple or standardised acupuncture technique and a moxibustion simulator to ensure reproducible results. On the other hand, blood flow volume (BFV) is known to be an important index to demonstrate the condition of organs and tissues. Thus, we employed BFV as a quantitative indicator to show the effects of traditional interventions on the human body.

We would like to summarise our 4 recent studies: first, a study of the effect of simple acupuncture on the BFV of extremities (Takayama et al., 2010); second, a study on the effect of the moxibustion simulator on the BFV of the abdominal organs and extremities (Takayama et al., 2009); third, a comparison study on the effects of herbal medicine and the moxibustion simulator on the BFV of abdominal organs (Takayama et al., 2010); and fourth, a study on the effects of standardised acupuncture in a patient with open angle glaucoma (Takayama et al., 2011).

2. TEAM theory and intervention characteristics

TM developed differently than modern biomedicine did in that it was based on many years of experience rather than experiments. Many TM theories are considered unproven by the modern biomedical concept. Traditional East Asian Medicine (TEAM) practiced in China, Japan, Korea, Mongolia, Tibet, and Vietnam originated in ancient China and spread throughout history. For instance, it came to Japan about 1,500 years ago and developed in its own way in Japan. It continues to exist as Kampo medicine (Traditional Japanese Medicine;

TJM) and uses different terminologies, interventions, and acupuncture points (acupoints) than does today's traditional Chinese medicine (TCM). It can be said that TM develops differently depending on the natural environment and culture and, thus, has different interventions.

2.1 TEAM meridians and organs

The TEAM theory states that meridians are connected to the organs and that acupoints on meridians affect organ function. It was historically very difficult to verify the meridian or acupoint structure and the connection between the organs and meridians.

According to TEAM, the main physiological functions of the stomach include receiving, digesting, transforming food and water, and taking charge of sending down food contents (Liu et al., 2006). Zusanli (ST36) and Tianshu (ST25) are located on the stomach meridian (Figure 1A). Zusanli is on outer side of just below the knee. Tianshu is located on the outside of the belly bottom on both sides. Those acupoints are known to be effective in the treatment of digestive system diseases, improving digestive function and decreasing abdominal pain (Liu et al., 2006). One of the functions of the liver is to regulate the free movement of Qi. Stagnation of liver Qi may impede blood circulation (Liu et al., 2006) and cause habitual limb chills. Taichong (LR3) is the source point of the liver meridian (Figure 1B) and stimulation on this point can regulate liver function (Liu et al., 2006).

However, the evidence of the functions explained above remains intangible.

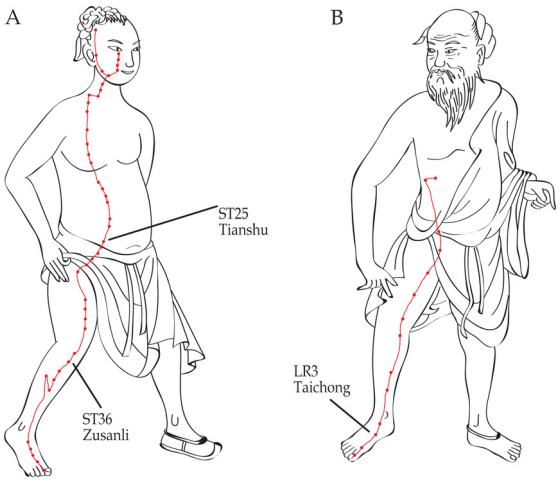

Fig. 1. (A) Stomach meridian. (B) Liver meridian. Modified from Liu et al., 2006.

2.2 Complexity of TEAM interventions

Acupuncture and moxibustion therapy are major TEAM interventions, and each has a wide range of treatment methods. This variation is due to the treatment method in clinical experiments, including acupuncture point; needle number, gauge, length, manufacturer, and materials; insertion depth; needle retention time and insertion technique; stimulation intent and duration; and the specific needling response be elicited (e.g., the de qi sensation in traditional acupuncture, the muscle twitch in trigger point treatment, or muscle contraction in

electroacupuncture). In order to standardise therapy methods, the STandards for Reporting Interventions in Controlled Trials of Acupuncture (STRICTA), which was organised by a group of British and American magazine editors, encourages practitioners to record needling data. In addition, for moxibustion therapy, therapy types, processing method, amount of moxa, and handling can vary. Due to the nature of the therapies that require treatment method variation, it is extremely challenging to ensure result reproducibility.

3. Reproducible simple or standardised intervention for the acupuncture and moxibustion therapy study

We reported some clinical experiments using simple or standardised acupuncture methods rather than using the method that is equivalent to actual acupuncture treatments. The report indicated that the results concluded that by the use of a simple acupuncture method, which involves inserting the needle perpendicularly into the skin surface on the acupoints, no additional stimulation with certain needle retention times was required. Example findings include improved swallowing in patients with swallowing problems and improved ambulatory function in patients with gait disorders (Seki et al., 2003; Seki et al., 2004; Seki et al., 2005). Intraocular pressure (IOP) changes in patients with glaucoma before and after 1 month of standardised acupuncture have also been reported (Kurusu et al., 2005). Clinical research conducted using the simple acupuncture method is a helpful tool for simplifying the complexity of clinical experiments for acupuncture treatments. Standardised acupuncture is a new treatment measure for some diseases. We used a simple or standardised acupuncture method with simple or no manipulation for the research shown below to investigate the effect of acupuncture.

Simple acupuncture employed in the studies is as follows:

A disposable fine stainless-steel needle (diameter, 0.16 mm; length, 40 mm; Seirin Co., Ltd., Shizuoka, Japan) was inserted bilaterally on an acupoint and maintained at a depth of 10 mm during the test. After insertion, stimulation (rotating the needles manually within an angle of 90°) was performed for 18 s.

Standardised acupuncture employed in the glaucoma study is as follows:

The acupoints were selected on the basis of the principles of TCM (Kurusu et al., 2005). Acupuncture was performed bilaterally for 15 min using disposable stainless-steel needles (0.16 mm or 0.20 mm × 40 mm; Seirin Co. Ltd., Shizuoka, Japan) at acupoints Cuanzhu (BL2), Taiyang (M-HN9), Sibai (ST2), Zusanli (ST36), Sanyinjiao (SP6), Taixi (KI3), and Taichong (LR3) while the patient was in the supine position, and bilaterally for 15 min at acupoints Fengchi (GB20), Ganshu (BL18), and Shenshu (BL23) while the patient was in the prone position. Each needle was simply inserted without any intention of eliciting specific responses (e.g., de-qi feelings) to a depth of approximately 20 mm at acupoints ST36, SP6, KI3, GB20, BL18, and BL23. The needles were inserted to a depth of approximately 3–10 mm for the BL2, M-HN9, ST2, and LR3 acupoints. Neither needle manipulation techniques nor other auxiliary interventions were used.

As stated above, reproducibility is the challenge for this type of moxibustion experiment. Several studies have assessed the effect of moxibustion (Freire et al., 2005; Joos et al., 2004; Joos et al., 2006; Yun et al., 2007), but controlling the temperature achieved by burning moxa has been difficult. The actual temperature depends on the practitioner's experience, and the temperature distribution in the target area is not uniform, making quantification of the effects of moxibustion difficult.

Moxibustion is a way of heating a local area in which moxa is burned at acupoints on the skin. Because the direct burning of moxa on the skin can cause burns, materials such as salt, ginger, or garlic may be used as a buffer between the skin and the moxa. To overcome such a challenge, we developed a heat transfer control device (HTCD) for local thermal stimulation that can be used to simulate the heat and mechanical pressure effects of moxibustion through collaboration with Prof. Shigenao Maruyama from the Institute of Fluid Science at Tohoku University (Figure 2) (Maruyama et al., 2009; Okajima et al., 2009; Takashima et al., 2007; Takashima et al., 2008). This device consists of a heating disk (10 cm in diameter) that can be set at a certain temperature with a precision of 0.1 °C that heats a small area to a uniform temperature, thus allowing us to quantify the effects of moxibustion. There are several acupoints located at the paraumbilical region. In particular, Shenque (CV8) and ST25 are considered by TEAM to influence the stomach, spleen, and intestines (Chen, 1981). This size of the disk can cover the important acupoints CV8 and ST25 for the treatment of digestive diseases. It also provides consistent area and temperature, both of which are crucial in this type of clinical research.

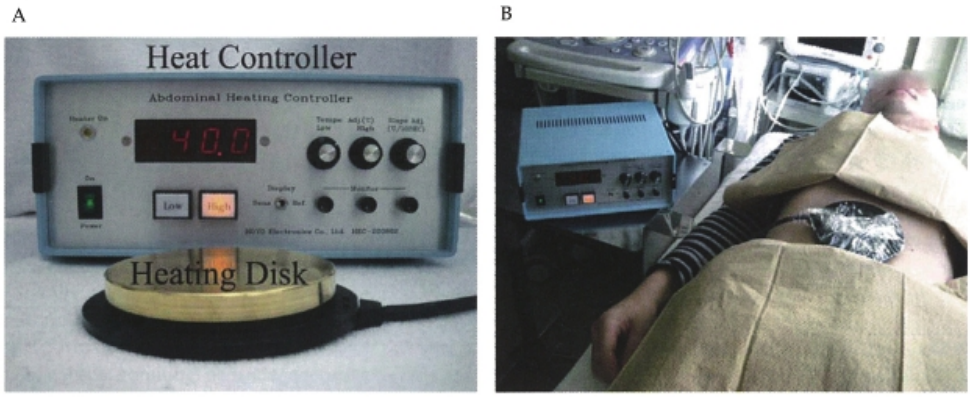

Fig. 2. (A) The heat transfer control device consists of a heating disk, a temperature sensor, and a heat controller. (B) Thermal stimulation to the paraumbilical region with the device for 20 min at 40 °C.

4. Haemodynamic evaluation method

Blood pressure, electrocardiogram, impedance cardiography (ICG), and ultrasound were employed to evaluate the haemodynamic changes in the study.

4.1 Blood pressure, electrocardiogram, and ICG

Blood pressure was measured using an oscillometer on the subjects' left upper arms. Three monitoring electrocardiographic electrodes were attached to the anterior chest.

ICG is a non-invasive monitoring method that allows for cardiac output measurements based on the thoracic resistance changes that result from variations in intrathoracic BFV (Albert et al., 2004; Perrino et al., 1994). Four ICG electrodes (BioZ ICG Module, Dash 3000®, GE Healthcare, Milwaukee, USA) were placed at the base of the neck and at the level of the xiphoid process in the midaxillary line.

4.2 Ultrasound system

Blood flow changes rapidly in the arteries of the extremities, especially in the peripheral arteries (Nimura et al., 1974). Pulse Doppler ultrasound is a non-invasive method for evaluating blood flow velocity. High-resolution ultrasound combined with pulsed Doppler ultrasound is useful for investigating small vessels such as the coronary, splenic, adrenal, and superior mesenteric artery (SMA) (Gembruch, 1996).

A Prosound α10® ultrasound system (Aloka Co., Ltd., Tokyo, Japan) was employed for measuring the haemodynamics of radial artery (RA), brachial artery (BA), and SMA. The haemodynamics of the retrobulbar vessels was assessed using a LOGIQ *e*® ultrasound system (GE Healthcare, Tokyo, Japan).

4.2.1 Measurements to compare RA and BA haemodynamics

Although the RA can be easily visualised by ultrasound, its diameter is far smaller than that of the BA. To compare the changes of haemodynamics in the RA and BA, we employed an e-Tracking system® (Aloka Co., Ltd., Tokyo, Japan) built into the Prosound α10® to obtain more minute data with a high-resolution linear array transducer (13 MHz). The e-Tracking system® is a computer-assisted analysis software developed for the measurement of flow-mediated vasodilatation, which is usually measured at the BA (Corretti et al., 2002; Deanfield et al., 2005). The software could automatically detect the vessel edge and continuously measure the vessel diameter and BFV with a precision of 0.01 mm (Soga et al., 2007).

Making the correct assessment of haemodynamics is more difficult and requires some skill. Fixing the probe at the optimal position and preventing upper-limb movement are crucial. The transducer was fixed with a special probe holder (MP-PH0001, Aloka Co., Ltd.) throughout the test at the site where the clearest B-mode image of the anterior and posterior vessel wall was obtained (Figures 3A and 3B). Compression of the artery was carefully avoided. The RA or BA diameter was automatically monitored when the tracking gate was placed on the intima of the vessel. The waveform of the vessel diameter changes over the cardiac cycle was displayed in real time using the e-Tracking system (Figure 3C). A Doppler angle of ≤60° was maintained to ensure accurate measurement. BFV was calculated automatically as the Doppler flow velocity (corrected for the angle) multiplied by the heart rate and the vessel cross-sectional area (Burns & Jaffe, 1985; Gill, 1985; Taylor & Holland, 1990). The use of this system avoids operator bias, increases reproducibility, and improves accuracy.

It is reported that changes in venous return due to respiration cause oscillation of the stroke volume and blood pressure (Hsieh et al., 2003). Thus, the arterial pulse should be modified by breathing (Korpas et al., 2006). The subjects were asked to breathe every 6 s during the test, and the haemodynamic parameters were calculated as average values for each 6-s period to minimise the influence of respiration in the present study.

4.2.2 Haemodynamic measurements to evaluate the effects of simple acupuncture in the SMA and BA and the effects of thermal stimulation and herbal medicine in SMA

A 5-MHz convex transducer was used to measure SMA haemodynamics and a 13-MHz linear transducer was used for the BA haemodynamics. The cross-sectional areas (CSAs) of the SMA and BA were calculated using the vessel diameter (VD) value, the distance from the anterior to the posterior intima (Figures 4A and 4C).

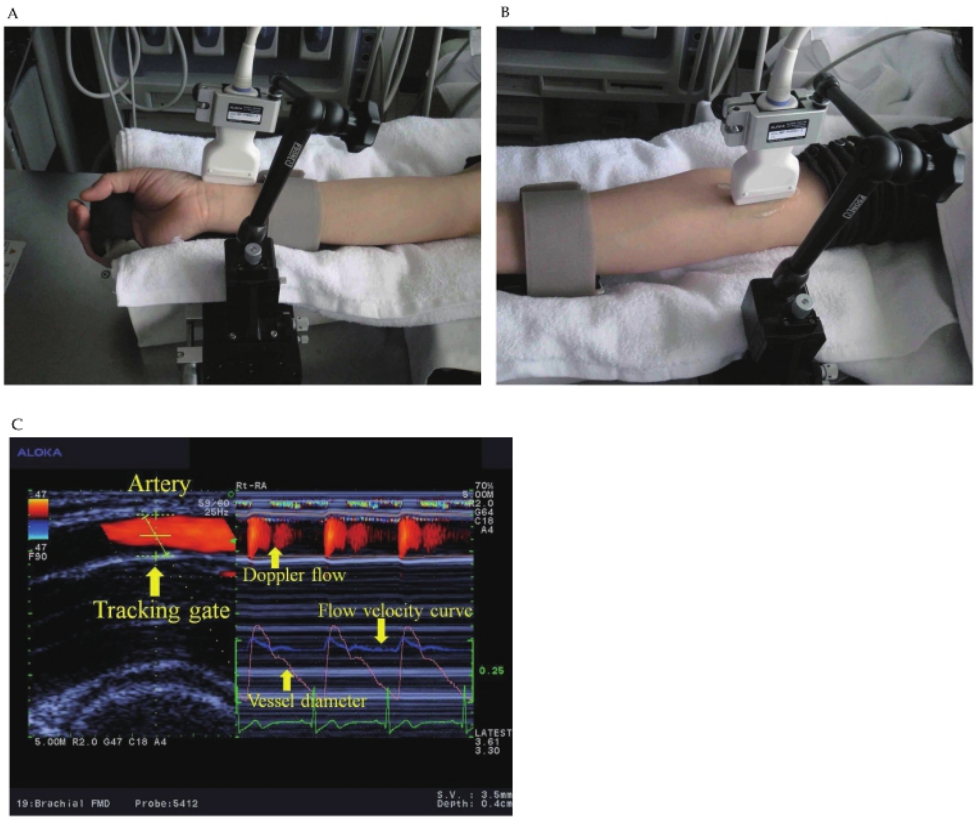

Fig. 3. (A) Ultrasound measurement of the radial artery with a special probe holder.
(B) Brachial artery measurement. (C) Left, the vessel image and the position of the tracking gate of the artery; Right, the changes in the vessel diameter, Doppler flow, and flow velocity determined using an automated edge detection device and computer analysis software (e-Tracking system ®, Aloka Co., Ltd., Tokyo, Japan).

SMA measurements were acquired within 2–3 cm of the arterial origin (Figure 4B) (Gill, 1985; Van Bel et al., 1990). Pulsed Doppler signals were obtained at the same site. The BA was monitored at a site above the elbow (Figure 4D).
To ensure accurate measurements, a Doppler angle of ≤60° was employed. Each Doppler waveform was drawn automatically and calculated using the ultrasound system software. The following haemodynamic parameters were determined.
Haemodynamic parameters: VD, CSA ($(VD/2)^2 \times \pi$), peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI = (PSV-EDV)/PSV), pulsatility index (PI = (PSV-EDV)/mean flow velocity (MV), MV, and blood flow volume (CSA × MV) (Gill, 1985; Van Bel et al., 1990).
Each parameter was recorded 3 times in 3 different cardiac cycles and averaged for each subject in an effort to minimise random errors (Gill, 1985).

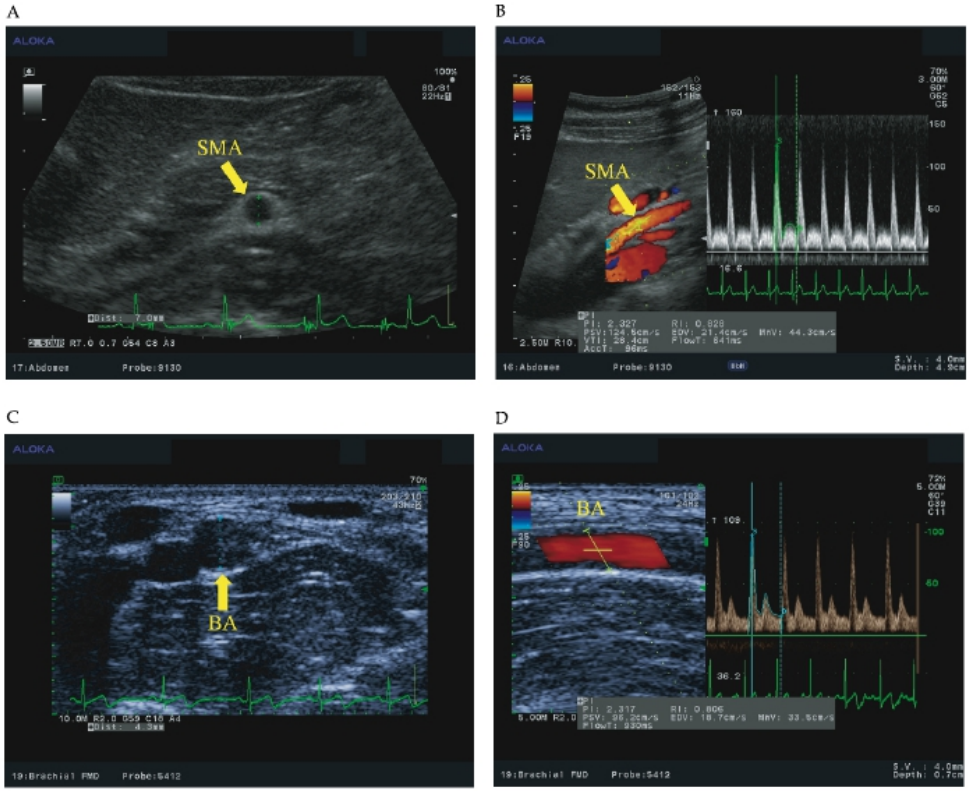

Fig. 4. Haemodynamic data obtained by ultrasound. Vessel diameter of the superior mesenteric artery (SMA) (A) and the brachial artery (BA) (C) were measured in B-mode. Blood flow velocity of the SMA (B) and the BA (D) were obtained by pulsed Doppler.

4.2.3 Haemodynamic measurement of the retrobulbar arteries

Real-time and non-invasive haemodynamic measurement using colour Doppler imaging (CDI) has been applied for measuring retrobulbar vessel haemodynamics, and its reproducibility has already been demonstrated (Matthiessen et al., 2004).

Ultrasound measurements were performed carefully to avoid any pressure on the eye. CDI was performed using a 13-MHz linear transducer for retrobulbar vessels such as the ophthalmic artery (OA), central retinal artery (CRA), and short posterior ciliary artery (SPCA). The OA was examined approximately 20 mm behind the globe (Figure 5A). The CRA was examined within 5 mm of the retrolaminar portion of the optic nerve (Figure 5B). The temporal SPCA was examined approximately 5–10 mm behind the globe (Figure 5C). All blood flow velocity waveforms were measured at the corrected Doppler angle. Resistive index (RI = (PDV-EDV)/PSV) was also measured in each retrobulbar vessel.

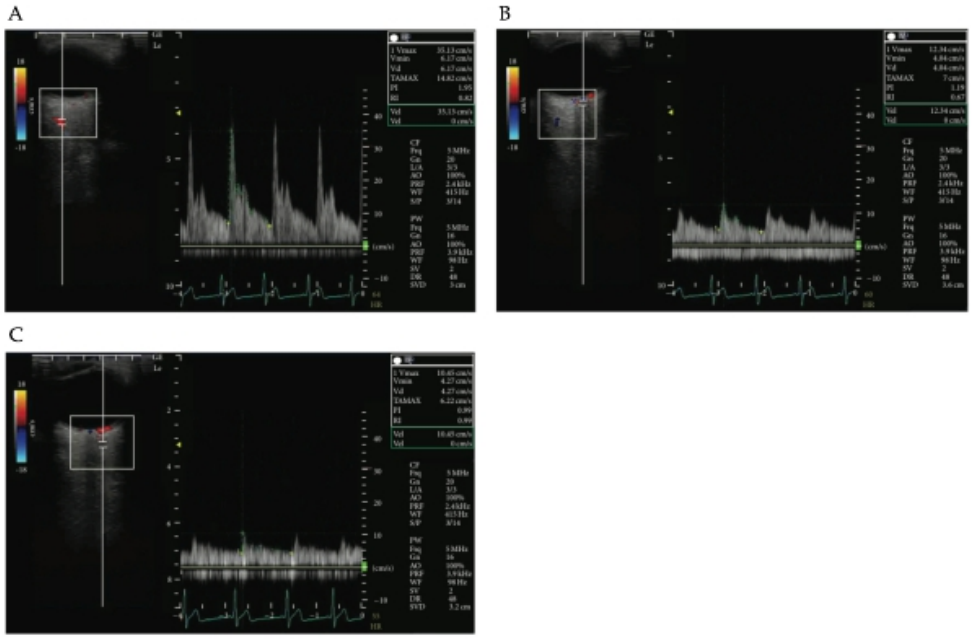

Fig. 5. Horizontal scans collected using colour Doppler imaging through the globe showing the (A) ophthalmic artery, (B) central retinal artery, and (C) short posterior ciliary artery.

5. Short-term effect of acupuncture on the peripheral arterial system of the upper limb and systemic haemodynamics

The radial, carotid, fibular, and posterotibial arteries have been used in pulse diagnosis. However, the radial pulse is more common in clinical pulse diagnosis. There have been some reports about the haemodynamic influences of acupuncture using a single acupoint (Haker et al., 2000; Syuu et al., 2001; Wan et al., 2000), but its effects on BFV changes in the peripheral artery have not been demonstrated. We reported the only preliminary study on the effects of simple acupuncture on the RA BFV (Takayama et al., 2010).
Furthermore, the relationship between peripheral artery blood flow and the cardiac index with regard to acupuncture has not been studied in human subjects.
We hypothesised that LR3, the primary acupoint in the liver meridian, could affect the peripheral artery BFV and the cardiac index. Blood flow to the RA is supplied via the BA.
The aim of this study is to clarify the effect of acupuncture on BFV of the RA and BA and the cardiac index in healthy subjects.

5.1 Methods

5.1.1 Subjects

Eighteen healthy volunteers (age, 32 ± 5 years; range, 24–40 years; 14 men and 4 women) were enrolled in the study. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. Written informed consent was given by all the subjects prior to participation.

5.1.2 Setting

All subjects were examined in the morning after an overnight fast. They were placed in a quiet, air-conditioned room (25–26 °C) and told to rest in the supine position.
Each subject's right arm was fixed and the right RA or BA was scanned longitudinally at a position in which the vessel diameter and Doppler wave readings were stable.

5.1.3 Protocol

An outline of the study is shown in Figure 6. Each subject underwent the test on the RA first followed by the BA at 7-day intervals. We performed acupuncture bilaterally on the LR3 and measured the haemodynamics of the RA or BA from rest to 180 s after acupuncture. Cardiac index was measured and the BA test was performed at same time.
After 10 min of resting in the supine position, haemodynamic measurements of blood pressure using the right RA or BA were taken (Corretti et al., 2002; Deanfield et al., 2005). Simple acupuncture was performed by a licensed acupuncturist. We measured RA or BA haemodynamics before and during acupuncture and at 30, 60, and 180 s after acupuncture (Figure 6). The haemodynamic parameters, including vessel diameter, BFV, cardiac index, and heart rate, were continuously recorded. Haemodynamic parameter values were calculated as averages for each 6-s period. Blood pressure was measured under resting conditions and 180 s after acupuncture.

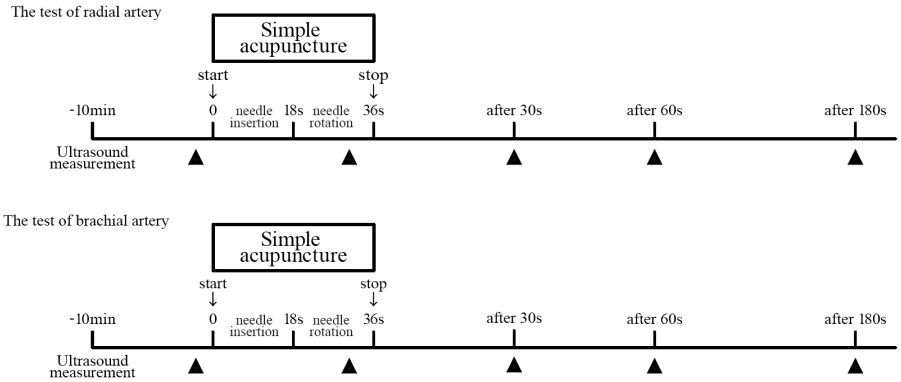

Fig. 6. Outline of the test. Ultrasound measurements were performed before, during, and 30, 60, and 180 s after acupuncture. Acupuncture consists of needle insertion and stimulation. An impedance cardiography monitor was used in the brachial artery test.

5.1.4 Statistical methods

Statistical analysis was performed using SPSS software (version 16.0; SPSS Japan Inc., Tokyo, Japan). Repeated-measure analysis of variance followed by Dunnett's post hoc test was used for statistical comparison between the measure points. Results are presented as the mean (SD) and $P < 0.05$ indicated significance for all statistical analysis.

5.2 Results

5.2.1 RA and BA haemodynamics

Neither the systolic nor the diastolic diameter of the RA or BA significantly changed in the test. The BFV was determined as millilitres per second per square metre and the percent

change at each time was calculated in relation to before acupuncture. BFV in the RA decreased significantly during acupuncture (mean (SD); 0.24 (0.23) mL/(s·m²), $P < 0.01$) but increased significantly at 60 s (0.62 (0.41) mL/(s·m²), $P < 0.05$) and 180 s after acupuncture (0.61 (0.31) mL/(s·m²), $P < 0.05$) compared with before acupuncture (0.51 (0.31) mL/(s·m²)). BFV in the BA also decreased significantly during acupuncture (0.56 (0.33) mL/(s·m²), $P < 0.05$) and increased significantly at 180 s after acupuncture (0.87 (0.36) mL/(s·m²), $P < 0.05$) compared with before acupuncture (0.73 (0.38) mL/(s·m²)). Figure 7 illustrates the profile of the percent changes in RA and BA BFV. BFV in the RA decreased significantly during acupuncture ($P < 0.01$) but increased significantly at 180 s after acupuncture ($P < 0.05$). BFV in the BA also increased significantly at 180 s after acupuncture ($P < 0.05$).

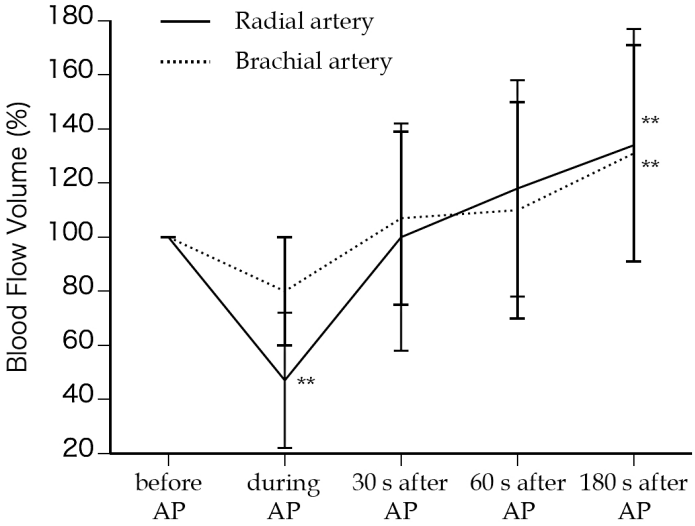

Fig. 7. Percent change in blood flow volume in the radial and brachial arteries. Each variable is relative to the 6-s period before acupuncture. Values represent the mean (SD). AP, acupuncture. **$P < 0.01$ versus before acupuncture.

5.2.2 Systemic haemodynamics

The percent change in cardiac index did not significantly change during acupuncture or at 30, 60, or 180 s after acupuncture compared with the values before acupuncture. Heart rate significantly decreased during acupuncture relative to that before acupuncture ($P < 0.01$). However, these values returned to before acupuncture levels 30 s after acupuncture. Neither systolic nor diastolic blood pressure changed significantly after acupuncture compared with before acupuncture. Systemic vascular resistance was calculated using the cardiac index and the mean blood pressure. The percent change in the systemic vascular resistance index decreased significantly after acupuncture compared with the value before acupuncture ($P < 0.05$).

5.2.3 Adverse events

There were no local complications such as bleeding, haematoma, or infection.

5.3 Discussion

To our knowledge, this is the first report to provide physiological evidence that acupuncture at only 1 acupoint has an effect on the peripheral and systemic haemodynamics in human subjects. The present result demonstrated that RA and BA BFV decreased immediately during acupuncture at LR3 and increased 180 s after acupuncture. While the cardiac index did not change significantly, the systemic vascular resistance index decreased significantly after acupuncture. We showed a decrease in BFV of the RA and BA during acupuncture and an increase in volume 180 s after acupuncture. We speculate that the response of acupuncture on peripheral artery haemodynamics during acupuncture was due to an increase in sympathetic tone as an extreme rapid response to the intervention caused by needle insertion.

These results suggest that the mechanism of increased blood flow by acupuncture is mainly due to decreased peripheral vascular resistance rather than to an increase in the cardiac index. The present findings also indicate that acupuncture at a single point increases peripheral blood flow without increasing the cardiac index, suggesting that acupuncture treatment can affect sympathetic tone in the upper limbs.

In conclusion, we measured the effect of acupuncture on the haemodynamics of the upper limb using high-resolution ultrasound.

5.3.1 Study limitations

This study had 2 primary limitations. The e-Tracking system cannot simultaneously show the pulsatility or resistive index, both of which reveal distal vascular resistance. However, continuous recording using this system showed that the blood flow velocity patterns changed gradually starting 60 s after acupuncture.

The test duration was <15 min, which may be insufficient for evaluating the effects of acupuncture. We attempted a longer test duration in a preliminary study, but fixing the right upper limb for more than 15 min was difficult due to muscle strain and cramps.

The present findings suggest that acupuncture at a single acupoint can alter both RA and BA haemodynamics in healthy subjects. However, these results should be treated with caution considering that this is a pilot study with no control intervention and with a limited sample size.

5.3.2 Future studies

In future studies, we plan to compare changes in peripheral BFV and cardiac index with control conditions (e.g., another acupoint).

6. BFV changes in the SMA and BA with abdominal thermal stimulation

In TEAM, a local thermal therapy known as moxibustion is widely used for conditions such as pain, nausea, vomiting, neurodegenerative diseases, inflammatory bowel diseases, and cerebrovascular and cardiovascular diseases (Giovanni, 1994; Cheng & Cheng, 2008; Wu et al., 2006).

Under normal conditions, SMA blood flow pattern and velocity show large variations due to the metabolic activity of the bowel (Kjeldsen & Schaffalitzky de Muckadell, 1993; Moneta et al., 1988; Perko, 2001). SMA BFV also changes in several diseases (Byrne et al., 2001; Erden et al., 1997; Sigirci et al., 2001). BFV is also strongly related to mesenteric ischaemia, especially chronic mesenteric ischaemia (Dietrich et al., 2007). To date, no reports have examined SMA blood flow changes in relation to moxibustion therapy.

The aim of this study was to evaluate SMA BFV changes in healthy subjects using abdominal ultrasound after thermal stimulation with an HTCD.

6.1 Methods

6.1.1 Subjects

Twenty-four healthy men (age, 31 ± 7 years; range, 21–44 years) were enrolled in the study. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. Written informed consent was given by all subjects prior to participation.

6.1.2 Setting

All subjects were examined in the morning after an overnight fast (at least 8 h). They were placed in a quiet, air-conditioned room (temperature: 25–26 °C) and told to rest in the supine position.

To quantify the heat delivered, we developed an HTCD instead of moxibustion for local thermal stimulation (Figure 2) (Takashima et al., 2008). The temperature of the heating disk could be increased incrementally to the target without exceeding it. Thermal damage occurs when the tissue temperature rises above 44 °C (Jiang et al., 2002; Ng & Chua, 2002). As a result, the disk temperature was set at 40–41 °C for safety. A patent for this device is pending in Japan.

6.1.3 Protocol

An outline of the study is shown in Figure 8. This is a prospective observational study. We performed abdominal thermal stimulation at the paraumbilical region using an HTCD for 20 min and measured SMA haemodynamics by ultrasound from rest until 40 min after thermal stimulation. To compare intestinal and peripheral BFV, we measured the haemodynamics of the BA simultaneously.

After the ultrasound system was positioned, the subjects rested in the supine position for 10 min. Abdominal thermal stimulation was done for 20 min at the paraumbilical region using the HTCD to a temperature of 40 °C. After 5 min, if the subjects were used to the heat, the temperature was increased to 41 °C. After thermal stimulation for 20 min, the device was removed. We measured the SMA and BA haemodynamics, heart rate, and blood pressure at rest (baseline), after 15 min of thermal stimulation, and at 10, 20, 30, and 40 min after the end of thermal stimulation (Figure 8).

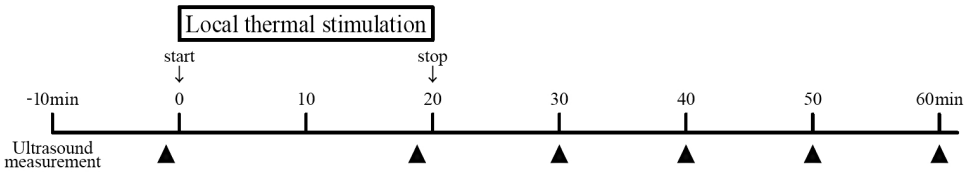

Fig. 8. Outline of the study. Thermal stimulation was applied to the paraumbilical region using a heat transfer control device for 20 min. Haemodynamics were measured at rest (baseline), during thermal stimulation (after 15 min), and at 10, 20, 30, and 40 min after the completion of thermal stimulation.

6.1.4 Statistical analysis

Statistical analysis was performed using SPSS software (version 16.0; SPSS Japan Inc.). Repeated measures analysis of variance with a Tukey post hoc test was used for statistical comparison with baseline. Although 24 subjects were enrolled in our study, data of 3 subjects were excluded because their examinations were technically unsuccessful (the SMA could not be identified clearly due to intestinal gas). As a result, data of 21 subjects were included in the final analysis. Results are presented as the medians and quartile (first and third), the mean and SEM, and 95% confidence intervals. $P < 0.05$ was used to indicate significance in all statistical tests.

6.2 Results

6.2.1 SMA and BA haemodynamics

The SMA diameter significantly increased during thermal stimulation ($P < 0.01$), as well as at 10 ($P < 0.01$), 20 ($P < 0.01$), and 30 min ($P < 0.05$) after thermal stimulation. The BA diameter also increased significantly during thermal stimulation ($P < 0.01$) and at 20 min ($P < 0.05$) after thermal stimulation. The MV in the SMA increased significantly during thermal stimulation ($P < 0.01$) as well as at 10 ($P < 0.01$) and 20 min ($P < 0.01$) after the end of thermal stimulation, but the MV in the BA decreased significantly at 30 ($P < 0.01$) and 40 min ($P < 0.01$) after thermal stimulation. The BFV in the SMA increased significantly during thermal stimulation ($P < 0.01$) as well as at 10 ($P < 0.01$) and 20 min ($P < 0.05$) after the end of thermal stimulation (Figure 9A). In the BA, however, BFV decreased at 40 min after the end of thermal stimulation ($P < 0.01$) (Figure 9B).

PSV in the SMA increased significantly during thermal stimulation ($P < 0.01$) but it decreased significantly in the BA at 30 ($P < 0.05$) and 40 min ($P < 0.01$) after the end of thermal stimulation. EDV did not change in the SMA, but it decreased significantly in the BA at 30 ($P < 0.01$) and 40 min ($P < 0.01$) after the end of thermal stimulation. RI in the SMA did not change, but it increased significantly in the BA at 40 min after the end of thermal stimulation ($P < 0.01$). However, the PI did not change significantly in either the SMA or the BA compared with baseline.

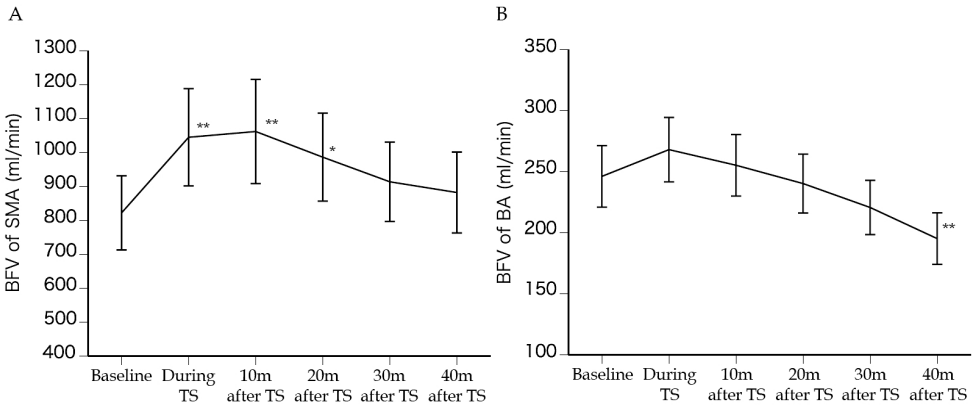

Fig. 9. Blood flow volume changes in the superior mesenteric artery (SMA) (A) and in the brachial artery (BA) (B). Values represent the mean and SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ versus baseline.

6.2.2 Systemic haemodynamics

There were no significant changes in heart rate compared with baseline. Systolic blood pressure showed a significant increase at 40 min after the end of thermal stimulation ($P < 0.05$), but no significant changes in diastolic blood pressure was observed between baseline and 40 min after thermal stimulation.

6.2.3 Adverse events

There were no complications such as local burns, pain, or discomfort that required treatment.

6.3 Discussion

To our knowledge, this is the first report to examine BFV changes in the SMA or BA after local thermal stimulation using an ultrasound system.

The present study showed that BFV in the SMA increased significantly during thermal stimulation and at 10 and 20 min after stimulation. BFV decreased significantly in the BA 40 min after stimulation. The thermal stimulation and pressure applied to the skin by the present method influences somatic afferent fibres. We speculate that the present method of stimulation increases SMA blood flow mediated by somatic group II, III, and IV afferent fibres and parasympathetic cholinergic nerves. Moxibustion is often combined with acupuncture; however, in Western countries, it is not often used because of the odour of the burning moxa. HTCD can heat the target area uniformly without any odour or fire-associated danger. Thus, abdominal thermal therapy using HTCD may be useful in place of moxibustion for patients with intestinal disorders.

In conclusion, we quantitatively measured the effect of local thermal stimulation using an HTCD and high-resolution ultrasound. Thermal stimulation of the paraumbilical region in healthy subjects increased blood flow in the SMA 20 min after stimulation.

6.3.1 Study limitations

This trial was a pilot study with a small sample size and no controls or randomisation. All subjects were men.

6.3.2 Future studies

In future studies, we plan to recruit men and women and use a larger sample size, a control group, and randomisation. We hope to study the effect of thermal stimulation on patients with disorders. We would also like to undertake further studies to clarify the effect of local thermal stimulation, for not only the abdomen but also other areas.

7. Effect of abdominal warming and herbal medicine on SMA blood flow

In TEAM, warming of the abdomen is performed to dispel cold and help Yang recovery via the use of moxibustion or warming herbal medicines (Liu et al., 2006). However, their physiological effect on abdominal blood flow has never been investigated.

Daikenchu-to is one of the warming herbal medicines that is used in TEAM to treat coldness in the Middle Jiao and abdominal pain (Liu et al., 2004). Daikenchu-to has traditionally been used in Japan for the treatment of intestinal obstruction and cold feeling in the abdomen. It is reported that Daikenchu-to stimulates colonic motility and increases portal blood flow in humans (Kawahara & Yanaga, 2009; Ogasawara et al., 2008).

The SMA supplies blood to the entire small intestine except for the superior duodenum, and it also supplies the caecum, ascending colon, and most of the transverse colon (Williams & Warwick, 1980). Our preliminary report was the first to show the effect of Daikenchu-to on the BFV of the SMA in humans (Takayama et al., 2009).
The aim of this partly randomised control study was to clarify the physiological effects of warming the abdomen in moxibustion-like stimulation and herbal medicine on SMA BFV in healthy subjects.

7.1 Methods

7.1.1 Subjects

Twenty-eight healthy men were randomly assigned to intervention groups A (age, 33 ± 7 years) and B (age, 35 ± 8 years). As a control group, another 14 healthy men (age, 33 ± 8 years) were assigned to group C. Since randomisation was performed in groups A and B, the study design was a partly randomised trial. We enrolled 42 subjects in this study. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. Written informed consent was given by all subjects prior to participation.

7.1.2 Setting

All subjects were examined in the morning after an overnight fast.
The herbal medicine Daikenchu-to (TJ-100; Tsumura, Co., Tokyo, Japan) was made in the form of a dry powder but is manufactured as an aqueous extract containing processed ginger (*Zingiber officinale*), ginseng (*Panax ginseng*), and zanthoxylum fruit (*Zanthoxylum piperitum*) in the ratio 5:3:2. These plants are all registered in the Pharmacopoeia of Japan. The 3 herbal medicines were extracted with purified water at 95 °C for 1 h and then spray-dried to produce a powder. TJ-100 was made by mixing Daikenchu-to extract powder and maltose syrup powder (Tsumura Co., Tokyo, Japan) in the ratio 1:8 (Tokita Y et al., 2007).

7.1.3 Protocol

An outline of the study is shown in Figure 10. Group A (n = 14) underwent abdominal thermal stimulation at the paraumbilical region using a HTCD for 20 min at 40 °C. Group B (n = 14) took 5.0 g of TJ-100 (Tsumura, Co., Tokyo, Japan) orally with 50 mL of distilled water (37 °C). As a control, group C (n = 14) took 50 mL of distilled water (37 °C) alone. Abdominal ultrasound was performed at rest and at 10, 20, 30, 40, and 50 min after the starting thermal stimulation, after oral administration of TJ-100, or after oral administration of water alone.

7.1.4 Statistical methods

Statistical analysis was performed using SPSS software (version 16.0; SPSS Japan Inc.). Characteristics and BFV changes between groups were compared using ANOVA. Repeated-measures ANOVA with a post-hoc Dunnett's test was used for statistical comparison to the resting state; $P < 0.05$ was considered significant.

7.2 Results

There were no significant differences in baseline characteristics between groups. SMA BFV increased significantly at 10, 20, 30 min ($P < 0.01$), and 40 min ($P < 0.05$) after the start of local thermal stimulation in group A compared to the resting value. SMA BFV also increased significantly at 10, 20, 30, 40, and 50 min after administration of TJ-100 ($P < 0.01$) in

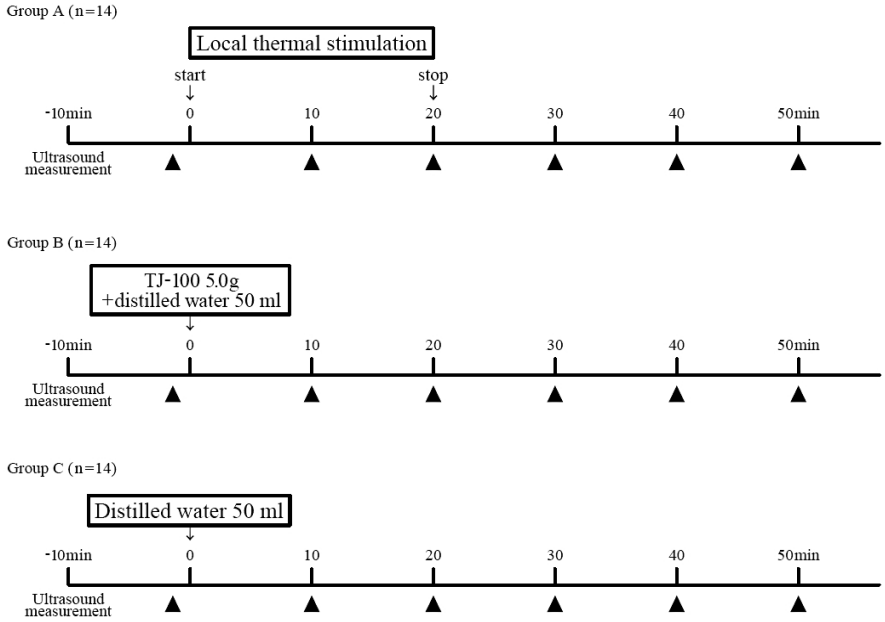

Fig. 10. Outline of the study. Group A: Thermal stimulation was applied to the paraumbilical region using the heat transfer control device for 20 min. Group B: Daikenchu-to (TJ-100; 5.0 g) in distilled water (37 °C; 50 mL) was administered orally. Group C: Distilled water (37 °C; 50 mL) alone was administered orally. Ultrasound measurements were performed at rest and at 10, 20, 30, 40, and 50 min after the start of thermal stimulation, oral administration of TJ-100, or oral administration of water alone.

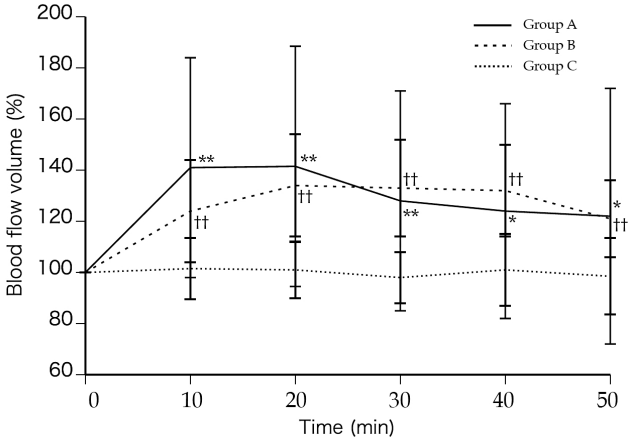

Fig. 11. Percent changes in superior mesenteric artery blood flow volume from the resting value. Values represent the mean (SD). Repeated-measures ANOVA with a post-hoc Dunnett's test was used for statistical comparison with the resting state. *$P < 0.05$, **$P < 0.01$ vs. the resting value in group A. ††$P < 0.01$ vs. the resting value in group B.

group B; however, it did not change significantly after administration of water alone in group C. There were significant differences between groups A and C ($P < 0.01$) and groups B and C ($P < 0.05$); however, no significant differences were observed between groups A and B ($P = 0.96$) with respect to BFV changes. Figure 11 displays the percent changes in SMA BFV.
No adverse events were observed in the study.

7.3 Discussion

To our knowledge, this is the first report to examine the physiological effects of warming the abdomen in moxibustion-like stimulation compared with an herbal medicine on SMA BFV in healthy subjects.
The present study showed that SMA BFV was increased by local thermal stimulation of the abdomen and by oral administration of TJ-100 compared with oral administration of water alone. The pattern of blood flow increase was similar in local thermal stimulation on the abdomen and oral administration of TJ-100.
The present findings indicate that warming the abdomen by thermal stimulation or the herbal medicine of Daikenchu-to has a physiological effect in increasing BFV in the SMA.
In conclusion, we evaluated the difference between thermal stimulation with HTCD and herbal medicine on the haemodynamics of SMA using a high-resolution ultrasound system.

7.3.1 Study limitations

Limitations of this study were as follows:
This was a partly randomised control study with a small sample size and enrolled only men.
HTCD was developed to simulate the heat and mechanical pressure effects of moxibustion. However, it does not simulate the smell of moxa or the smoke of moxibustion.
The subjects could identify the Daikenchu-to because this medicine is spicy and a little sweet. Thus, the test was not perfect, even though the examiners and subjects were blinded to the treatment.
Intake of food prior to the study was controlled by asking the subjects not to eat before the study. However, the patients fasted at home and were not monitored. Thus, there is a very small chance that an increase in SMA BFV could have occurred due to the subject's diet.

7.3.2 Future studies

Evaluation of the clinical effects of these therapies in patients with SMA or IMA ischaemia is required. In future studies, we would like to include both genders and patients with low SMA blood flow.

8. Short-term effects of acupuncture on open-angle glaucoma in retrobulbar circulation: additional therapy to standard medication

Glaucoma is one of the causes of blindness (K. Nakae et al., 2005). The main treatment strategy for glaucoma is to control IOP (Weinreb & Khaw, 2004). Although IOP reduction is currently the main target for the treatment of glaucoma, treatment modalities that enhance retrobulbar haemodynamics in addition to reducing IOP may have a beneficial effect on glaucoma therapy. It has been reported that glaucoma is associated with reduced blood flow velocity and elevated RI in the retrobulbar vessels (Akarsu & Bilgili, 2004; Costa et al., 2003;

Kaiser et al., 1997; Rankin, 1999). Impaired ocular circulation contributes to the progression of glaucomatous damage (Satilmis et al., 2003; Schumann et al., 2000; Yamazaki & Drance, 1997). Acupuncture has also been used for the treatment of ocular diseases, including glaucoma, in TEAM (Li, 1999).
The aim of this study was to evaluate haemodynamic changes in the retrobulbar vessels using CDI to investigate the effect of acupuncture on open-angle glaucoma (OAG) eyes.

8.1 Methods

8.1.1 Subjects

Eleven patients with OAG (20 eyes with OAG; 1 man and 10 women; mean age, 63 ± 11 years) were enrolled in the study. Each underwent standard medical treatment for at least 3 months. Patients who underwent laser trabeculoplasty, any ocular surgery, or inflammation within the past year were excluded from the present study. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. Written informed consent was given by all the subjects prior to participation.

8.1.2 Setting

The ultrasound measurements were performed in an air-conditioned room with the patients in the supine position. On the trial days, patients arrived having taken their regular medications.
Each patient received standardised acupuncture therapy in the morning as described above. Five licensed acupuncturists and 1 physician-acupuncturist with >5 years of acupuncture experience administered the acupuncture treatment.

8.1.3 Protocol

An outline of the study is shown in Figure 12. To minimise the effects of diurnal variation, all measurements were recorded at the same time of day (10–11 AM) for each patient by the same examiner. As a control, subjects underwent measurements of the systemic haemodynamics, retrobulbar vessel haemodynamics, and IOP that were performed at rest and 1 hour after rest. One month later, each patient underwent the same measurements before and after acupuncture treatment.

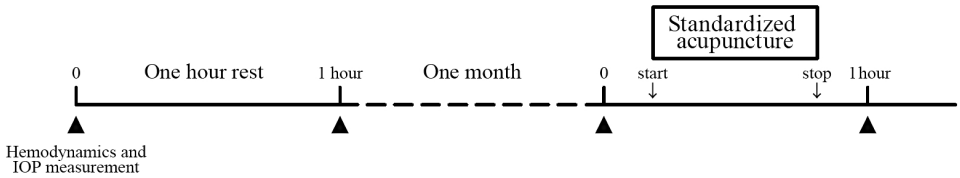

Fig. 12. Outline of the study. Standardised acupuncture was applied at the second visit more than 1 month after the first visit.

8.1.4 Statistical methods

Statistical analysis was performed using SPSS software (version 16.0; SPSS Japan Inc.). The parameters between before and after acupuncture or between control and acupuncture were compared by paired t-test.

8.2 Result

The blood pressure and heart rate did not change significantly by acupuncture.

The IOP level significantly decreased by acupuncture compared with before acupuncture ($P < 0.05$). The Δ value of IOP also significantly decreased by acupuncture compared with control ($P < 0.01$).

Retrobulbar vessel RI in the OA, CRA, and SPCA is shown in Table 1. The RI in the CRA and SPCA decreased significantly by acupuncture compared with that before acupuncture ($P < 0.05$). The Δ value of RI in the SPCA also decreased significantly by acupuncture compared with that of the control ($P < 0.01$) (Table 1).

No adverse events were observed in the study.

Resistive index	Control			Acupuncture		
	Rest	After 1 hour	Δ value	Before	After	Δ value
Ophthalmic artery	0.74 ± 0.04	0.75 ± 0.05	0.006 ± 0.037	0.74 ± 0.04	0.74 ± 0.04	−0.006 ± 0.036
Central retinal artery	0.75 ± 0.09	0.72 ± 0.03	−0.027 ± 0.085	0.72 ± 0.05	0.68 ± 0.04*	−0.036 ± 0.059
Short posterior ciliary artery	0.68 ± 0.05	0.68 ± 0.04	0.004 ± 0.038	0.67 ± 0.04	0.64 ± 0.06*	−0.032 ± 0.054 ††

Table 1. Resistive index in the ophthalmic artery, central retinal artery, and short posterior ciliary artery. The values represent the mean and SD. *$P < 0.05$ versus before acupuncture. ††$P < 0.01$ versus control.

8.3 Discussion

To our knowledge, this is the first report to examine haemodynamic changes in retrobulbar vessels related to acupuncture in OAG eyes.

The present findings suggest that acupuncture can alter vessel resistance in the SPCA despite treatment with standard medications.

Decreased distal vascular resistance in the SPCA indicates increased blood flow in the choroid. It has been reported that the blood flow in the eye is controlled by sympathetic and parasympathetic nerves and is related to the release of nitric oxide or calcitonin gene-related peptide (Shimura et al., 2002; Wiencke et al., 1994). It has also been reported that the regulation of regional blood flow by somatic afferent stimulation is based on somatoautonomic reflex mechanisms in the choroidal blood flow of the eyeball (Shimura et al., 2002). Haemodynamic changes in the SPCA induced by acupuncture may be related to these mechanisms. Acupuncture may be applied as an additional therapy to treat OAG.

In conclusion, we measured the effect of standardised acupuncture on the retrobulbar haemodynamics in OAG eyes using high-resolution ultrasound.

8.3.1 Study limitations

We should interpret these results cautiously because the present study was a case series study in which intervention was provided only once.

8.3.2 Future studies

Longer observation of acupuncture therapy in future studies is needed to investigate the progression of glaucomatous damage associated with impaired ocular circulation.

9. Conclusion

TM therapies affect the autonomic nervous system and the human body. Invasive measurement methods may also affect the autonomic nervous system. As a result, invasive measurement methods might not accurately evaluate the effect of TM therapies.
These studies suggests that use of non-invasive and real-time methods like blood pressure, ECG, ICG, and ultrasound to evaluate haemodynamics could assess the effects of TM therapies on various organs in humans in detail. This type of method may be an ideal indicator for the assessment of the effects of TM therapies in humans.

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8

Degeneration/Regeneration as a Mechanism Contributing to the Effect of Manual Acupuncture-Induced Injury on Rat Skeletal Muscle

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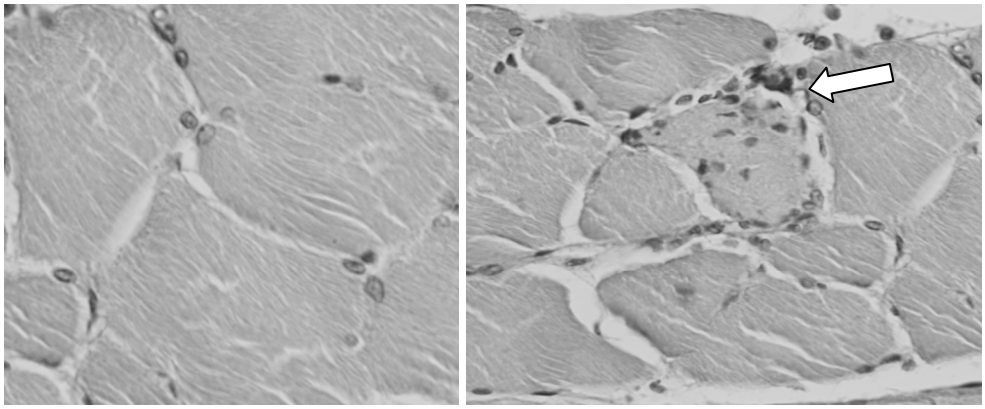
U.S.A.

1. Introduction

This book chapter is to further improve our understanding of the underlying mechanism of local injury that occurs after manual acupuncture needle manipulation that initiates muscle degeneration/regeneration process, which is essential for muscle maintenance and adaptation. Muscle injury triggers a sequence of events that begins with the host inflammation, secretion of myogenic transcription factor and various growth factors that are important for the activation and proliferation of muscle satellite (stem) cells.

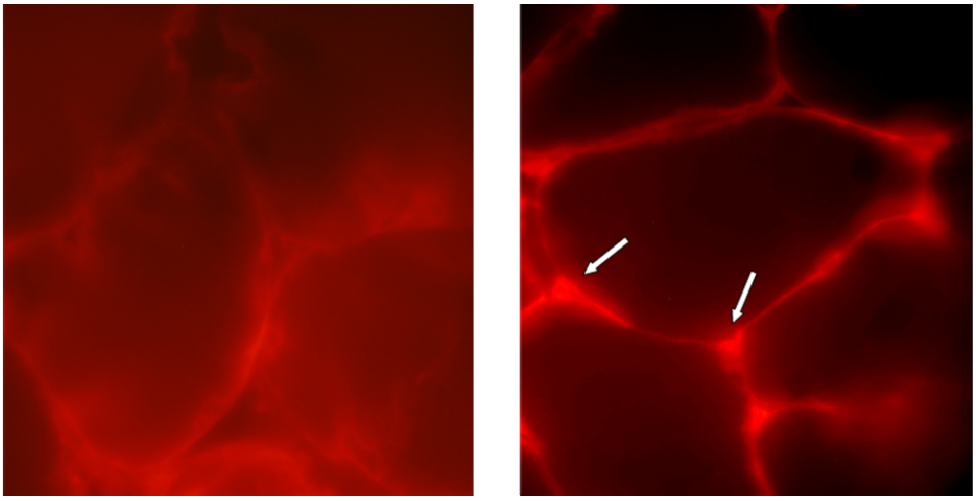
Skeletal muscle is maintained by resident stem cells call muscle satellite cells because of their location on the periphery of the myofiber under the surrounding basal lamina (Mauro, 1961). Satellite cells are quiescent mononuclear cells that are present in different types of skeletal muscle and are associated with all muscle fiber types, although the distribution might be unequal. For instance the percentage of satellite cell in adult tibialis anterior muscle is larger than other muscles (Pavlath et al., 1998). Satellite cells are responsible for post natal growth, hypertrophy and repair of skeletal muscle (Grounds, 1999).

In normal undisturbed muscle, these cells remain in a quiescent stage (Schultz et al., 1985). After muscle injury, satellite cells become mitotically active, proliferate, differentiate and eventually fuse together to regenerate new muscle fiber, which is well-innervated, fully vascularized, through a process that resembles in some aspects embryonic (Seale et al., 2001). During muscle differentiation, satellite cells exhibit increased expression of the myogenic transcription factor MyoD (Cooper et al., 1999). Analysis of muscle regeneration has previously been studied using experimental models that induce muscle injury by various protocol such as eccentric contraction (Rathbone et al., 2003) or treadmill exercise (Smith et al., 2001). Although exercise induces injury-related satellite cell activation (Smith et al. 2001), its use is limited in patients with restricted exercise capacity during long periods of bed rest. Instead, such injury can be induced during acupuncture needle manipulation, which has been shown to induce muscle injury on acupuncture point (ST-36) in the tibialis anterior (TA) muscle (see Figure 1) and produce muscle regeneration via up-regulation of MyoD (see Figure 2 and Figure 3).



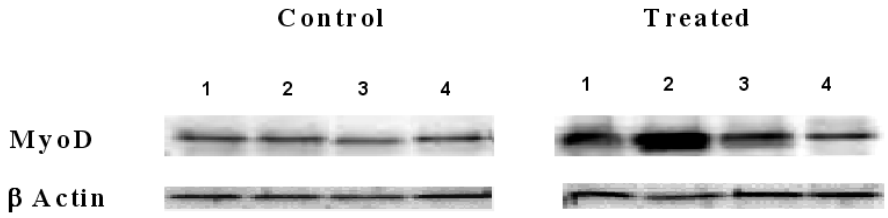
1.A Control TA Muscle **1.B Treated TA Muscle**

Fig. 1. **Acupuncture needling-induced signs of muscle injury**
Characteristic sagittal sections of hematoxylin-stained TA muscle. (**A-left slide**) Non-treated control TA muscle reveals a uniform labeling of compact myofibers and resident nuclei. (**B-right slide**) Acupuncture needling of the right TA muscle induced loss of myofiber integrity and apparent myonecrosis. Needling also stimulated the infiltration of mononucleated cells to the injured sites within the muscle tissue (arrow). Bar = 100 microns. (Ameis et al. 2008)



2.A Control TA Muscle **2.B Treated TA Muscle**

Fig. 2. **Evidence of myogeneic potential with TA muscle. (A)** Serial sections of control TA muscle incubated with antibodies specific for MyoD routinely displayed low levels of immunoreactivity. **(B)** MyoD-positive cells (arrows) were consistently observed in subsets of cells within the acupunctured TA muscle tissue. Immunoreactive profiles identified dense, nuclear staining that was localized at the periphery of the fiber membrane (arrows). Bar = 50 microns. (Ameis et al., 2008).



Western Blot Analysis

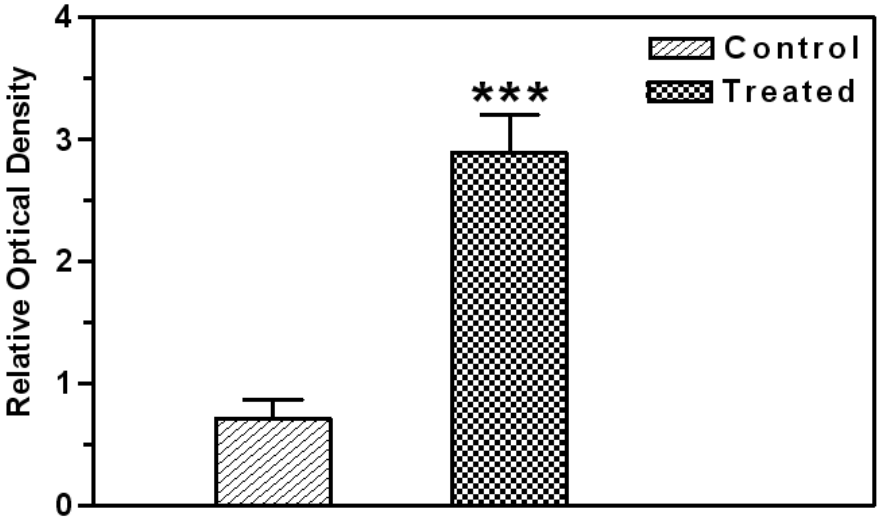


Fig. 3. Acupuncture needling-induced MyoD protein level as determined by Western Blot analysis. (**A**) The gel image of MyoD and beta-actin in control and treated TA muscle tissue. Lane (1, 2, 3 and 4 represent the different leg muscles) (**B**) Relative optical density. The results indicated a significant increase in treated compared with control (*** $p<0.001$) using Student t test. (Ameis et al., 2008)

2. Acupuncture and skeletal muscle regeneration

Acupuncture is an ancient Chinese therapy with a mode of action that is controversial. In traditional Chinese medicine (TCM) theory, needle manipulation is an important element associated with the efficacy of manual acupuncture. The acupuncture needles are manually manipulated after their insertions into a specific acupuncture point. Needle manipulation typically consists of rapid back-and-forth rotation, which can be brief (a few seconds) or prolonged (several minutes). Traditionally, in humans, needle manipulation is performed to elicit the characteristic reaction of acupuncture needling known as *de'qi* (Park et al., 2002). *De'qi* has a sensory component that is perceived by the patient as a pinching-type pain or heaviness in the area surrounding the needle. The acupuncturist administering treatment

perceives *de'qi* by needle grasp, or resistance to further manipulation. *De'qi* is widely viewed as essential to acupuncture's therapeutic effectiveness. It is possible that the *de'qi* reaction is a sign of muscle fiber injury or degeneration with subsequent regeneration.

3. Muscle injury and the degenerative phase

The degenerative phase of skeletal muscle injury involves inflammatory cells which is a critical component for successful regeneration to occur (Chargé & Rudnicki, 2004). Among inflammatory cells is a macrophages that infiltrate the injury site to phagocytose muscle debris (Robertson et al., 1993) and release interleukin-6 (IL-6) (Allen et al., 1995). IL-6 has the potential to regulate many critical process related to skeletal muscle including the regulation of cell proliferation, cell differentiation and overall tissue regeneration response (Baeza-Raja & Muñoz-Cánoves, 2004; Cantini et al., 1995; Kami et al., 2000). It has been shown that IL-6 plays an essential role in reguilation of satellite cell (Serrano et al., 2008). Loss of IL-6 results in reduced satellite cell proliferation and migration (Serrano et al., 2008). Although IL-6 is classified as a pro-inflammatory cytokine it has a variety of biological functions. IL-6 can also act as in anti-inflammatory manner to inhibit the inflammatory response (Bunn et al., 2004; Xing et al., 1998).

The expression of IL-6 mRNA was detected in different injury models of muscle regeneration (Balasubramaniam et al., 2009; Sheriff et al., 2009; Warren et al., 2002). For example, Bunn et al. (2004) investigated the increase of IL-6 during regeneration following crush injury in rat muscle at different time points: 2, 4, 8, 16, and 24 days. The results showed a high level increase on day 2 post injury, decrease on day 4 and then increase again on day 8. Our current, unpublished data using acupuncture induced injury showed the expression of IL-6 mRNA was elevated rapidly in response to manual acupuncture in the treated rats TA muscle than the control at day 1. The expression was decreased at day 3 and increased again at day 7. It decreased again at day 21 post treatment (see Figure 4).

Taken together, the results of the studies discussed show that the inflammatory response seen after acupuncture injury is in many ways similar to those reported after other types of skeletal muscle injury. However acupuncture-induced model of injury has the added benefit that it could be applied in humans to study the recovery process following injury. Muscle injury common in contact sport has been studied in animal models and recently particular attention has been paid to skeletal muscle regeneration after injury. Attention has also focused on various treatment modalities in order to speed up the recovery process as well as to facilitate anti-inflammatory actions.

Although it is known that the immune system plays a major role in the skeletal muscle regeneration after injury, little detailed research has been done on the immune components of physiological response to injury so that conclusions are currently quite generalized or even conflicting. This makes it difficult to suggest incontrovertible evidence-based recommendations for treatment. Acupuncture has been accepted as a technique which can be used in humans, in contrast to other muscle-injury models (such as injection of toxins, freezing or crushing).

4. Muscle injury and regeneration

The regeneration phase, in which muscle stem cell are activated and rapidly up-regulate the expression of mygenic regulatory factor (MyoD). This cellular and molecular phenotype is a

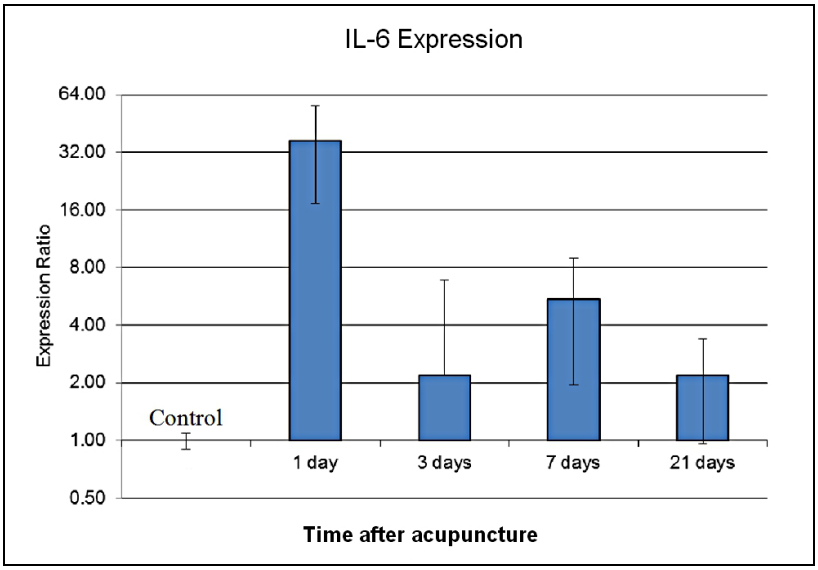


Fig. 4. **IL-6 Expression** Data represent fold changes in *IL-6* mRNA as assessed by RT-PCR after 0 (control), 1, 3, 7 and 21 days post acupuncture treatment of TA muscle. Differences in expression between groups were assessed by two-sided Pair Wise Fixed Reallocation Randomization Test. Level of probability was set at $P < 0.05$ as statistically significant. Values are mean +/- SEM (19.44, 4.74, 3.53, 1.23 folds) different from control. (unpublished data, Ameis et al.)

presumed prerequisite for muscle regeneration and repair. At the molecular level, activation of satellite cells is characterized by the expression of MyoD (Cooper et al., 1999), which is routinely associated with cell determination, and is often highly expressed in actively proliferating myoblasts. Moreover, MyoD expression may be critical for the differentiation of myoblasts in response to stimuli that are produced during muscle injury (Seale and Rudnicki, 2002; Sabourin and Rudnicki, 2000) and remodeling. The absence of MyoD adversely affects muscle regeneration, delaying the transition of satellite cell-derived myobast from proliferation to differentiation (Yablonka-Reuveni et al., 1999). Increased expression of MyoD has been shown to occur in both adult and aged skeletal muscle in response to injury (Becker et al., 2003; Krainak et al., 2006). It has been expressed also in young skeletal muscle following manual acupuncture treatment applied in acupuncture point ST36, located at the tibialis anterior (TA) muscle (Ameis et al., 2008).

The capacity of muscle regeneration is related to several factors, including the available pool of resident satellite cells in muscle fiber. TA muscle reportedly contains a greater pool of resident satellite cells than other muscle tissues (Kadi et al., 2004; Pavlath et al. 1998) and TA muscle regeneration and remodeling after injury is initiated at faster rates (Pavlath et al. 1998). These characteristics of the TA muscle are most likely related to its functional demand; TA muscle helps coordinate the control of balance and foot stability during normal walking, and is therefore active for extended periods daily. ST36 is the most commonly targeted acupuncture needle point in both clinical settings (Li et al., 2004), and experimental studies (Ameis, 1991).

Several pathways, including insulin-like growth factor (IGF-1) play vital role in regulating muscle regeneration (Machida & Booth, 2004). IGF-1 is a member of the family of insulin-related peptides. It is a widely distributed trophic hormone capable of mediating autocrine, paracrine or endocrine effect. IGF-1was originally referred to as "somatomedin" to reflect its expression response to growth hormone stimulation and its ability to mediate some of the growth-promoting effect of growth hormones (de Moor et al., 1994). IGF-1 plays important roles in both embryonic and post-natal mammalian growth (vanBuul-Offers et al., 1994). Early studies compared the metabolic effect of IGF-1 and insulin on muscles including stimulation of protein metabolism, glucose transport and glycogen and triglyceride synthesis.

IGF-1 has been associated specifically with increasing proliferation of satellite cells (Hill & Goldspink, 2003). IGF-1 has been showed to be increased following acute muscle damage or chronic aerobic exercise (Bamman et al., 2001; Hambrecht et al., 2005). Several pathways, including IGF-1 regulate muscle regeneration. *IGF-1* has been specifically associated with increasing proliferation of satellite cells (Machida & Booth 2004). The local up-regulation of IGF-1 mRNA has been induced in skeletal muscle by various models of muscle injury, such as myotoxin injection (Hill & Goldspink, 2003; Keller et al., 1999; McKoy et al. 1999) or eccentric contraction (Hill & Goldspink, 2003; Hill et al., 2003; Jennische & Hansson, 1987).

In our published study using acupuncture-induced injury, IGF-1 was higher in the TA muscle after manual acupuncture treat than control (see Figure 5). IGF-1 remained higher than control at day 1 of post acupuncture treatment and peaked at day 3. On day 7 and day 21, *IGF-1* had started to return to basal normal.

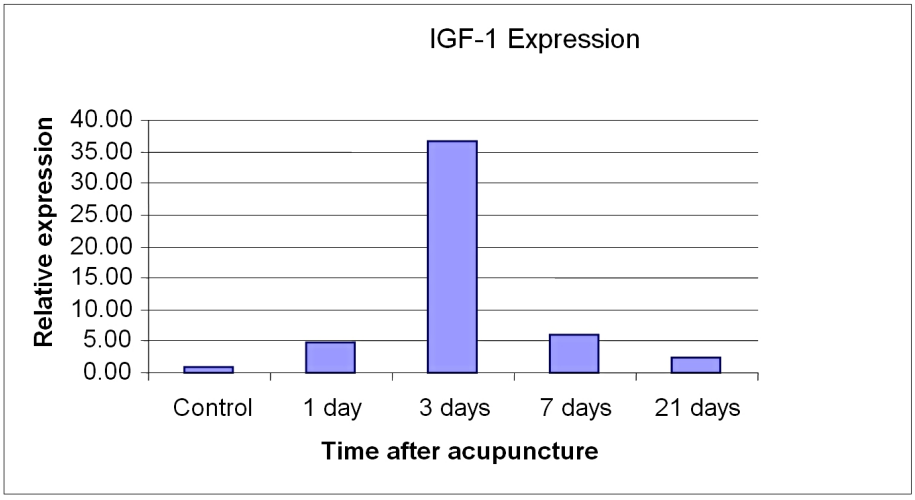


Fig. 5. **IGF-1** Data represent fold changes in *IGF-1* as assessed by RT-PCR after 0 (control), 1, 3, 7 and 21 days post acupuncture treatment of TA muscle. Differences in expression between groups were assessed by two-sided Pair Wise Fixed Reallocation Randomization Test. Level of probability was set at $P < 0.05$ as statistically significant. Values are mean +/- SEM (0.6, 0.31, 0.96, 0.19 folds) different from control. (unpublished data, Ameis et al.)

5. Acupuncture as a model of muscle injury

After pathological or experimental injury, the muscle undergoes regeneration processes. This regenerative capacity is due to the presence of a tissue-specific population of myogenetic stem cells. These specialized somatic stem cells are termed satellite cell because of their location on the periphery of the myofiber under the surrounding basal lamina (Mauro, 1961). During muscle development, satellite cells also mediate the post natal growth and are the primary means by which the mass of adult muscle is formed (Grounds, 1999). During post natal muscle development, satellite cells fuse with new or pre-existing myofiber. In adult muscle, in response to stress induced by weight-bearing exercise or trauma, satellite cells become mitotically active, proliferate, differentiate, and eventually fuse together to regenerate new muscle fiber (Seale et al., 2001; Chargé and Rudnicki, 2004). The essential role of satellite cells in muscle regeneration is well documented. However, an understanding of the molecular mechanism that regulates the activation and foundation of muscle stem cells has remained elusive and is a topic of extensive research targeting the crucial involvement of satellite cells in muscle reconstruction.

At the molecular level, activation, proliferation and differentiation of satellite cells is characterized by the up regulation of myogenic regulatory factor (MRFs). MRFs are skeletal muscle-specific transcription factor. The family consists of MyoD, Myogenin, MYF-5, MRF-4 to a larger basic-helix-loop-helix (bHLH) class of transcription factor. MyoD has been shown to up-regulate at the early stage of satellite cell proliferation. Myogenin and MRF-4 is up-regulated in cells, beginning their terminal differentiation program (Chargé and Rudnicki, 2004, review).

Analysis of muscle regeneration has previously been reported using different treatments to first induce degeneration of the muscle tissue, such as freezing (Warren et al., 2002) or snake toxins (such as notexin or cardiotoxin) (Mendler et al., 1998; Yan et al., 2003). Instead, such muscle injury can be induced using acupuncture, a minimally invasive technique. A study showed that a single session of manual acupuncture, consisting of needle manipulation in rat TA muscle, lead to focal injury and activation of satellite cell via up-regulated MyoD protein within muscle fiber (Ameis et al., 2008). A recent unpublished study has shown that manual acupuncture-induced injury in rat TA muscle up-regulated the expression of myogenic regulatory factor MyoD mRNA and peaked at 24 h post manual acupuncture treatment (see Figure 6). MyoD mRNA expression that peaked at 24 h has also been reported in rat pantais and soleus muscle in response to mechanical overload and stretch (Owino et al., 2001; Peviani et al., 2007).

In contrast to those using non-physiological models of muscle damage, other injury models have shown different time peak of MyoD. For example, Mendler et al. (1998) investigated the mRNA expression of MyoD during muscle regeneration following injection of myotixin in rat muscle found an increase of MyoD mRNA at 72 h post injury induced by injection of these myotoxin in rat muscle. Yan et al. (2003) using the same injury model using snake venom extract observed similar peak time point at 72h post injury in mice muscle. The reason for the difference in the time course of expression of MyoD mRNA between our study and those using venom toxin is unclear, but may be related to the severity of the insult that may lead to a partial loss of the satellite cell population (Polesskaya et al., 2003). However, whether this holds true is a topic for further investigation.

An alternative method to myotoxin which is considered more physiologically relevant is freeze injury (Chargé and Rudnicki, 2004). It has been shown that after freezing injury,

MyoD mRNA expression increased in mice TA muscle and peaked at 72 h time point (Warren et al., 2002).

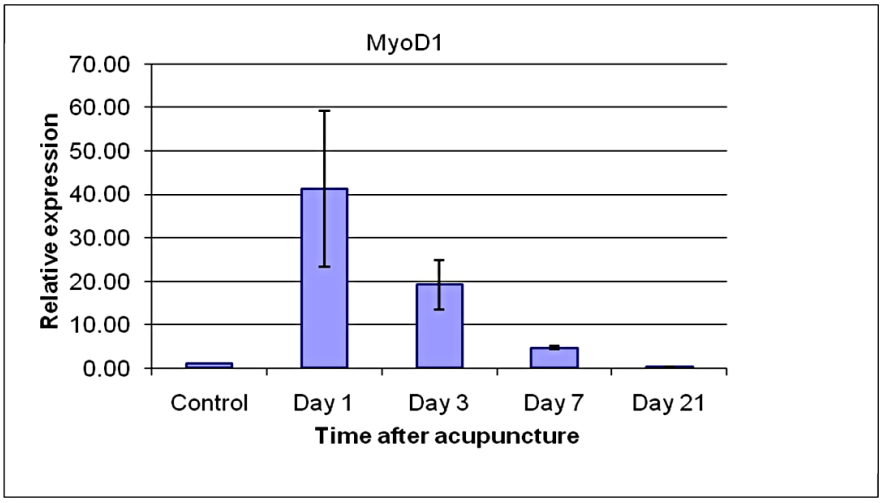


Fig. 6. ***MyoD1*** Data represent fold changes in *MyoD1* mRNA as assessed by RT-PCR after 0 (control), 1, 3, 7 and 21 days post acupuncture treatment of TA muscle. Differences in expression between groups were assessed by two-sided Pair Wise Fixed Reallocation Randomization Test. Level of probability was set at $P < 0.05$ as statistically significant. Values are mean +/- SEM (0.00, 17.88, 5.62, 0.34 folds) different from control. (unpublished data, Ameis et al.)

Comparing the effect of eccentric contraction and freezing shows that eccentric contraction exhibits a higher expression of MyoD mRNA than freezing (Warren et al., 2007). This might be explained by the observation that freezing induced freezing of satellite cells (Irintchev et al., 1997; Pastoret and Partridge, 1998).

The earlier peak expression of MyoD mRNA may be due to the nature of the intervention in the acupuncture needling model of muscle injury, which is similar to mechanical overload and stretch. The intervention involves a single insertion of a needle into acupuncture point ST36 in the rat TA muscle followed by a slight back-and-forth manipulation of the needle. During acupuncture needle manipulation, the muscle would be stretched and for normal muscle it is enough to hold a stretch for 15 to 30 seconds and repeat it for 3 to 5 times (Pezzullo & Irrgang, 2001). Therefore acupuncture needling model of injury, the needle is manipulated for 20 seconds every 3 minutes over duration of 15 minutes total. Therefore, this technique induces immediate and distant muscle injury--the needle insertion causes focal muscle injury at the point of insertion and the needle manipulation which stretches muscle fiber causes more distant muscle fiber injury.

There are several potential research advantages of acupuncture induced injury versus mechanical overload and stretch. The acupuncture injury model can be used to understand the process of muscle regeneration that will allow more precise comparison in future investigations of regenerative differences between age groups. It can also be used to study the regenerative process. Knowledge of the sets of genes associated specifically with the

nature of the injury may have application for developing new strategies for acceleration of the recovery process in injury skeletal muscle.

6. Possible role of acupuncture used as a countermeasure to forced inactivity

Forced inactivity during bed rest (Fitts et al., 1986) and space flight (Taylor et al., 2002) result in decrease of muscle regeneration. During activity, skeletal muscles are damaged and repaired repeatedly throughout life. Muscle regeneration maintains locomotor function during aging and delays the appearance of clinical symptoms of several conditions of muscle wasting. In humans, satellite cell function declines during aging (Gnocchi et al. 2008). However, in response to exercise, satellite cells increase in both young and old men and women, with significant increase particularly in adults (Dreyer et al., 2006; Kadi et al., 2004). In aged rats, satellite cells displayed a longer lag period before entering the cell cycle compared to adult rats (Gopinath and Rando, 2008). During inactivity (ex. hind limb unloading), the satellite cell mitotic activity is significantly reduced, especially in aged rats (Darr and Schultz 1989; Mozdziak et al., 2000; Siu et al., 2005). Therefore, it is suggested that activation of satellite cell may inhibit the loss of muscle function under atrophic conditions. Prevention of skeletal muscle atrophy during prolonged inactivity is important. A variety of potential counter measures have been tested and in many cases proved ineffective or unsatisfactory. In more recent study, daily injection of the complex IGF-1 during hind limb suspension or recovery showed to be beneficial (Chakravarthy et al., 2000; Zdanowica & Teichberg, 2003).

Because of the importance of IGF-1 to muscle regeneration, therapeutic applications are beginning to be explored. For example, experimentation on aged rat recovering from hindlimb immobilization shows that exogenously administration IGF-1 stimulates muscle recovery and an increase in satellite cell proliferation potential in hindlimb musculature (Chakravarthy et al., 2000). IGF-1 has been naturally expressed following manual acupuncture, therefore we suggest that acupuncture may be used as an approach to enhance muscle regeneration during forced inactivity such as bed rest.

This book chapter has sought to enhance our understanding of the underlying mechanism of local injury that occurs after manual acupuncture needle manipulation that initiates the degeneration/regeneration process. Muscle injury induced by acupuncture needling triggers a sequence of events that begins with the host inflammation, secretion of myogenic transcription facto and various growth factors that are important for the activation and proliferation of muscle satellite (stem) cells.

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9

A Neurovascular Blood-Flow Modulation Model via Acupuncture Induced Nitric Oxide

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1. Introduction

Acupuncture is a practice of inserting needles into the body to reduce pain or induce anesthesia. More broadly, acupuncture is a family of procedures involving the stimulation of anatomical locations on or beneath the skin by a variety of techniques. Employing acupuncture to treat human illness or to maintain body health has been practiced for thousands of years. Recently, models able to describe the mode of action of acupuncture have aroused scientists' curiosity. Scientific interest in acupuncture has led numerous investigators to conduct clinical trials that have tested the efficacy of acupuncture at various acupuncture points. However, the mechanism(s) of action of acupuncture at the various meridians are still poorly understood.

The traditional Chinese medical theory says: 'Qi acts as the commander of blood and blood acts as the mother of Qi'. This is a general description of the effects of Qi on blood and this theory can be extended to include the ideas that Qi promotes blood circulation and controls the blood functioning. Furthermore blood conveying Qi and blood nourishing Qi.

Previous studies have provided a variety of information regarding the physiological effects of acupuncture on animal and human bodies. Most of them have indicated that acupuncture is able to increase blood flow [1], and at the acupuncture points and meridians have a high electrical conductance [2, 3]. A relationship has also been suggested that among those acupuncture points and meridians with connective tissue planes [4] and the perivascular space [5]. A number of possible mechanisms by which acupuncture acts have been reviewed [6].

The results obtained from human and animal studies have shown that acupuncture enhances the generation of nitric oxide (NO) and increases local circulation [7]. Kim, et al. (2006) pointed out that employing acupuncture on stomach 36 point (ST-36) is able to reduce blood pressure by activating NO signaling mechanisms [8]. Ma (2003) showed that NO content and Nitric Oxide Synthase (NOS) expression were consistently higher at skin acupuncture points/meridians [9]. Chen et al. (2005) showed that L-arginine-derived NO synthesis appears to mediate the noradrenergic function of skin sympathetic nerve activation and that this contributes to skin electrical resistance of the acupuncture points and meridians [10].

NO is known to exert an effect on a number of functions including the regulation of blood pressure, contributing to the immune response, the control of neurotransmission and participation in cell differentiation and other physiological functions [11]. NO, a diffusible

signaling gas is synthesized by three NOS isoforms, namely neuronal NOS (nNOS), inducible NOS (iNOS) [12] and endothelial NOS (eNOS) [13, 14]. In the cardiovascular system, NO is tonically released by the endothelial cells in response to shear stress to maintain vascular tone [15]. This effect is due to the relaxation of the vascular smooth muscle cells in the medium layer (tunica media) of the arterial wall. However, NO is also involved in the regulation of synaptic neurotransmission, platelet aggregation, inflammation, appetite, peristalsis, renal metabolism, respiratory function, lipid metabolism and glucose metabolism.

It is supposed that the gas (NO) is part of Qi, then the proposed model can be annotated as this part of Traditional Chinese Medical theory. This hypothesis involves a blood-flow modulate model. Briefly, the acupuncture stimulus is able to induce a burst of NO production through mechanotransduction at the local acupuncture point and this NO diffuses and changes the blood flow either at the local and/or organ microcirculation level. Erythrocytes in blood vessel detect the oxygen and modulate vascular tone via controlled NO processing, and this is able to change the frequency of resonance and modulate the vascular tone.

Chinese acupuncture theory has long been recognized that there are twelve main meridians or energy channels that relate to the internal organs. These include the lungs, large intestine, stomach, spleen, heart, the pericardium (the sac around the heart), etc.

Acupuncture induced differential production of NO in various meridian organs, which are connected via tissue/cells coupled to the cyclically strained blood vessel; vascular blood flow and its distribution among different vascular beds, are regulated by the changes in micro vascular tone.

2. A neurovascular blood-flow modulation model of acupuncture

In vascular wall, most of the bioavailable NO is believed to be derived from eNOS and diffuses into vascular smooth muscle and the blood stream, where it rapidly reacts with the hemoglobin (Hb) of the red blood cells. Of course, it is also able to transport oxygen and carbon dioxide. The blood carries the oxygen complex with Hb to all parts of human body where it is required for metabolism and also returns with carrying carbon dioxide back to the lungs, where gaseous exchange with the atmosphere. The peripheral chemoreceptors located in the carotid bodies respond primarily to hypoxaemia. Central chemoreceptors located in the region of the brainstem respond to hypercapnia. Activation of either the hypoxic or hypercapnic chemoreflex elicits both hyperventilation and sympathetic activation [16]. Evidences from animal and human studies have revealed that NO may play a role in hypercapnia induced vasodilatation [17,18].

Recently, nNOS has been identified as a source of NO in the vicinity of microvessels and has been shown to participate in vascular function. Thus, NO can be produced and transported to the vascular smooth muscle cells from endothelial cells and perivascular nerve fibers, mast cells and other NOS-containing sources [19].

In Chinese terms, "acupuncture is a healing art of inserting a needle into an acupuncture point in the meridian to regulate an imbalance of Qi. The aim of acupuncture is to stimulate the flow of Qi through that meridian."

To explain the Qi phenomenon, we proposed a neurovascular blood-flow modulation model via acupuncture induced NO.

2.1 Acupuncture induces mechanotransduction in connective tissue

Immediately after a needle is inserted into connective tissue, the mechanical force is transferred to the extra cellular matrix (ECM). The ECM is a multi-component tissue that is able to transduce the internal and external mechanical signals into changes in the tissue structure and function through a process termed mechanochemical transduction [20,21].

2.2 Mechanical force modulates NO production, and mediates the local vascular circulation

The availability of NO in arteriolar endothelium and mast cells appeared to be maintained mainly by nNOS, whereas that in venular endothelium greatly depends on eNOS. Through the ECM, the mechanical force stimulus can travel across the acupoint into the local tissue cells including arterioles, nerve terminals and mast cells, and this will trigger nNOS induced NO production [9] which diffuses into the vascular smooth muscle and change blood flow and local circulation.

2.3 NO regulates central nervous system and cardiovascular activities

Sympathetic cardiovascular rostral ventrolateral medulla (rVLM) neurons that respond to both visceral (reflex) and somatic (acupuncture) nerve stimulation during stimulation of specific acupoints [22]. Nitric oxide (NO) syntheses (NOS)-containing neurons in the rVLM are activated during cardiac sympathoexcitatory reflexes. NO, specifically nNOS mediates sympathetic cardiac-cardiovascular responses through its action in the rVLM.[23]
Acupuncture stimulation increases blood flow of the stimulated area by causing axon reflex and increased cardiac output. [24] and regulate the cardiovascular system.

2.4 Blood flow distributing among different vascular beds are regulated by changes of vascular tone via controlled NO processing

Mechanical forces, comprising both unidirectional laminar and oscillatory shear, induce an increase in NO production through increased activation and expression of eNOS. The nNOS and eNOS have distinct local roles in the physiologic regulation of human coronary and peripheral microvascular tone in vivo. Whereas eNOS-generated NO facilitates dynamic alterations in blood flow distribution, the tonic generation of NO by nNOS may be important for the regulation of basal vasomotor tone and blood flow [25,26].
Hemoglobin, albumin, and glutathione carry and release nitric oxide (NO) within microvessels. NO moves in blood vessels from upstream to downstream resistance vessels which allow larger vessels that generate large NO to influence vascular tone in downstream vessels in response to both flow and receptor stimuli [27].
When acupunctured at a specific acupuncture point, the local mechanical stress produced is coupled with a cyclic strain of blood vessel and changes the resonance frequency of arterial trees [28]. Vascular blood flow and its distribution among different vascular beds are regulated by changes in microvascular tone.

3. Evaluation of the proposed blood flow modulation model

Traditional acupuncture theory proposes that the needling of appropriately selected acupuncture points has effects that are remote from the site of needle insertion, and this is mediated by means of the meridian system [29]. To date, physiological models attempting to

explain these remote effects have either invoked systemic mechanisms involving the nervous system, or involved signal transduction through connective tissue combining with the involvement of other systems including the nervous system [30,31].
It has been found that the mean of laser Doppler flowmetry (LDF) signals were significantly larger at the acupoints than in their surrounding tissues, which indicated a large volume of blood delivery to the microvascular beds of acupoints. These results indicate that the physical properties of the vascular structure of acupoints may affect the perfusion resistance, and modulate the microcirculatory perfusion [32].

3.1 Mechanotransduction between the acupuncture point and connective tissue

In order to understand the acupoint/meridian system, all the studies were mainly looked for distinct histological features that might differentiate acupuncture points from the surrounding tissue for past 30 years.
Various structures, such as neurovascular bundles [33-36], neuromuscular attachments [37-39] and various types of sensory nerve endings [37, 40, 41-45], have been described at acupoints. Nevertheless, acupoints and meridians are frequently located along connective tissue planes, that is between muscles or between muscle and bone or tendon[46-48].
When needles were inserted into acupoints, the connective tissue around the acupuncture needle is wounded. This allows the needle is able to pull and distort the surrounding tissue and this movement delivers a mechanical signal into the tissue at the cellular level [49,50]. Such mechanical signals are recognized as important mediators of information at the cellular level [51] and could be transduced into bioelectrical and/or biochemical signals [52,53] and lead to downstream effects, including cellular actin cytoskeleton polymerization, signaling pathway activation, changes in gene expression, protein synthesis and extracellular matrix modification [54,55].
It has been suggested that the mechanism underlying needle grasp in acupuncture is caused by the muscle contraction [57,58]. Since neurovascular bundles are located along connective tissue planes, the same amount of needle grasp may have more powerful downstream effects at acupoints due to the mechanical matrix deformation caused by tissue winding. Acupuncture has been shown to improve cutaneous microcirculation and tissue healing in musculoskeletal flaps in rats [59,60] and to increase circulation in the skin above the parotid glands [61]. Mechanical forces are also able to initiate complex signal transduction cascades, including the nuclear factor-kappaB and mitogen-activated protein kinase pathways and these lead to functional changes within the cell [62].

3.2 Mechanical force modulates NO production, and mediates the local vascular circulation

Changes in ECM composition can modulate the transduction of mechanical signals to, between and within cells [56]. Acupuncture needle manipulation, thus, may cause lasting modification of the ECM surrounding the needle, which may, in turn, influence the various cell populations sharing this connective tissue matrix such as fibroblasts, sensory afferents, immune and vascular cells. Vascular cells are equipped with numerous receptors that allow them to detect and respond to the mechanical forces generated by pressure and shear stress. The cytoskeleton and other structural components have an established role in mechanotransduction and are able to transmit and modulate tension within the cell via focal adhesion sites, integrins, cellular junctions and the ECM.

Traditionally, eNOS, which is primarily a membrane-bound protein [69], is considered the principal source of bio-available vascular NO. In recent years, the role of nNOS-generated NO in vascular function has been clearly demonstrated [63-68]. Several studies [73, 74] reported that nNOS-containing nerve fibers, which innervate arterioles and nerve terminals, are the major sources of arteriolar NO. The availability of NO in arteriolar endothelium and mast cells appeared to be maintained mainly by nNOS, whereas that in venular endothelium greatly depends on eNOS.

Through the ECM, the mechanical force stimulus can travel across the acupoint into the local tissue cells including arterioles, nerve terminals and mast cells, and this will trigger nNOS induced NO production [9] which diffuses into the vascular smooth muscle and change blood flow and local circulation.

3.3 Acupuncture regulates central cardiovascular activities by NO production

When acupuncture stimulates forelimb, hindlimb, chest, and abdomen of human body, all the heart rate of above parts decreases significantly which were paralleled by the decreasing in cardiac sympathetic nerve activity. The reflex pathway to decrease heart rate by acupuncture-like stimulation consists of mainly group IV muscle afferent fibers whose activity leads to the activation of GABA-ergic neurons in the brainstem and an inhibition of sympathetic outflow to the heart [93].

When electro-acupuncture applied on the acupoint ST-36, the functional magnetic resonance imaging (fMRI) activity in the hypothalamus, the dorsal raphe nucleus, the periaqueductal gray, and the rVLM showed significant correlation with the heart rate variability (HRV) data [94]. EA at acupoints overlying deep and superficial somatic nerves leads to point-specific effects on cardiovascular reflex responses. Sympathetic cardiovascular rVLM neurons that respond to both visceral (reflex) and somatic (EA) nerve stimulation manifest different level of responses during stimulation of specific acupoints [22]. Acupuncture stimulation seems to reduce sympathetic nervous system activation via activating of the cholinergic system or opioid receptors in the rVLM [4,39]. Nitric oxide (NO) synthase (NOS)-containing neurons in the rVLM are activated during cardiac sympathoexcitatory reflexes. NO, specifically nNOS mediates sympathetic cardiac-cardiovascular responses through its action in the rVLM [23].

Acupuncture stimulation increases the blood flow of the stimulated area by causing axon reflex and increased cardiac output, as well as a decrease in total peripheral resistance via CNS at the same time [24].

3.4 Blood flow distribution among different vascular beds is regulated by changes in vascular tone via controlled NO processing.

The organ blood flow affected differently by stimulating different points, and different methods or regions of acupuncture stimulation also influence blood pressure and autonomic nerves differently [24]. The functional magnetic resonance imaging (fMRI) studies have shown that acupuncture stimulation, when associated with sensations comprising deqi, evokes deactivation of a limbic-paralimbic-neocortical network, which encompasses the limbic system, as well as activation of somatosensory brain regions [97,98]. Among acupuncture-like stimuli delivered to the body, cortical cerebral blood flow was increased by stimuli to face (brain stem), forepaw (the spinal cord at cervical) and hindpaw (the spinal cord at cervical). The afferent pathway of the responses is composed of somatic groups III

and IV afferent nerves and whose efferent nerve pathway includes intrinsic cholinergic vasodilators originating in the basal forebrain [99]. Activation of parasympathetic nitrergic nerves innervating renal vasculature contributes to vasodilatation in renal arteries and pre- and postglomerular arterioles, an increase in renal blood flow. NO from neurons in the brain acts on the paraventricular nucleus of the hypothalamus and the rVLM and inhibits the central sympathetic nerve activity to the kidney, leading to renal vasodilatation and increased renal blood flow [100].

In a knee joint microcirculation study, a dynamic balance between the autonomic nervous system and the release of NO is the primary mechanism mediating the electro-acupuncture stimulation (EAS) effects has been suggested [95]. When acupuncture was given at ST36 of rat, the blood perfusion in the stomach increased significantly, the blood perfusion in the blood vessels and microcirculation of other body parts significantly increased. [96]

Generation of free NO from nonenzymatic reservoir in circulation including *S*-nitrosothiols and hemoglobin(Hb) has been recognized as a major physiological source of NO in biological systems including vasculature [70-72].

NO is produced by endothelial cells and diffuses into vascular smooth muscle and into the flowing blood, where it rapidly reacts with Hb in red blood cells (RBC). RBC has an active nitric oxide synthesizing mechanism which has properties similar to eNOS syntheses. This RBC-NOS activity contributes to the NO export from RBC. RBC-NO generating mechanisms can be stimulated by exposing red cells to shear stress and that calcium plays a role in this stimulation [73].

Mechanical forces, comprising both unidirectional laminar and oscillatory shear, are being recognized as important inducers of vascular NO generation. Laminar shear induces an increase in NO production through increased activation and expression of eNOS. Precisely, laminar shear activates eNOS through both Ca2+-dependent and Ca2+-independent mechanisms [74,75]. Oscillatory shear has also been shown to be able to stimulate an acute increase in NO production and up-regulation of eNOS [76]. Periodic bursts of intracellular free Ca^{2+} in response to a constant agonist concentration have been observed in a number of non-excitable cell types including endothelial cells [77-79]. There is controversy regarding shear stress-induced Ca^{2+} transient release in endothelial cells with some investigators reporting multiple Ca^{2+} transient events [80-83], whereas others report only their irregular appearance or nothing at all [84-86]. Furthermore, both transient and sustained release of NO has been reported in response to this shear stress. It is seems that shear stress regulates NO release in a Ca^{2+}-independent mode through phosporylation of eNOS, which results in a sustained basal NO production irrespective of the presence or absence of the Ca^{2+} transient effect [87-89]. Kutchan and Frangos [90] measured the NO end-oxidation products (NO_2^- and NO_3^-) released from endothelial cells exposed to laminar flow and reported the presence of transient Ca^{2+}-dependent NO release at the initial moment but sustained release in the presence of a constant shear stress level. Buerk and Riva [91] observed the presence of spontaneous low-frequency NO oscillations in the cat optic nerve head, which were attributed to a natural variation in shear stress. Kanai et al. [92] measured NO release from endothelial cells exposed to constant shear stress using of NO-sensitive microelectrodes and reported shear stress induced periodic Ca^{2+} transient release and a concomitant release of NO.

Several studies have indicated nNOS to be present in perivascular nerve fibers [101]. Vascular blood flow and its distribution among different vascular beds are regulated by

changes in microvascular tone. NO plays a key role in the local paracrine regulation of vessel tone both under resting conditions and when blood flow increases in response to agonist stimulation or increased shear stress. The nNOS plays an important role in the local regulation of vessel tone, independent of the effects of nNOS-derived NO in the central nervous system [28]. The nNOS and eNOS have distinct local roles in the physiologic regulation of human coronary and peripheral microvascular tone in vivo and then, that these isoforms may therefore subserve distinct functions. Whereas eNOS-generated NO facilitates dynamic alterations in blood flow distribution, the tonic generation of NO by nNOS may be important for the regulation of basal vasomotor tone and blood flow [25,26].

If Blood vessels are continuously subjected to mechanical forces in the form of stretching and this encompasses cyclic mechanical strain due to the pulsatile nature of blood flow and shear stress. These are accompanied by phenotypical modulation of smooth muscle cells and endothelial cells, which then produce structural modifications of the arterial wall. The hemoglobin, albumin, and glutathione carry and release NO may have influenced the movement of NO by blood within microvessels. NO can move in blood from upstream to downstream resistance vessels. This mechanism allows larger vessels that generate large amount of NO to influence vascular tone in downstream vessels in response to both flow and receptor stimuli [27].

A meridian is a hypothetical or functional line linking various arterial trees (acupuncture points) that have a similar resonance property [28]. The meridian selected frequency may be the same as the resonant frequency of its related internal organ. From the pressure wave propagation equation, the resonance frequency of the organs (or tissue's) main artery coupled system will decide the blood pressure energy distribution. Needling at an acupoints is a disturbance that will cause an impedance mismatch and therefore influence the efficiency of the resonance. Stimulating an acupuncture point, which is a tissue-rich with small arteries, is an effective way to cause the redistribution of blood pressure energy and therefore influence the blood perfusion.

From above studies, it is suggested that when acupuncture is applied at a specific acupoint, the local mechanical stress produced from needle grasp is able to trigger a burst of NO production and couple with the cyclic strain of the blood vessels, then there is a change in the microvascular tone of blood vessels. NO in the central nervous system plays a very important role in the control of sympathetic outflow and regulation of cardiovascular activities. This has exerted an effect on the artery tree changing the various meridian organs' blood distribution and perfusion in our body. The mechanism underlying the increased blood flow resulting from acupuncture rely on the activation of thin nerve fibers and these release vasoactive neuropeptides and NO from their peripheral terminals upon activation. A dynamic balance between the autonomic nervous system and the release of NO is the primary mechanism mediating the acupuncture stimulation effects in the local microcirculation.

4. Discussion

Acupuncture has been shown to improve cutaneous microcirculation and tissue healing in musculoskeletal flaps in rats [59,60] and to increase circulation in the skin above the parotid glands [61]. The mechanism underlying increased blood flow by acupuncture has been suggested to rely on the activation of thin nerve fibers, which release vasoactive

neuropeptides and NO from their peripheral terminals upon activation; this leads to vasodilatation and increased blood flow [1,102,103]. It is noted that because NO is involved in multiple body functions, its presence in the peripheral blood or in an acupuncture point is supportive of our hypothesis.

According to Traditional Chinese Medical theory, acupuncture points are functionally related to certain visceral organs and these relationships have been partially proved by clinical application of acupuncture therapy. Acupuncture point ST36 (stomach 36) is the most commonly used acupuncture point for the purpose of immune strengthening and immune regulation in oriental medical clinics (specifically strengthening and regulating Qi and blood in oriental medical terminology) [104]. Lee's morphological study [105] suggest that there is a commonality of CNS cell groups in brain controlling the stomach (viscera) and the Zusanli point (ST36). A fMRI study was able to demonstrate that stimulation of ST36/SP6 specifically activated the orbital frontal cortex and de-activated the hippocampus [106]. Alternatively, stimulation of GB34/BL57 activated the dorsal thalamus and inhibited various primary motor areas and the premotor cortex. These results strongly support the existence of acupoint specificity.

The modulatory effect of acupuncture on the cardiovascular and the sympathetic system has been referred to somatoautonomic reflexes [107]. Manual acupuncture stimulation elicited a transient increase similar to skin sympathetic nerve activity (SSNA) and that this increase is dependent on the baseline of the SSNA [108]. EA can reduce sympathetic nerve activity (SNA) and arterial pressure (AP). Specifically, short-term EAe of ST36 is able to resets the neural arc to a lower SNA, which moves the operating point toward lower AP and SNA under baroreflex closed-loop conditions [109]. L-arginine-derived NO synthesis appears to mediate noradrenergic function during skin sympathetic nerve activation, which probably contributes to skin electrical resistance of the acupuncture points and meridians [10].

It has been speculated that acupuncture stimulates some sensory nerves or the autonomic nervous system and induces the recovery of blood circulation [110-112]. NO content and nNOS expression have been shown to be consistently higher at the skin acupuncture points/meridians [9]. EA at ST-36 is regulated through NOS in those organs on the stomach meridians [8]. ST-36 is used to promote blood flow and to treat cardiovascular disease; therefore, ST-36 possibly induces its beneficial antihypertensive effect by activating NOS in the microcirculation. Meanwhile, acupuncturing ST-36 alone has been shown to produce different results when used to treat a renal hypertensive hamster compared to a sham-operated hamster. However, this is not universal for all acupoints and there was no response in organs that are not responsible for hypertension illness.

According Traditional Chinese Medical theory, the effects of acupuncture at a specific point are expressed differently. Here, a blood-flow modulation model starting from a local perspective was proposed and this model is able to suggest physiological processes that can bridge from the traditional nerve model of acupuncture to the vascular model. Based on this, we suggest that when target organs located on and not located on the same meridian as, for example, ST-36, it should be possible to identify the physiological mechanisms responsible for individual organ behavior. However, while acupuncturing ST-36 alone has produced different results between renal hypertensive hamster and sham-operated control [8], there is also need to consider differences in the operation methods. Finally, we suggest that the best approach to a physiological study of acupuncture point either alone or combined with other acupuncture points should involve a broad range of measurements in addition to studying NOS.

The Traditional Chinese medical theory says: 'Qi acts as the commander of blood ,and blood acts as the mother of Qi' This is a general description of the effects of Qi on blood and it can be expanded to include the ideas that Qi promotes blood production, and controls the blood circulation. Furthermore blood conveying Qi and blood nourishing Qi. If we suppose the NO is Qi, then the model we propose can be annotated as this part of Traditional Chinese Medical theory.

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10

Acupuncture – An Electrical Phenomenon

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1. Introduction

In his article: "Acupuncture for everyone?", in "Deutsches Ärzteblatt" (2006;4:C157-8) designated IRNICH the acupuncture as a peripher stimulation therapy and pointed to the difficulties to show point-specific effects for certain indications in comparison with sham- or minimal acupuncture. However in the author's opinion, the acupuncture is not a peripher stimulating therapy, but a regulation therapy. His view of the acupuncture points, their importance for the normal bioelectric situation of the organism and his hypothesis of the mode of functioning of the acupuncture will be presented in this article.

2. The hypothesis

Acupuncture points, which normally exhibit a lower skin resistance as its surrounding, are, just like receptors, free nerve ends and other conductive structures, which are there, components of the information and energy network. This system and our regulation system, needs not necessarily to be perceived as an energy circuit in which our life energy "Qi" circulates, how it accepts the ancient Chinese acupuncture theory, but this system could also serve for the supply and for the flow of information and electrical energy out of the us surrounding air-electric field, the ambient potential, which in my view might represent the so called "cosmic energy" of the old Chinese.
As the electric current always follows the way of the slightest resistance, also this "c*osmic* energy" enters the body at points which exhibit the lowest skin resistance and which are concurrent with acupuncture points. I suppose that for these electro receptors the ambient potential is the adequate stimulus which triggers a receptor potential, which then runs as a forward conducted action potential from cell to cell via excitable or conductive structures and nerve tracks. After the repolarisation of the receptor the next receptor potential follows and so on. Probably also natural electromagnetic waves such as atmospheric impulse radiation enters the body at that points, and follows structures with "cable like" properties, running there and possibly leaves the body again at other points, perhaps at acupuncture points or reflex zones at the feet, which are regarded by the followers of reflexology as organ-specific.

On account the similarity of frequencies and the form of discharge between atmospheric impulse radiation and the electric impulses in the nervous system, one assumes connections between both. In addition it can be assumed that the continuous supply and the flow of this "cosmic energy" through the body are necessary for the maintainance of the normal bioelectric situation and to the conservation of our life energy and for the functioning of the organism, and that organs receive their information and energy via the nerve and functional connections with its electro receptors.
Ancient Chinese illustrations show some acupuncture meridians running in cranio-caudal direction (in a upright standing person, in vertical direction), the longest are extending from the head to the feet. FISCH (6) reported:

"*The Chineses say: Humans swim in energy like a fish in the water*".

PORKERT(22) wrote on acupuncture points:

''*According to Chinese teachings view, they represent to openings in the surface of the body, through which the Qi can pass especially easy*",

and BISCHKO(2):

" *...in special research method, acupuncture points appear histologically as cumulation of ending structures with receptor and effector characteristics.*"

HEINE (7) described the morphology of acupuncture points histologically as perforations of bundles of vessel- nerve- and connective tissue-bundles of the body surface, and makes therewith a physical explanation of the electric measurement of acupuncture points possible. Therewith the relation between skin points and remote Iying organs could be explained anatomically.
The following data are suitable to support the conceptions of the author: The Viennese physicist MARESCH found when his investigations of humans weather sensitivity, that in the air-electric field an adult of normal size is exposed to a tension of 200 V. He also found skin areas with especially electrical behaviour, which he called electrically excellent spots of the skin. At these points he could measure in disturbances of the air-electrical field changes of the electrical values. (18) KÜGLER (17) mentions, that the atmospheric impulse radiation, the so called atmospherics are regarded as a weather-active agent and that in the bioassay by in true-to-nature copied electromagnetic impulses a clear change of the motoric was caused and by simulation of bad weather signals a reduction in tissue respiration resulted.
KÖNIG,(16) writes over the air-electrical field among other things:
"The electrical conductivity of the human body or a plant is largely enough, in order to reduce the electrical field within the body very strongly. Nevertheless show measurements by means of electrodes deeply within from plants and animals, that within the ULF- range [electromagnetic processes of natural origin with frequencies of under one Hertz are called Iying within the ULF - range (Ultra Low Frequenzy)] the internal fields mostly in temporal phase synchronously pulsate. It is assumed that peripheral receptors take up the outside field, whereby the internal field is to be understood as a kind answer of the outside irritations. Independently of it a static or slowly variable magnetic field reaches each part of the human body" and in other place: "Electromagnetic fields can be

determined in the membrane of nerve cells, and therefore it is not excluded that with different frequencies a direct component of the parallel electric current which is generated by the outside fields leads to a Stimulation of the cells or a change of their adressability".
Electromagnetic fields can affect the mobility of ions, involved in the process of nerve excitation. Vibrations of ions with frequencies of the externally operating field influence their ability to penetrate the nerve membranes. Therewith they steer the excitability of the nerves. Electromagnetic fields can probably also influence the so-called spontaneous activity of receptors. Possibly the external electromagnetic fields determine the internal electromagnetic regulation, which is effective in various frequency ranges. POPP and STRAUSS (21) wrote, among other things:
"Modulated electromagnetic waves mediate as biosignals communication between cells and regulate the cell growth. (...) We know that there are electromagnetic waves in cells and we convinced ourselves of the fact that a fundamental biological role comes to them. RUTH could prove electromagnetic waves and especially biophotons in cells. Also other researchers found such radiates at many living systems."

3. Ear-acupuncture, acupuncture, acupressure, neural therapy, reflexology, reflex zone therapy, percutaneous regulation therapy

All living cells are excitable. Prerequisite for the excitability and operability of the cell is the passive (rest) membrane potential. This is the difference of voltage which results at the cell membrane, among other things by the concentration differences of potassium and sodium ions, which exists between the cell inside and outside of the cell. For example the rest membrane potential of an unexcited nerve cell membrane amounts to 70-90 mVs, thereby the outside of the membrane is positive opposite the interior of the cell. During the nerve excitation the cell membrane becomes more permeable for certain ions than in a passive state. Now these ions can follow their concentration gradient, the sodium ions stream into the cell inside and the potassium ions outward. This very soon leads to a depolarization and a reversal of the potential difference in the action potential. After the re-establishment of the rest membrane potential, the repolarization, the cell is excitable again. This ion migrations through the cell membrane accompany all life processes, so f. i. the conduction of excitation in the nervous system, the brain activity and the transport of substances through the cell membrane etc. The cell remains however only so long excitable and functioning, as after each excitation process the repolarization follows. As long as these bioelectric processes run off normally, the continuous information flow in the nerve and regulation system is undisturbed.
According to the conceptions of the traditional Chinese medicine then the life energy "Qi" circulates regularly in the acupuncture meridians, YIN and YANG are in balance. When this balance is disturbed, it comes to disturbances of the cell functions and depending on their localization different disease pictures develop. Such cell malfunctions can appear as consequence of an oxygen deficiency, f.i. with blood circulation disturbances or as consequence of poisons, which intervene in the oxidation processes of the cells. Thus it comes according to H.ISKRAUT(13) to an extreme

depolarization of the affected cell, that is, to an extraordinary voltage drop at the cell membrane and thus the function fails with a "cathode block". Thus the cell became unexitable.
Such a cathode block spreads from cell to cell or via nerve and functional connections and reflects itself finally at the external ears and other functionally associated skin areas (reflex zones) and skin spots (acupuncture points).
A markedly hyperalgesia develops or an away-Ied pain occurs there; in addition changes in electric skin resistance and potential as a sign of the available disturbances of the ion household and the homeostasis in the tissue. Such changes were proven at reflex zones and acupuncture points. The nervous system of humans is a functional unit, in which the loss of a subrange causes always also a disturbance of the whole system. Nerve and functional connections via which the cathode block spreads towards the periphery can in reverse direction also serve for the treatment of this health trouble. One can cancel a cathode bloc in terms of “Minus and Plus cancel each other out” by artificially setting of an anode bloc in the affected spots (13). The fact that we can act therapeutic frorn acupuncture points and reflex zones by acupuncture, ear acupuncture, acupressure, neural therapy, reflexology, or the percutaneous regulation therapy on the disturbed organ, respectively on the there reflected disease, permits the conclusion, that all these treatments work like an anode bloc, which can cause the repolarization of extremely depolarized cells, apparently even cells which are affected by the loss of function (cathode block).

4. Neural therapy

In the case of neural therapy the repolarization of the extremely depolarized cells affected by the loss of function is effectuated by the injected local anaesthetic. It is known that local anaesthetics can influence membrane permeability and increase the membrane potential. With the local anaesthesia the membrane potential increases so far, that it could not break down any longer, the cell becomes hyperpolarized and thus unexitable (anode block). Thus it falls out for the pain line. According to DOSCH (5) the charge of a normally polarized cell of 90 mV is increased by the injection of local anesthetic procaine to 290 mV. Thus this cell is inexcitable. After 20 minutes, the membrane potential falls back to baseline by 90 mV. The cell is polarized normal again, exitable and functional. Also, the membrane potential of a cell in an constant of 10 mV depolarized interference field is by such an injection initially hyperpolarized to 290 mV (local anesthesia) and normalizes then after 20 minutes to 90 mV. In this way also the "Second phenomenon" HUNEKEs (12) becomes true.

5. Acupuncture

With the acupuncture the repolarization of extremely depolarized cells, affected by the loss of function, is caused by the electric current, which develops with the puncture of the needle into the body, probably in addition, by the electric current, which is supplied from the us surrounding air-electrical field, from the ambient potential, by way of the needle. This leading connection exists during an acupuncture for several minutes, with so-called

'permanent needles' even over days. According to WOLFF(23) develops with the puncture of the needle in the body always an electrical potential respectively a micro current, which is measurable and apparently able to increase the membrane potential even of extremely depolarized cells till to their repolarization. This normalization then spreads probably from cell to cell or by functional and neural connections, or by the intercellular substance, the system of the "Grundregulation" according to PISCHINGER (19) from the skin to the disturbed organ, so that ideally also its malfunction is corrected. The superiority of the acupuncture of traditional points opposite needle punctures into non acupuncture points could explain itself by the fact that between the traditional points and the disturbed organ respectively the organ, which should be treated, nerve and functional connections exist, which are especially conductive, while the "wrong points" do not have such good connections to the disturbed organ. Therefore they could not be directly reached by the electric current, which is supplied by the acupuncture needle ("acupuncture current"), but possibly only after a detour via the intercellular substance, the system of the "Grundregulation" according to PISCHINGER (19). [According to HEINE (7) one understands by it a network of high-polymere sugar protein complexes, which like a molecular sieve traverses the entire extracellular area.
By the intercellular substance informations can be passed on very fast from cell to cell. Changes of charge are transported for example over "information bridges" made of water and sugar polymers. HEINE described the morphology of acupuncture points histologically as perforations of bundles of vessel- nerve- and connective tissue-bundles of the superficial "body fascia", and makes therewith a physical explanation for the electric measurement of the acupuncture points possible. Therewith the relation between skin points and remote Iying organs could be anatomically explained.]

6. Acupressure, reflexology

The Akupressur and the reflexology of the feet probably cause the repolarization of the cells affected by the loss of function due to extreme depolarization by a provoking of the pressure receptors or with pressure on the nerve membrane:

"A sufficient fast deformation of the nerve membrane by local pressure leads to an away-led action potential" KEIDEL W.D. (14).

The "Percutaneous regulation therapy by normalizing of disturbed body potentials and cell functions over acupuncture points and reflex zones" abbreviated: PR, according to HELMBOLD,K. (11), bases on theories and experiences of ear acupuncture, acupuncture, neural therapy and reflexology and acts similar to the acupuncture, but with the application of pharmaceutical preparations, which are apparently able to increase the membrane potential even of extremely depolarized cells, when they are applied to the disturbed acupuncture points and reflex zones (painful or hyperalgetic skin points and skin zones); indexed acupuncture points can also be treated) and always the external ears, which apparently acts due their innervation as reflex zones for the whole organism (the vagus nerve, the trigeminal nerve and the cervical plexus, between which connections exist). When an acupuncture needle puncture into the body, then develops a micro-current that is measurable and apparently able to increase the membrane potential of

depolarized cells, till to their repolarization and to an ion exchange through the cell membranes and thus to the normalization of the disturbed cell function. This normalization then spreads, probably, as already described above, from the skin to the disturbed organ, so that ideally also its disfunction is corrected. With the PR could be achieved in suitable cases similar successes as with the ear acupuncture, the acupuncture or the neural therapy, but without pain and without the risk of dangerous side effects. Treatment trials showed that this is also possible with ointments, which contains as active substances only salts respectively their ions, which are necessary for the repolarization and in our body needed for the normal functioning of all life processes, that are going on with an ion exchange through the cell membrane and certain electrical phenomena.
These trials led to the "Jonen-Salbe" and the "Jonen-Salbe 3,5 g," both ion ointments contains as active substances calcium chloride, potassium chloride and sodium chloride, respectively its ions. Sometimes stepped the PR a rapid improvement with Lumbago and other painful malfunctions of the movement apparatus, with hay fever and functional diseases, also with MS-patients with spastic paralyses (only to the mitigation of thespastic), here is to that a case example: My patient, 54 years old, suffered from multiple sklerosis with spastic tetraparesis and decubital ulcers at both inner ankles. For the relief of his spasticity he needed 4 times daily 25 mg Lioresal. By applying the "Jonen-Salbe" on the outer ears disappeared the spasticity within a few minutes for hours. [Such rapid success has been designated as "minute phenomenon"(9) analogous to the "second phenomenon" according to HUNEKE because in both ultimately happens apparently the same, only different rates.] This success was reproducible. The patient used during the last 3 years of his life for the relief of his spasticity only this ointment 2 times dayly on the outer ears. (Sorry I could not reach a clinical review with similar cases. Therefore: if anywhere such treatment trials should be made, I would be very grateful for the release of these experiences helmbold – sw@t-online.de www.jonen-salbe.de).
The PR with ion ointments causes the normalization of the disturbed cell function apparently by the contained salt ions. Ions are small electrically charged particles, in which f. i. salts disintegrates, when dissolved in water. Of the sodium ions reported H.ISKRAUT (13) already 1954:

"*They set up the membrane potential like local anesthetics without that there an anesthesia must occur.*"

and:

"*Even with lack of sodium ions, the nerve loses its excitability, without the necessity that the membrane potential change significantly. Intake of sodium ions raises this state again.*"

Therefore, it is incomprehensible, that physiological saline solution continues - more than 5 decades (!) - has been considered as "drug free" and used as a placebo and may be still used till today. One should, check all studies, which were made with NaCl as placebo, and then correct it accordingly. In cases where the alleged placebo has shown to be particularly effective, one should clarify, whether it in such cases can be with used as a therapeutic agent. But now to the mode of function of the ion ointments: apparently formed by the application of these ion-ointments by the contained ions at the depolarized cell membrane a concentration gradient or an electro-chemical potential gradient, which increases the membrane potential till to the repolarization of the depolarized cells and to

the ion exchange through the cell membrane and thus to the normalization of the cell function. That membrane potentials are caused by concentration differences of sodium and potassium ions is natural-scientifically understandable. Therefore speak the with the PR with ion ointments achieved successes, in my view, not only for the effectivity of this therapy but also for the correctness to the hypothesis: Acupuncture - an electrical phenomenon. The ancient Chinese recommendation, during a thunderstorm not to acupuncture, suggests, that this also the ancient Chinese had already recognized.

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Part 4

Current Scenario

11

Acupuncture Analgesia Research and Clinical Practice in Taiwan

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1. Introduction

Traditional Chinese acupuncture has a history of over 2,500 years. It is now considered to be a type of "complementary medicine" because it is effective in the treatment of many conditions with fewer side effects compared with other medical procedures, such as surgery or treatment with pharmaceuticals (Wu, 1996). The WHO has published guidance describing the efficacy of acupuncture in the cure or relief of 64 different symptoms (2003). For example, acupuncture has been successfully applied in cases of chronic pain, nausea, arthritis, and digestive problems, among others.
Two different strategies have been commonly used when performing acupuncture therapy, i.e., manual acupuncture (MA) and electroacupuncture (EA). EA is a modified form of traditional MA. The advantage of EA is in its combined therapeutic effects of transcutaneous electric nerve stimulation (TENS) and MA.

2. General concepts of the mechanism of acupuncture

Many studies in animals and humans have demonstrated that acupuncture can cause multiple biological responses (Wang, et al., 2001). MA and EA are capable of triggering a chain of events that can be understood through controlled experiments. The best known mechanism is via endogenous opiates and their receptors. Early works have demonstrated the role that endogenous opiates play in the central nervous system in acupuncture analgesia. Different kinds of endogenous opiates, such as β-endorphin, enkephalin, endomorphin, and dynophin, have been reported to act as frequency-dependent factors in EA.
In the 1970s and early 1980s, naloxone, an opiate receptor antagonist, was found to attenuate the analgesic actions of acupuncture in humans (Mayer, et al., 1977) and in mice (Pomeranz&Chiu, 1976). The release of a morphine-like substrate in the central nervous system was hypothesized to be a possible mechanism of this analgesic action. Soon afterwards, β-endorphin and enkephalin were purified and it was suggested that these opiates play a role in acupuncture in humans and animals (Clement-Jones, et al., 1980; Kiser, et al., 1983; Pert, et al., 1981). In humans, elevated levels of β-endorphin in the cerebrospinal fluid and of plasma enkephalin were observed after acupuncture treatments. The relationship between acupuncture analgesia and different kinds of endogenous opiates was explored in detail (Cheng, et al., 1979).

This chapter is focused on the basic and clinical studies of acupuncture analgesia performed by us and our colleagues in Taiwan in recent years. Descriptions of our personal experiences in providing acupuncture treatments for analgesia are also included.

3. Basic research

3.1 Effects of EA analgesia on central monoaminergic neurons

3.1.1 Relationship of EA with serotonergic neurons

During the past decades, investigations of the mechanisms of EA analgesia have focused on the effects of endogenous opiates. In addition to opioids, serotonin has been a subject of particular emphasis (Cheng, et al., 1979). We attempt to explore the relationship between EA and serotonergic neurons in the central nervous system. We found that tail pressure pain thresholds were increased by EA and 5-hydroxytryptophan (5-HTP, a precursor of serotonin) in rats, but decreased by administration of *p*-chlorophenylalanine (PCPA, an inhibitor of serotonin synthesis) and naloxone (a μ-opioid antagonist). The changes in pain threshold produced by EA were reduced by pretreatment with PCPA. These results have revealed that EA analgesia activates serotonergic neurons in the central nervous system (Tsai, et al., 1989).

3.1.2 Relationship between EA and adrenergic neurons

Further, we explored the relationship between EA analgesia and other monoaminergic neurons in mice. The involvement of adrenergic neurons was tested. The writhing responses induced by acetic acid and the pain induced by formalin were found to be inhibited by EA at 2 Hz and 10 Hz (0.5 msec, 3–5 V). Analgesia induced by EA was potentiated by intracerebroventricular (i.c.v.) injections of serotonin (5-HT) and norepinephrine (NE) and was attenuated by intraperitoneal administration of inhibitors of monoamine synthesis (PCPA and α-MT), as well as reserpine, a monoamine depletor. The study revealed that the effects of EA analgesia are also related to the adrenergic neurons in the central nervous system (Kuo, 1995).

3.1.3 Relationships of different frequencies of EA analgesia with monoaminergic neurons

We performed a study to characterize the relationships of different frequencies of EA analgesia with monoaminergic neurons and opioid receptors. The formalin test was performed in ICR mice. The brain concentrations of endogenous monoamines were determined by HPLC. The evidence suggests the following. (1) Exogenous 5-HT and NE enhance the analgesic effect of the different frequencies of EA, especially at 100 Hz. (2) The antinociception of EA at different frequencies of stimulation (2, 10, and 100 Hz) were found to be attenuated by PCPA, and were potentiated by 5-HTP. (3) Prazosin (an adrenergic α_1 receptor antagonist) and clonidine (an adrenergic α_2 receptor agonist) were found to be capable of potentiating the antinociception of different frequencies of EA whereas yohimbine (an adrenergic α_2 receptor antagonist) was found to reverse 2-Hz and 10-Hz EA analgesia and potentiate 100-Hz EA analgesia (4). Pindobind-5-HT_{1A} (a 5-HT_{1A}-directed antagonist) and LY-278584 (a 5-HT_3 antagonist) were found to reverse the 3 different frequencies of EA analgesia, and ketanserin (5-HT_2 receptor antagonist) was found to potentiate 100-Hz EA analgesia. The concentrations of endogenous brain monoamines were influenced by different frequencies of EA. These results reveal that the analgesic effect of EA

is related to serotonergic and adrenergic neurons with different frequencies of stimulation. In the serotonergic pathway, EA analgesia may be mediated via 5-HT_{1A} and 5-HT_3 receptors. Furthermore, 5-HT_2 may be involved in high frequency EA analgesia. In the adrenergic pathway, both adrenergic α_1 and adrenergic α_2 receptors were found to be involved in EA analgesia and may provide opposing functions (Yu, 1995).

3.1.4 Intermittent-alternating mode of administering EA stimulation postpones the development of EA tolerance

As mentioned above, EA produces analgesia that is mediated by a variety of central neurochemical substances including opioid peptides and serotonin. However, regardless of the applied frequency, prolonged EA stimulation for several hours inevitably leads to a decrease in the analgesic effect. This phenomenon is known as "tolerance to EA analgesia" or "EA tolerance" and is known to discourage the use of EA anesthesia during major surgery. Clinical experience has revealed that the effect of EA analgesia can last longer if EA is applied intermittently.

We conducted a study to test whether changes in the parameters and mode of stimulation can prevent, or at least postpone, the development of EA tolerance. EA stimulation was applied to bilateral Zusanli (ST36) and Sanyinjiao (SP6) acupoints in rats using a simultaneous mode (S mode) to both legs or an alternating mode (A mode) between the 2 legs. A similar degree of analgesic effect was obtained in S and A modes of stimulation. However, tolerance usually developed within 5 h in the S mode, and was postponed for 10 h in the A mode. The results revealed that EA with the A mode rather than the S mode provides the benefit of postponing or avoiding EA tolerance without affecting the potency of the EA-induced analgesia (Lin, et al., 1993). The conclusion of this study is of particular importance. The EA machines currently used in the clinics worldwide employ the A mode theory.

4. Clinical research

Everyone has experienced pain related to complicated physiological and psychological reactions. Pain can make patients feel uncomfortable and become sleepless or agitated. To demonstrate the efficacy of acupuncture analgesia, a series of studies was conducted by our group in Taiwan and are described below.

4.1 Effect of high and low frequency EA on pain after lower abdominal surgery

Postoperation pain is a very subjective phenomenon. Patients recovering from the same surgical procedures individually experience a different extent of postoperative pain. The pain occurring during the first few hours after the operation is usually quite intense. Pain can be felt after the operation and when the use of analgesic drugs is diminished, it can be a sensation involving intense pain for a few hours before it stops. In addition, pain stimulates the sympathetic nervous system and causes increases in the heart rate, blood pressure, sweat production, and endocrine hyperfunction, and delays the patient's prognosis (Edwards, et al., 2007; Kumar&Wilson, 2007).

To date, only a few studies have investigated the effects of acupuncture on postoperative pain and have shown conflicting results (Christensen, et al., 1989; Christensen, et al., 1993; Galloway, et al., 1984; Martelete&Fiori, 1985). A critical difference among these studies was the stimulation modality and the lack of a sham control. None of these studies examined the

effect of pre-stimulus acupuncture on postoperative pain and concomitant side effect profiles.

We performed a study to evaluate whether preoperative application of different frequencies of EA stimulation can be effective in relieving postoperative pain, as well as postoperative opioid-related side effects. We examined the effects of preoperative EA at classical bilateral acupuncture points (Zusanli; ST36) on postoperative pain and opioid-related side effects. One hundred healthy consenting women undergoing lower abdominal surgery were randomly assigned to 4 treatment regimens: Group I (n = 25), control; Group II (n = 25), sham-EA (needle insertion without electrical stimulation); Group III (n = 25), low EA (2 Hz of electrical stimulation); and Group IV (n = 25), high EA (100 Hz of electrical stimulation). EA groups received needle insertion with or without electrical stimulation 20 min prior to anesthesia. All patients received patient-controlled analgesia (PCA) of morphine after the operation. Postoperative pain was evaluated by recording the (1) time of the first self-administration of an analgesic, (2) number of instances of PCA self-administration, (3) total amount of morphine required for PCA, and (4) patients' visual analogue scale (VAS) pain score. We found that the first analgesic requested was 10, 18, 28, and 28 min in the control, sham-, low-, and high-EA groups, respectively. During the first 24 h, the total amount of morphine required was decreased by 21%, 43%, and 61% in the sham-, low- and high-EA groups, respectively. The incidence of nausea and dizziness during the first 24 h after surgery was significantly reduced in both the low- and high-EA groups compared with the control and sham-EA groups. We also found that sham-EA exerts a beneficial effect with respect to its pain relieving quality but not on the side effect profiles. Our findings demonstrate that preoperative treatment with low- and high-EA can reduce postoperative analgesic requirements and associated side effects in patients undergoing lower abdominal surgery (Lin, et al., 2002).

4.2 Effects of acupuncture on pain after cesarean section

We performed a study to characterize the effects of acupuncture or EA on the acuppoint Sanyinjiao (SP6) to alleviate pain after operation in conjunction with PCA. In this study, our subjects were pregnant women who had a cesarean section for their childbirth. Sixty women, who had had spinal anesthesia during the cesarean section, were randomly assigned to the control, acupuncture, and EA group. After the operation, acupuncture or EA was provided on the bilateral acupuncture point Sanyinjiao (SP6), and PCA was made available. From the point of the first morphine request, the frequency of PCA demands in 24 h and the doses of PCA used were recorded under double-blinded conditions. In addition, the subjects' vital signs, opioid-related side effects, and pain scores were monitored.

The results showed that the members of the acupuncture and EA groups were able to delay the time before morphine was requested by up to 10–11 minutes relative to the control group. The total dose of PCA used within the first 24 h was 30%–35% less in the acupuncture and EA groups when compared with the control group. This difference was statistically significant. However, there was no significant difference between the acupuncture and EA groups. The pain scores of the EA and acupuncture group were lower than the pain score of the control group within the first 2 h, and these differences were statistically significant. However, 2 h later, there were no significant differences of the VAS scores between either of the treatment groups and the control group. Finally, the incidence of opioid-related side effects, such as dizziness, was lower in both the acupuncture and EA groups relative to the control group. This study reveals that the application of acupuncture and EA could delay the time required before a request for pain relief medication after a cesarean section and decrease the PCA doses used within the first 24 h (Wu, et al., 2009).

4.3 Immediate effects of acupuncture on gait patterns in patients with knee osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease in the elderly population, with the knee being the most commonly affected joint in the lower limbs. Clinical symptoms of knee OA may include pain, swelling, stiffness, muscle weakness, limited range of motion (ROM), and deformity (Kindynis, et al., 1990). Any one of these symptoms will affect the function of the joint and lead to abnormal gait patterns. Pain relief is of primary urgency at clinics where efforts are made to reduce the functional disabilities caused by pain (Caldwell, et al., 2002).

In previous reports, the pain and discomfort arising from OA can be directly and quickly reduced, and the ROM of the joint can be improved via acupuncture (Berman, et al., 1999; Christensen BV, et al., 1992; Ezzo, et al., 2001; Gaw, et al., 1975). However, these studies were based mainly on data obtained from subjective evaluations. No objective assessment of the efficacy of acupuncture in treating knee OA by evaluation of gait improvement has been reported in the literature. Three-dimensional gait analysis has been widely used in the diagnosis of various kinds of neurological and musculoskeletal diseases and in the assessment of subsequent treatment (Barr, et al., 1994; Goh, et al., 1993; Huang, et al., 2008a, 2008b; Lu, et al., 2008; Messier, et al., 1992; Powers, et al., 1999). Therefore, gait analysis techniques can be very helpful for an objective and quantitative assessment of the treatment effects of acupuncture on knee OA. There have been several studies on the effects of knee OA on walking patterns using gait analysis. Patients with knee OA were found to have altered temporal-distance variables, including slower walking speed, decreased stance knee flexion and ROM (Barr, et al., 1994; Gok, et al., 2002; Lin KH, et al., 2008), increased knee abductor moments (Childs, et al., 2004; Goh, et al., 1993; Lin, et al., 2008), decreased knee extensor moments (Goh, et al., 1993; Mundermann, et al., 2005), and decreased peak vertical force during push-off. However, the use of gait analysis for the assessment of treatment effects in patients with knee OA has been limited.

We conducted a study to investigate the short-term effects of acupuncture treatment on patients with knee OA by comparing the lower extremity kinematics and kinetics during gait before and after acupuncture stimulation, as this would form a basis for subsequent long-term follow-up studies. Twenty patients with bilateral medial knee OA were assigned evenly and randomly to a sham group and an experimental group. During the experiment, the experimental group underwent a 30-min formula EA treatment while the sham group received a sham treatment. Before and after treatment, subjects were evaluated for knee pain using VAS and then their performance of level walking was assessed using gait analysis. We found that the VAS scores were decreased significantly after acupuncture in both groups, and mean change in the VAS values of the experiment group was 2-fold greater than that of the sham group. After formula acupuncture stimulation, while no significant changes were found in all the gait variables in the sham group, the experimental group exhibited significant increases in the gait speed, step length, as well as in several components of the joint angles and moments. The results suggest that improved gait performance in the experimental group may be associated with pain relief after treatment, but the relatively small decrease in pain in the sham group was not enough to induce significant improvements in gait patterns (Lu, et al., 2010).

4.4 Comparison of the pain relief effect of EA, regional nerve block, and EA plus regional nerve block in cases of frozen shoulder

Frozen shoulder is a type of spontaneous, progressive periarthritis that occurs over the shoulder joint, and its etiology is not yet clear. Traditional treatments for frozen shoulder have included conservative medical therapy, physical therapy, nerve block, and acupuncture.

We performed a study to determine the pain relief effect of EA, regional nerve block (RNB), and a combination of EA+RNB for frozen shoulder. A total of 150 patients with newly acquired frozen shoulder were randomly divided into 3 groups. Group I patients (n = 50) were given RNB with stellate ganglion block and suprascapular nerve block by treatment with 10 mL of 1% xylocaine. Group II patients (n = 50) underwent EA with local acupoints Jianyu(LI15), Jianjing(GB21), Jianqian (EX-UE12), and ouch points treatment. Group III patients (n = 50) underwent a combination of EA+RNB, which was performed with acupuncture first, followed by RNB. Six vectors of movements were investigated in all methods. A 4-grade Bromage score was used for pain assessment with Grade 1 indicating completely painless; Grade 2, slight pain (i.e., pain on motion); Grade 3, moderate pain (i.e., pain without motion); and Grade 4, severe pain (with requests for analgesics). The range of the shoulder joint was also recorded. Patients were requested to ask for a second treatment if pain recurred (Barr, et al., 1994).
The onset (time from injection to maximal pain relief), duration (time from injection to Grade 3 Bromage score), and side effects were recorded. The result showed that the combined EA and RNB method had significantly high pain control quality, longer duration, and a better range of movement of the shoulder joint than that of EA or RNB performed alone (Lin, et al., 1994).

4.5 Comparative evaluation of EA and TENS for the treatment of myofascial pain syndrome

Myofascial pain syndrome is a neuromuscular dysfunction that arises from a trigger point in skeletal muscle. This disorder can occur in any skeletal muscle. It is usually caused by trauma, inflammatory disease, overwork, or fatigue. It can also be caused by the cumulative effects of longstanding repetitive minor trauma or longstanding muscle tension due to poor posture, occupational disease, or emotional stress. Cervical myofascial pain syndrome is defined by the specific neck muscle site and the resulting pain and stiffness of the neck is accompanied by disturbances in autonomic conditional phenomena, such as headache, dizziness, nausea, vomiting, and insomnia. This affects the daily activities and lives of patients. We performed a study to evaluate quantitatively and to study objectively the immediate effect of the treatment of trigger points with several physical medicine modalities in order to improve our understanding of the clinical application of EA stimulation and TENS on myofascial pain. In total, 66 patients with cervical myofascial pain were randomly divided into 3 groups for the administration of 3 different combinations of physical medicine modalities.
In the first group, a cervical hot pack and a stretching exercise was performed. In the second group, a cervical hot pack and 100-Hz EA at bilateral Fengchi (GB20) and Jianjing (GB21) acupoints as well as a stretch exercise were performed. In the third group, a cervical hot pack and 100-Hz TENS as well as a stretching exercise were performed. The improved degree of VAS, pain threshold, and pain tolerance values at trigger points were recorded for the comparison of the immediate effect between the 3 groups. A paired t-test and one-way ANOVA were used for statistical analysis of the data.
The results revealed the following: (1) The 3 groups obtained significant improvement in pain reduction by evaluation of VAS after treatment, but there was no significant difference in the immediate therapeutic effect among the 3 groups. (2) There was significant improvement in the pain threshold in the EA group after treatment, but not in the control and TENS groups. (3) There was no statistically significant improvement in pain tolerance among the 3 groups after treatment. The results suggest that EA could effectively alleviate the pain threshold when trigger points are addressed as a treatment for myofascial pain syndrome (Chen, 1998).

4.6 Assessment of the effect of postoperative acupuncture stimulation on pain relief after total knee replacement

Postoperative pain, which occurs during the hours immediately after a surgical operation, is very intense and diminishes gradually. We performed a study to estimate the effect of 100-Hz frequency EA stimulation combined with PCA to relieve pain after an operation. We selected total knee replacement (TKA) patients as subjects to examine the effects of 100-Hz EA in conjunction with PCA on alleviation of postoperative pain. The subjects were randomized into 3 groups: a control group, sham EA group, and 100-Hz frequency EA group. Each group consisted of about 30 patients. The study results show that the total dose of PCA used within the first 24 h was 29% less in the 100-Hz EA group relative to the control group. This was also statistically significant. Our results revealed that treatment with 100-Hz EA postpones the initial demand for pain control and decreases the total PCA dose within the first 24 h. When comparing pain scores, the patients in the 100-Hz EA group had lower scores than those of the control and sham EA groups within 6 h. These were statistically significant. However, 6 h later, all 3 groups had similar pain scores. Finally, opiate-related side effects were lowest in the low- and high-frequency EA groups. In conclusion, the results suggest that 100-Hz EA can postpone the initial demand for pain control and decrease the total PCA dose requested within the first 24 h. These effects can help to reduce opiate-related side effects (Chen, 1992).

4.7 Conclusions for clinical research

Our results suggest that acupuncture/EA has significant efficacy with respect to pain relief and reduction in the side effects of morphine administration after surgery. Furthermore, acupuncture/EA also has efficacy against pain due to frozen shoulder, knee-joint OA, and cervical myofascial pain.

5. Evidence-based medicine in acupuncture

In Western medicine, evidence-based medicine (EBM) is popular. EBM adopts methods of epidemiology and statistics, in order to analyze data from an enormous medical database, to define effective treatments. EBM is dependent on the use of randomized controlled trials, as well as systematic reviews of a series of trials and meta-analysis. Practitioners are thereby able to optimize patient care by referring to the best research. Promotion of EBM will certainly improve the quality of clinical research and medical care.

Literature indicates that acupuncture can be used for treatment of many diseases and health cultivation, but is it really effective? We performed a literary review that investigated its effectiveness using EBM. We invited scholars who specialized in each of the systems. These scholars shared their opinions, formulated questions, and helped to define the standards. In total, 234 papers were divided into 10 groups and were evaluated by 29 reviewers. The information is classified into the following 10 groups.

1. Pain
2. Immune
3. Nerve
4. Respiration and Circulation
5. Gastrointestinal
6. Urinary
7. Muscle and skeleton
8. Obstetrics/gynecology and pediatrics
9. Metabolism

10. miscellaneous

The following 5 steps of EBM were used to evaluate the information.

1. Formulating answerable clinical questions
2. Searching for the best evidence
3. Critical appraisal
4. Applying evidence to patients
5. Auditing

We found that higher strength of evidence for curative effects was centralized in the muscular and skeletal systems. OA and lower back pain were among the conditions most successfully treated by acupuncture. Similarly, significantly high strength of evidence was identified for the treatment of symptoms, such as asthma and vomiting. Safety and cost-effectiveness of acupuncture were also supported by higher strength of evidence (Lin JG&Lin, 2008).

6. Clinical practice

Dr. Jaung-Geng Lin has performed acupuncture on many world leaders and has received numerous awards. Regarding the selection of acupoints for acupuncture analgesia, Dr. Lin, according to his experience, has the following suggestions.

6.1 Therapeutic principles for pain relief

1. For acute strain myofibrositis or myofascial pain syndrome, Yanglingquan (GB34) is the principal acupoint for pain relief. In the traditional theory, Yanglingquan (GB34) is one of the eight meeting points, and is where the qi of the tendon gathers. The tendon in the classical literature indicates the soft tissue, such as muscle, tendon, and fascia in modern medicine. The classical literature also indicates that soft tissue diseases are usually treated at the Yanglingquan (GB34) acupoint.
2. If the pain is caused by a sprain, Yanglingquan (GB34) is the first choice of acupoints to relieve pain.
3. Following the meridian from the painful location to identify appropriate acupoints is important.
4. The trigger area or tenderness points are appropriate points to perform acupuncture. These points are ouch points as described in "Beiji Qian Jin Yao Fang; Essential Recipes for Emergent Use Worth A Thousand Gold" (Sun, Simiao; 581 AD).
5. If the pain symptom induces anxiety or unstable emotion, acupoints for sedation such as Shenmen (HT7) could be performed.

6.2 For specific areas of pain, we have the following suggestions.

6.2.1 Headache

6.2.1.1 Frontal headache (Yangming meridian headache)

Selection of distant points: Hegu (LI4; Figure 1).
Selection of adjacent points: related acupoints.
If the headache is caused by allergic rhinitis: combine with Yingxisng (LI20; Figure 2).

6.2.1.2 Lateral headache (Shaoyang meridian headache)

Selection of distant points: Yanglingquan (GB34; Figure 3)

Selection of adjacent points: Xuanlu (GB5; Figure 4)

6.2.1.3 Occipital headache (Taiyang meridian headache)

Selection of distant points: Lieque (LU7; Figure 5)
Selection of adjacent points: Fengchi (GB20; Figure 6) and Tianzhu (BL10; Figure 7)

6.2.1.4 Parietal headache (Liver meridian headache)

Selection of distant points: Taichong (LR3; Figure 8; combined with liver meridian); Yougquan (KI1; Figure 9; combined with kidney meridian)
Selection of adjacent points: Baihui (GV20; Figure 10)

6.2.2 Neck pain

Selection of distant points: Lieque (LU7; Figure 5)
Selection of adjacent points: Fengchi (GB20; Figure 6)

6.2.3 Neck sprain

Yanglingquan (GB34; Figure 3) can be added

6.2.4 Frozen shoulder

Selection of distant points: Yanglingquan (GB34; Figure 3), Tiaokou (ST38; Figure 11), and Chengshan (BL57; Figure 12)
Selection of adjacent points: Jianliao (TE14; Figure 13), Jianjing (GB21; Figure 14), and ouch point.

6.2.5 Hypochondriac pain

Neiguan (PC6; Figure 15), Zhigou (TE6; Figure 16), and Sanyanglou (TE8; Figure 17)

6.2.6 Abdominal pain

Zusanli (ST36; Figure 18)

6.2.7 Dysmenorrhea:

Zusanli (ST36; Figure 18) and Sanyinjiao (SP6; Figure 19)

6.2.8 Waist pain, back pain, and sciatica

Selection of adjacent points: Shenshu (BL23; Figure 20) and Mingmen (GV4; Figure 21)
Selection of distant points: Weizhong (BL40; Figure 22), Yanglingquan (GB34; Figure 3), Huantiao (GB30; Figure 23) and Kunlun (BL60; Figure 24)

6.2.9 Knee joint pain

Yanglingquan (GB34; Figure 3) and Yinlingquan (SP9; Figure 25)

6.2.10 Ankle pain

Kunlun (BL60; Figure 24), Taixi (KI3; Figure 26), and Yanglingquan (GB34; Figure 3)
It is suggested that patients with neck pain, frozen shoulder, waist pain, and back pain should follow individual exercise programs as described in our previous book (Lin JG, 1988).

The above mentioned acupoints are of particular importance and can be applied in clinical practice. These acupoints are described because they are the most important acupoints for analgesia. In addition, the rules for combinations of acupoints and for acupuncture should be taken into consideration. In brief, the following should be considered as the rules for acupoints combinations.

1. Using single acupoint only: treat with only one acupoint.
2. Using two acupoints at one time: use the same acupoint on both the sides of the body.
3. Using a combination of anterior and posterior points: choose the acupoints on the anterior and posterior side of the local origin of the disease.
4. Using a combination of exterior and interior points: choose the acupoints on both sides of the local origin of the disease.
5. Using a combination of upper and lower points: choose the acupoints on the upper and lower extremity at the same time.
6. Using a combination of near and distant acupoints: choose the acupoints near the local disease area and a distant area.
7. Using a combination of "master" and "guest" acupoints: choose the Yuan point of the main meridian first and the Lou point of the opposite meridian later.
8. When applying a treatment for lack of vigor and symptoms thereof, use the vigorous points to treat weak bodies and choose acupoints according to symptoms.

With regard to the rules for acupuncture prescription, the chosen acupoints can be divided into 4 groups according to the functions of: principle, associate, adjuvant, and messenger for clinical applications. (1) The principle is the most important point for therapeutic effects. (2) The associate assists and enhances the effects of the principle. (3) The adjuvant assists the principles to treat the complication. (4) The messenger harmonizes the effects of the others.

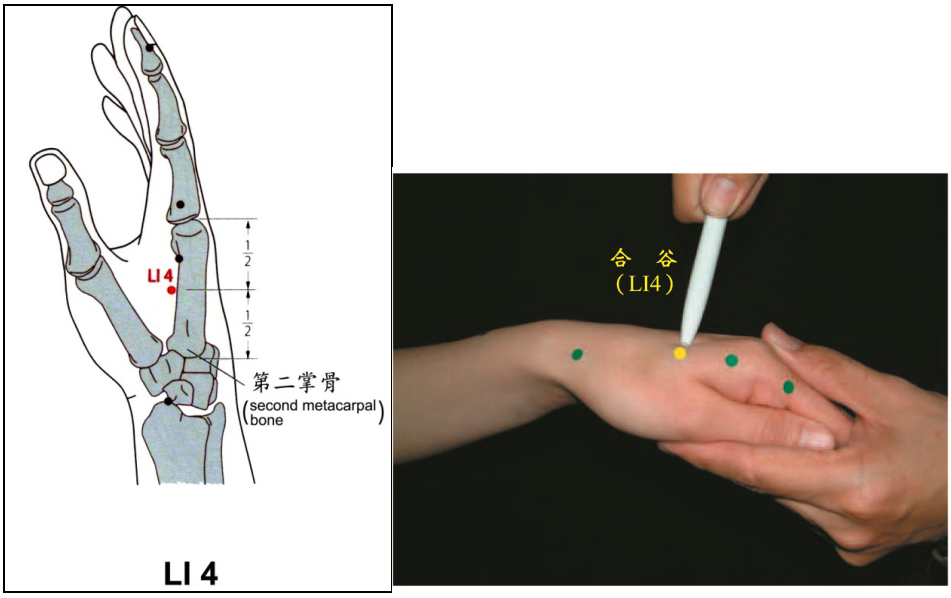


Fig. 1. Hegu(LI4). On the dorsum of the hand, radial to the midpoint of the second metacarpal bone (WHO 2008).

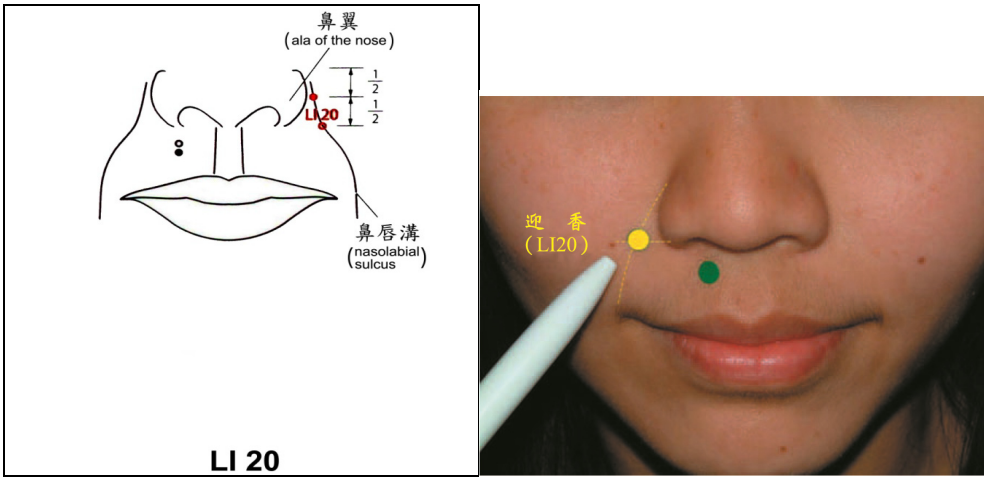


Fig. 2. Yingxiang (LI20). On the face, in the nasolabial sulcus, at the same level as the midpoint of the lateral border of the ala of the nose. (WHO 2008)

Remarks: Alternative location for LI20 - On the face, in the nasolabial sulcus, at the level of the inferior border of the ala of the nose.

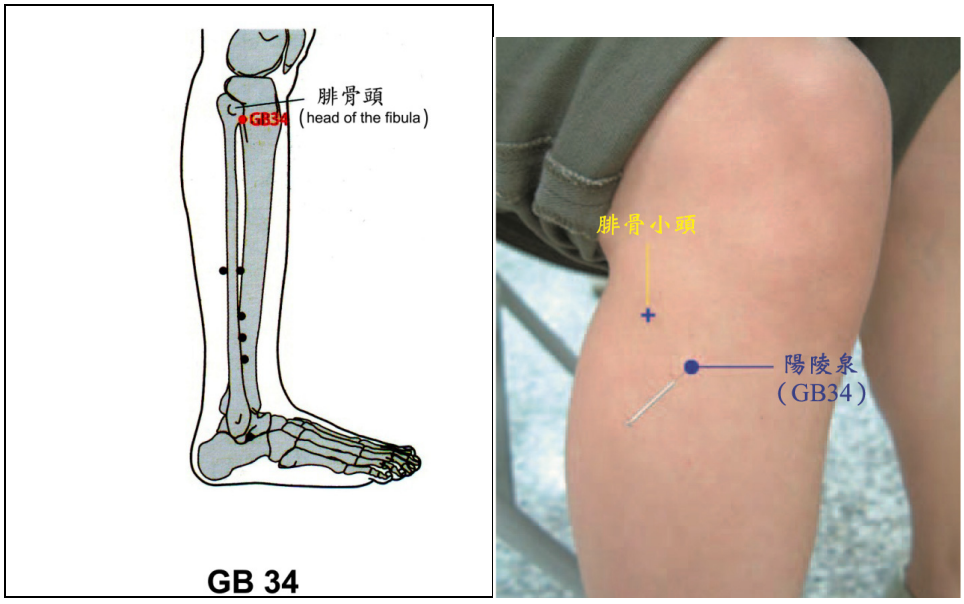


Fig. 3. Yanglingquan (GB34). On the fibular aspect of the leg, in the depression anterior and distal to the head of the fibula. (WHO 2008)

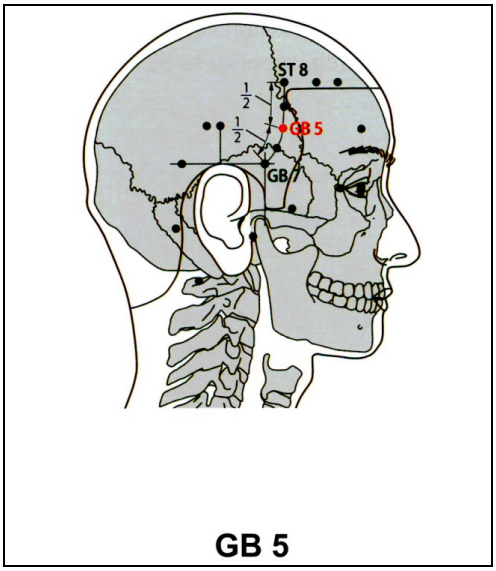


Fig. 4. Xuanlu (GB5). On the head, at the midpoint of the curved line from ST8 to GB7.

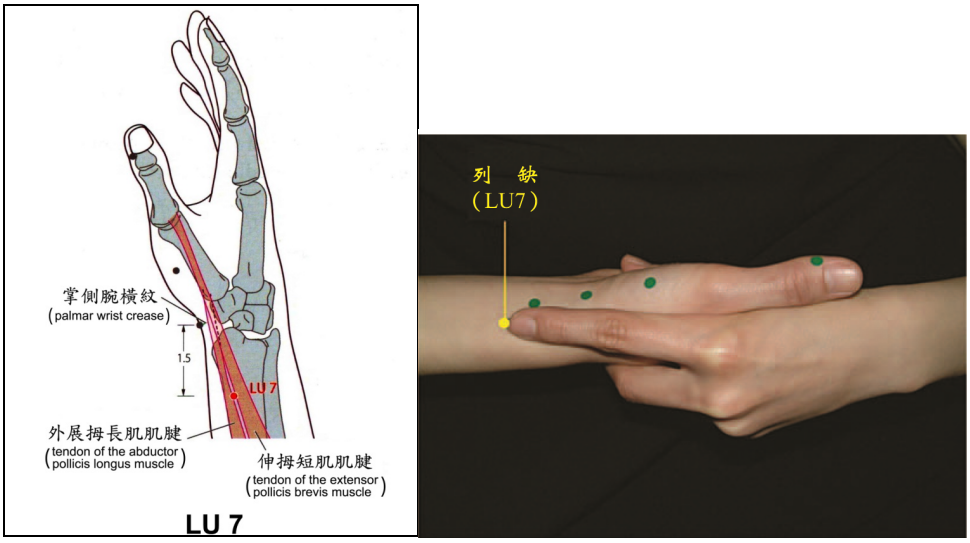
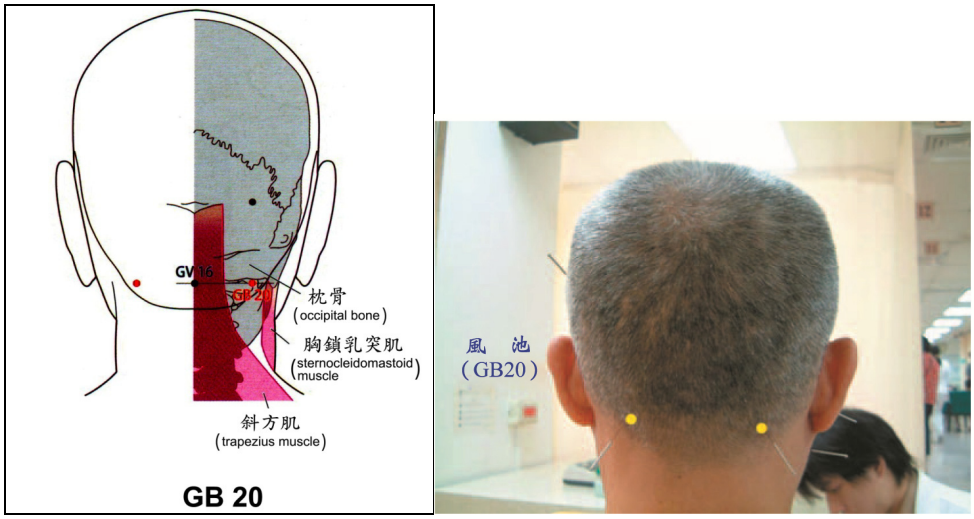


Fig. 5. Lieque (LU7). On the radial aspect of the forearm, between the tendons of the abductor pollicis longus and the extensor pollicis brevis muscles, in the groove for the abductor pollicis longus tendon, 1.5 B-cun superior to the palmar wrist crease. (WHO 2008)



Note: GB20 is at the same level as GV16.

Fig. 6. Fengchi (GB20). In the anterior region of the neck, inferior to the occipital bone, in the depression between the origins of sternocleidomastoid and the trapezius muscle. (WHO 2008)

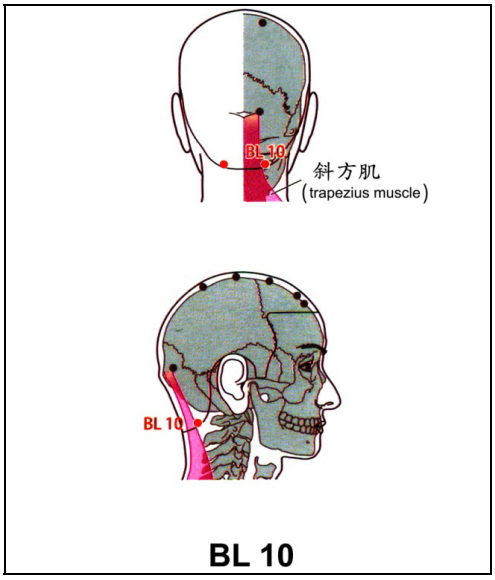
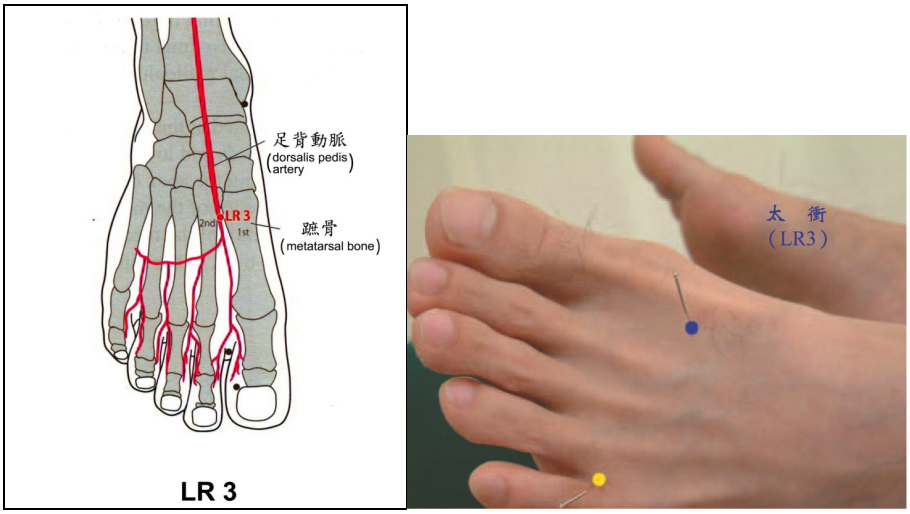
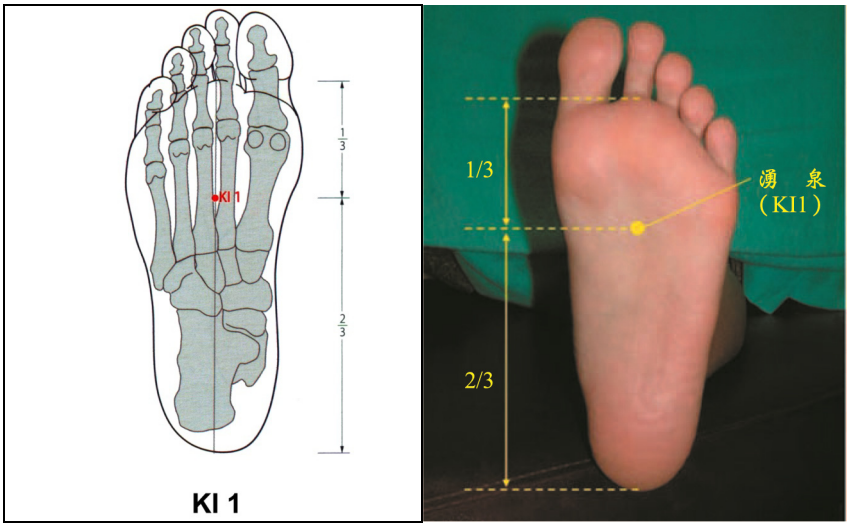


Fig. 7. Tianzhu (BL10). In the posterior region of the neck, at the same level as the superior border of the spinous process of the second cervical vertebra (C2), in the depression lateral to the trapezius muscle.(WHO 2008)



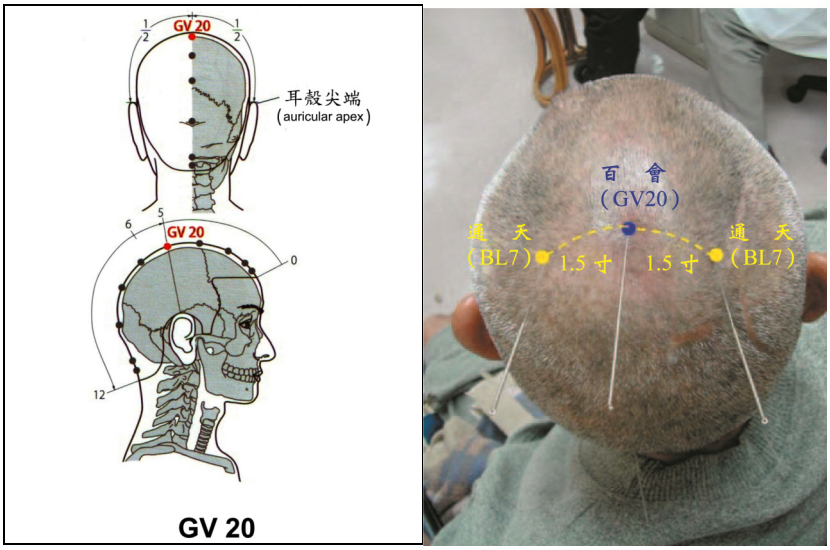
Note: LR3 can be felt in the depression when moving proximally from LR2 in the gap between the first and second metatarsal bones towards the base of two metatarsal bones

Fig. 8. Taichong (LR3). On the dorsum of the foot, between the first and second metatarsal bones, in the depression distal to the junction of the bases of the two bones, over the dorsalis pedis artery. (WHO 2008)



Note: When the toes are flexed, KI1 is located approximately in the depression at the junction of the anterior one third and the posterior two thirds of the line connecting the heel with the web margin between the bases of the second and third toes.

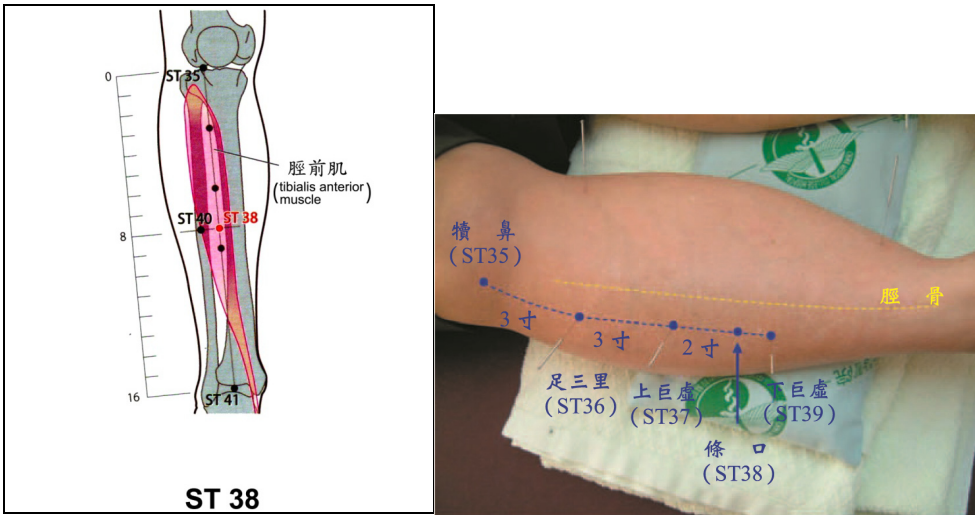
Fig. 9. Yougquan (KI1). On the sole of the foot, in the deepest depression of the sole when the toes are flexed . (WHO 2008)



Note 1: GV20 is located in the depression 1 B-cun anterior to the midpoint of the line from the anterior hairline to the posterior hairline.

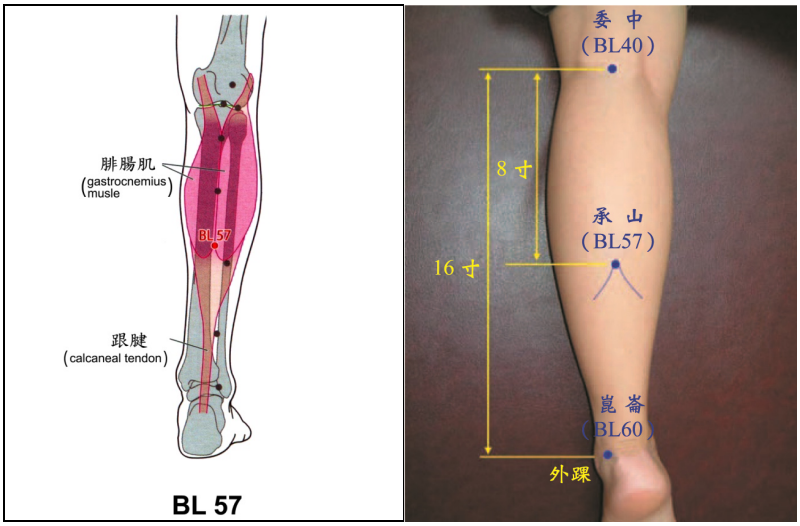
Note 2: When the ears are folded, GV20 is located at the midpoint of the connecting line between the auricular apices.

Fig. 10. Baihui (GV20). On the head, 5 B-cun superior to the anterior hairline, on the anterior median line. (WHO 2008)



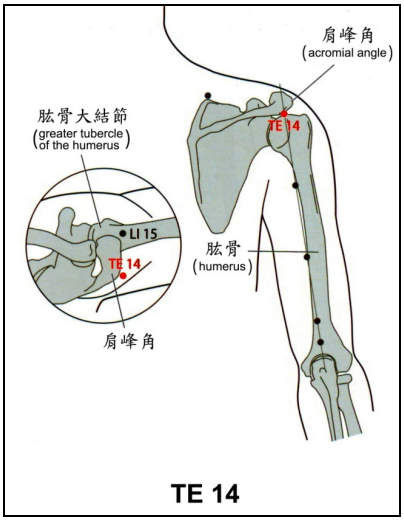
Note: ST38 is located on the tibialis anterior muscle, at the same level as ST40.

Fig. 11. Tiaokou (ST38) On the anterior aspect of the leg, on the line connecting ST35 with ST41, 8 B-cun inferior to ST35. (WHO 2008)



Note: With the leg stretched (plantar flexion) or the heel up, BL57 is located at the sharp angled depression inferior to the muscle belly of the gastrocnemius muscle. The two heads of the gastrocnemius muscle are separated to make a lambda shape (Λ)

Fig. 12. Chengshan (BL57). On the posterior aspect of the leg, at the connecting point of the calcaneal tendon with the two muscle bellies of the gastrocnemius muscle. (WHO 2008)



Note: When the elbow is flexed and the arm is abducted, two depressions appear respectively anterior and posterior to the acromion. LI15 is located in the anterior one, deeper than the posterior one, in which TE14 is located.

Fig. 13. Jianliao (TE14). On the shoulder girdle, in the depression between the acromial angle and the greater tubercle of the humerus. (WHO 2008)

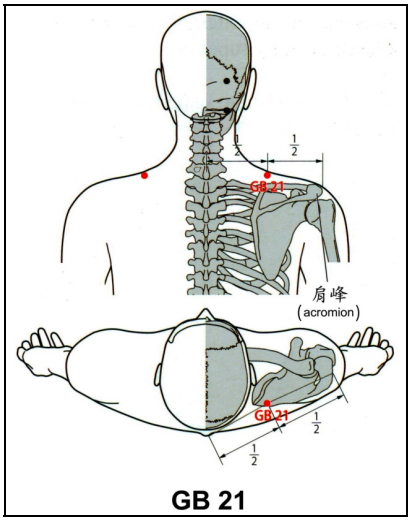
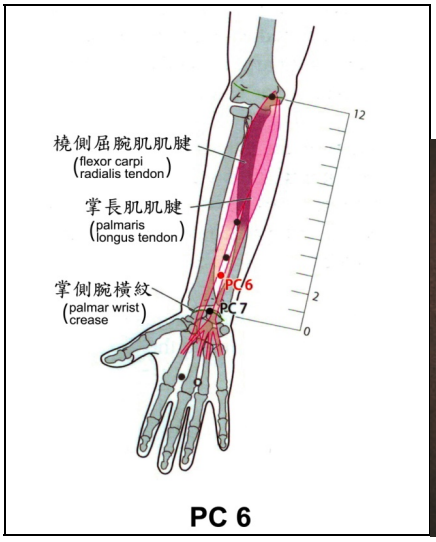
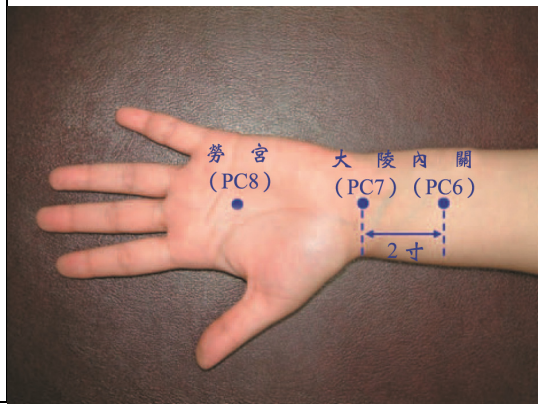


Fig. 14. Jianjing (GB21). In the posterior region of the neck, at the midpoint of the line connecting the spinous process of the seventh cervical vertebra (C7) with the lateral end of the acromion.(WHO 2008)

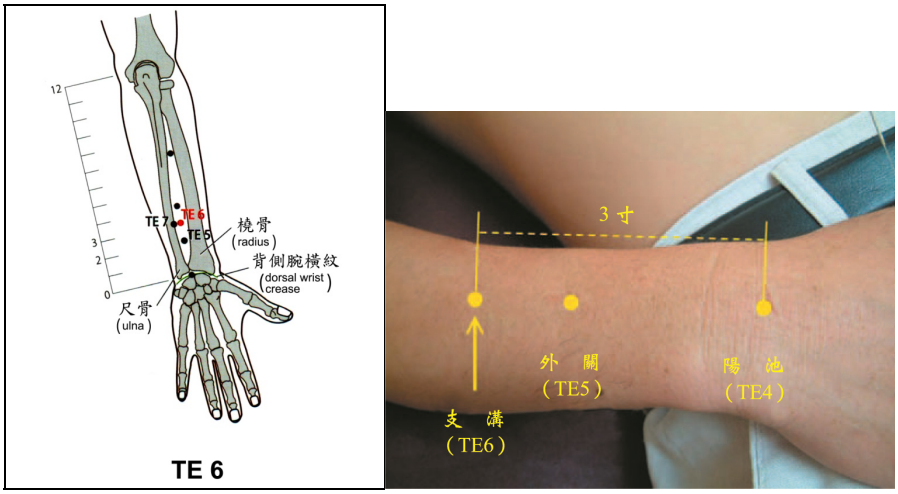




Note 1: With the fist clenched, the wrist supinated and the elbow slightly flexed, the two tendons become more prominent. PC6 is located 2 B-cun proximal to PC7. The posterial point corresponding to PC6 is TE5.

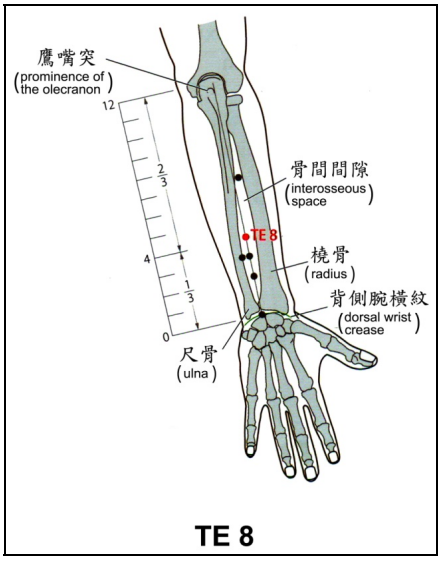
Note 2: If the palmaris longus tendon is not present, PC6 is medial to the flexor carpi radialis tendon.

Fig. 15. Neiguan (PC6). On the anterior aspect of the forearm, between the tendons of the palmaris longus and the flexor carpi radialis, 2 B-cun proximal to the palmar wrist crease. (WHO 2008)



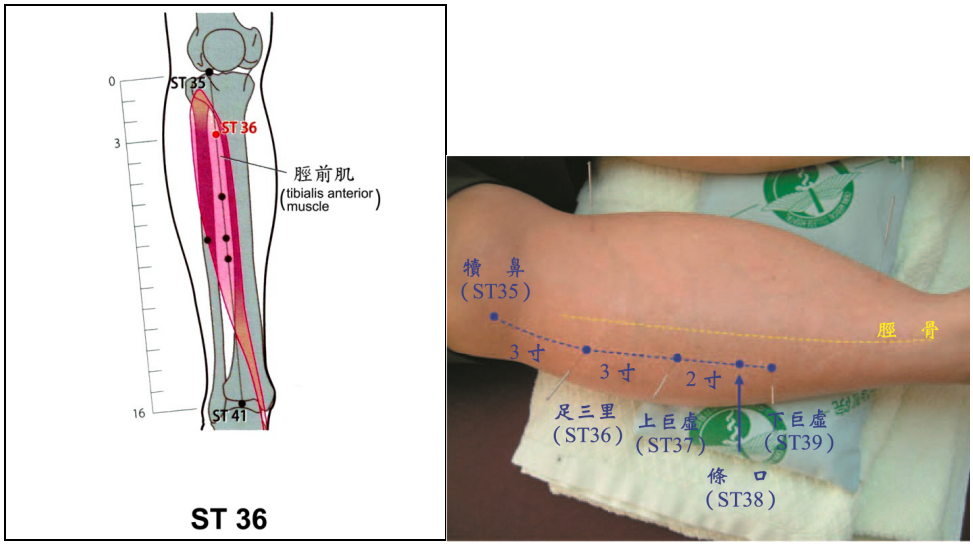
Note: 1 B-cun proximal to TE5, between the radius and the ulna, at the same level as TE7.

Fig. 16. Zhigou (TE6). On the posterior aspect of the forearm, midpoint of the interosseous space between the radius and the ulna, 3 B-cun proximal to the dorsal wrist crease. (WHO 2008)



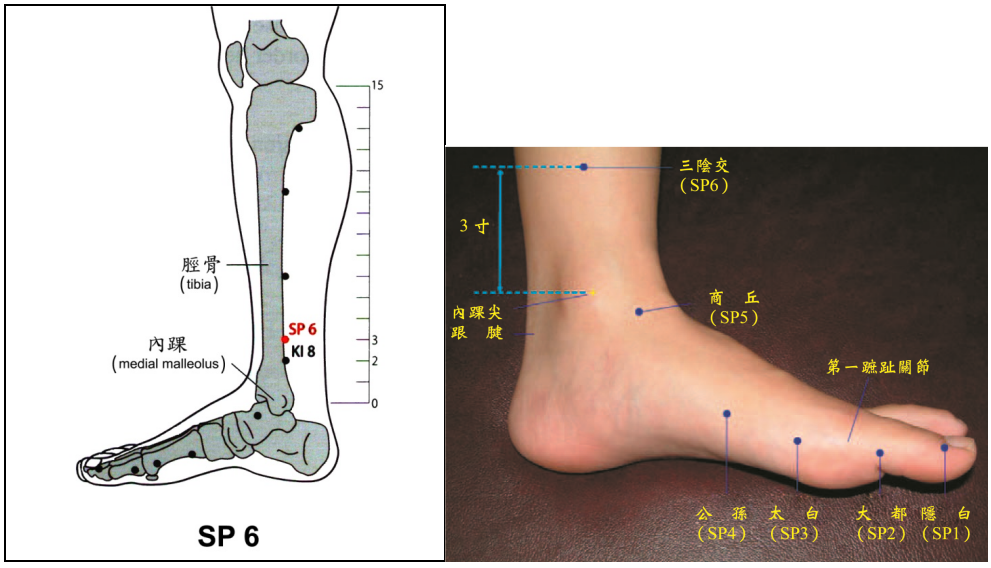
Note: At the junction of the upper two thirds and lower one third of the line connecting TE4 with the tip of the elbow.

Fig. 17. Sanyanglou (TE8) On the posterior aspect of the forearm, midpoint of the interosseous space between the radius and the ulna, 4 B-cun proximal to the dorsal wrist crease. (WHO 2008)



Note: ST36 is located on the tibialis anterior muscle.

Fig. 18. Zusanli (ST36). On the anterior aspect of the leg, on the line connecting ST35 with ST41, 3 B-cun inferior to ST35. (WHO 2008)



Note: 1 B-cun superior to KI8.

Fig. 19. Sanyinjiao (SP6).On the tibial aspect of the leg, posterior to the medial border of the tibia, 3 B-cun superior to the prominence of the medial malleolus. (WHO 2008)

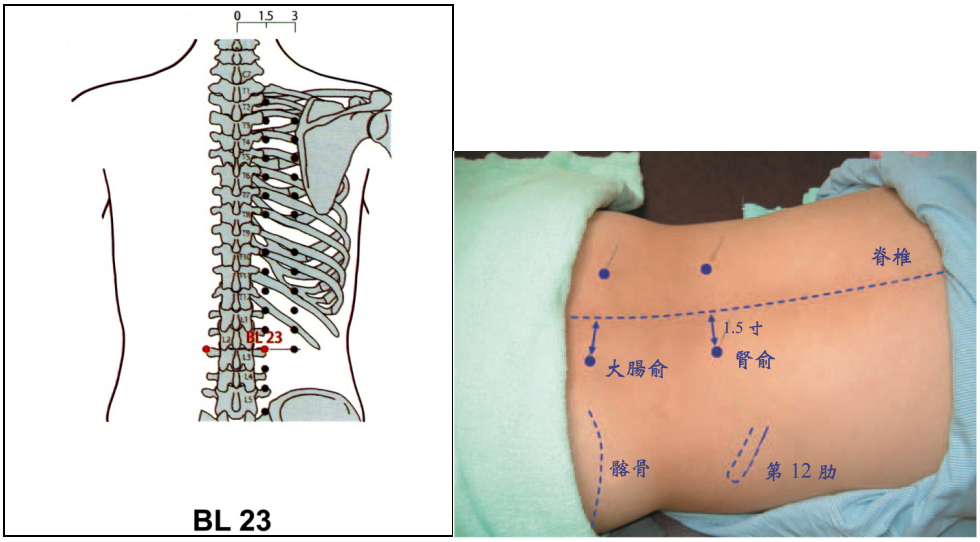


Fig. 20. Shenshu (BL23). In the lumbar region, at the same level as the inferior border of the spinous process of the second lumbar vertebra (L2), 1.5 B-cun lateral to the posterior median line. (WHO 2008)

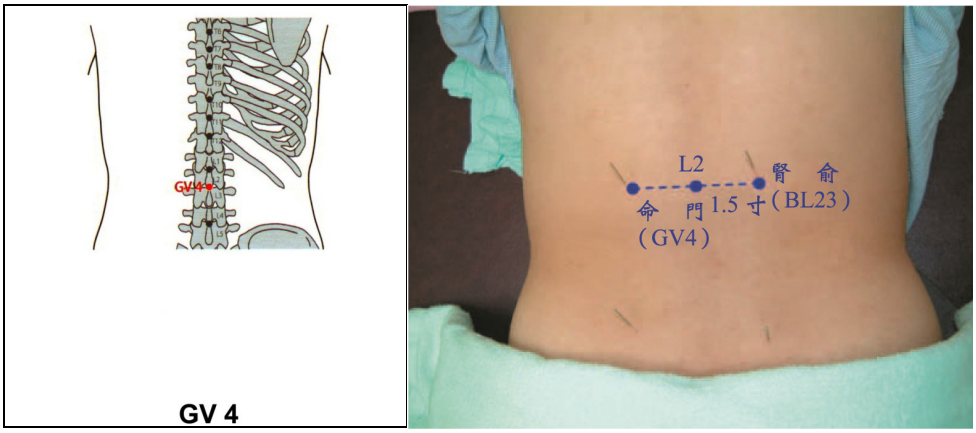


Fig. 21. Mingmen (GV4) In the lumbar region, in the depression inferior to the spinous process of the second lumbar vertebra (L2), on the posterior median line.(WHO 2008)

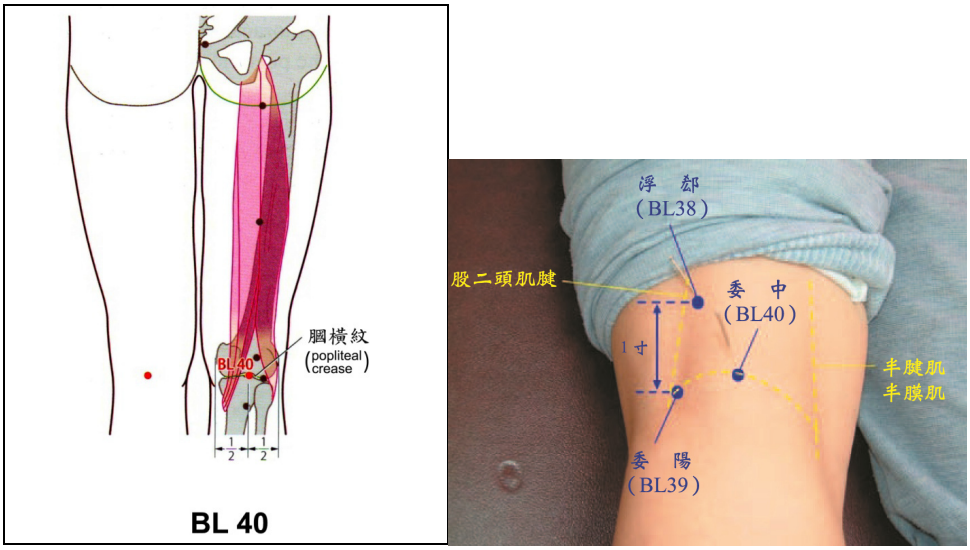
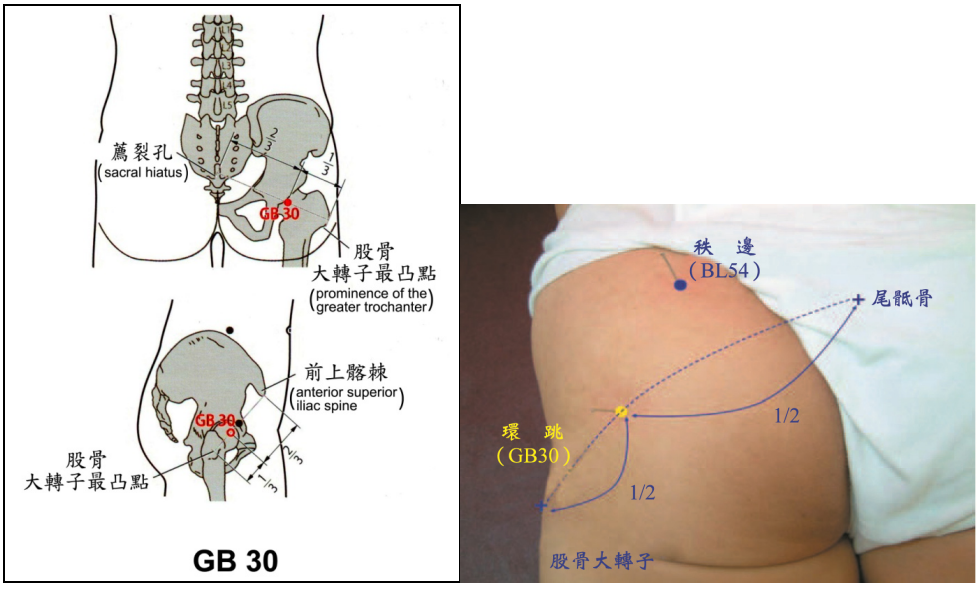


Fig. 22. Weizhong (BL40). On the posterior aspect of the knee, at the midpoint of the popliteal crease. (WHO 2008)



Note: GB30 is easier to locate when the subject is lying on the side with the thigh flexed. (WHO 2008)

Fig. 23. Huantiao (GB30). In the buttock region, at the junction of the lateral one third and medial two thirds of the line connecting the prominence of the greater trochanter with the sacral hiatus.

Remarks: Alternative location for GB30- In the buttock region, at the junction of the lateral one third and medial two thirds of the distance between the prominence of the greater trochanter and the anterior superior iliac spine.

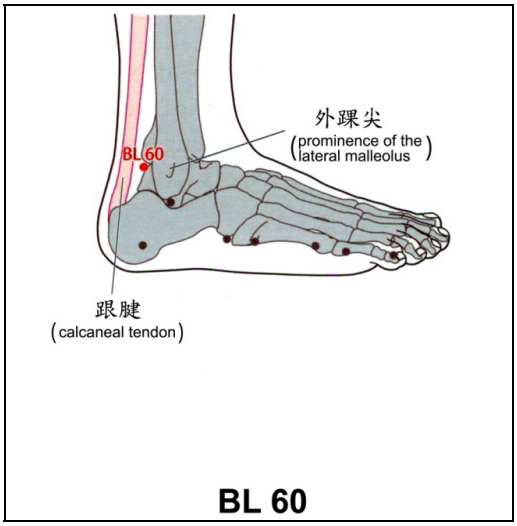
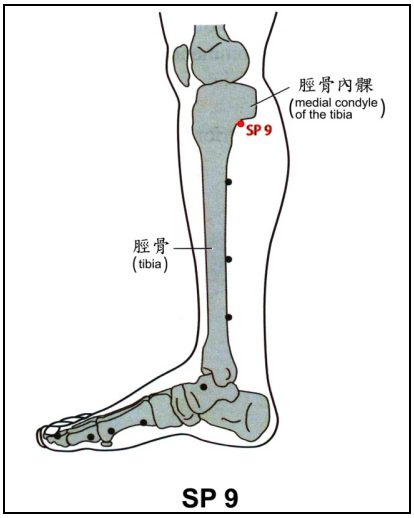


Fig. 24. Kunlun (BL60). On the posterolateral aspect of the ankle, in the depression between the prominence of the lateral malleolus and the calcaneal tendon. (WHO 2008)



Note: A depression can be felt inferior to the knee joint when moving proximally along the medial border of the tibia. SP9 is located in a depression at the angle formed by the inferior border of the medial condyle of the tibia and the posterior border of the tibia. (WHO 2008)

Fig. 25. Yinlingquan (SP9). On the tibial aspect of the leg, in the depression between the inferior border of the medial condyle of the tibia and the medial border of the tibia.

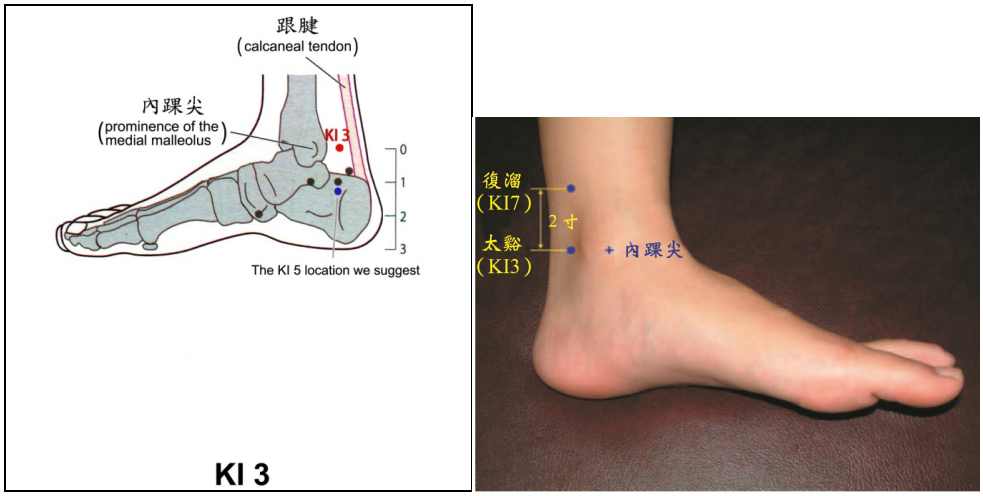


Fig. 26. Taixi (KI3). On the posteromedial aspect of the ankle, in the depression between the prominence of the medial malleolus and the calcaneal tendon. (WHO 2008)

7. Acknowledgment

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12

A Spatial Analysis of Acupuncture Practitioners in Ontario, Canada: Assessing Regional and Intra-Metropolitan Trends

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1. Introduction

Regional disparities in health care supply are typically measured in terms of accessibility to family doctors, specialists and other services associated with the conventional medical (CM) sector. Although progress is being made, research on the geographic properties of complementary and alternative medicine (CAM) remains underdeveloped by comparison. CAM's long history of use, continued popularity and commercial success (via chiropractic, massage, acupuncture, homeopathic, naturopathic and other approaches) and slow but insistent integration with CM makes continued study not only logical but necessary. To gain a better understanding of a location's endowment of medical resources, or indeed to compare health care supply amongst areas, it is important to assess both CM *and* CAM activity. To this end, study needs to evaluate the many diverse sources of medical supply from a geographical perspective.

This paper appends the literature by considering the location properties of acupuncture establishments and does so at two scales: regionally throughout the Canadian province of Ontario and locally within the Greater Toronto metropolitan area. While the emphasis of this study is to describe the spatial patterns of offices listing acupuncture as its main purpose (as classified by standard industrial classification codes), for perspective these primary function acupuncture (PFA) offices are compared to the location tendencies of both CAM collectively (chiropractic, massage, acupuncture, homeopathic, naturopathic and holistic) and 'total' health care supply (CAM plus medical doctor offices, physiotherapists, clinics and hospitals or more generally CM). The analysis reveals that acupuncture offices have strong clustering tendencies and that the intra-Toronto concentrations occur in close proximity to Chinese ethnicity enclaves. These spatial outcomes have wider ramifications in terms of: health care policy, the increasingly debated possibility for greater integration between acupuncture, and other CAM approaches, with conventional (Western, biomedical, allopathic) medicine and in understanding the location-specific criteria that are conducive to attracting CAM activity and perhaps in fostering places of healing.

2. Acupuncture/CAM empirical research: general directions in the literature

Along with herbology, food therapy, manual treatments and therapeutic exercise (such as t'ai chi), acupuncture is a main discipline of Chinese traditional medicine (Giordano et al.,

2004). Research on acupuncture continues to develop and Li et al. (2010) provide a useful synopsis of the literature. They summarize that the active fields of acupuncture study include: 1) clinical research, such as the safeness of acupuncture, the needle 'placebo' effect, and effectiveness in relieving chronic pain, 2) use of acupuncture (based on client surveys and ethical guidelines of acupuncture experiments) and 3) experimental research on acupuncture mechanisms (that critique and assess acupuncture procedures and the utilization of subsidiary technologies such as functioning magnetic resonance imaging). It is noteworthy that spatial research of any type, whether from the perspective of the patient/client or practitioner, is absent from their summary.

In wider terms, the literature on the geography of health care supply over space is emerging. The body of work is heavily biased towards the conventional medicine sector, with study on the location of, and accessibility to, medical doctor's offices and hospitals being the most common. The geographic properties of complementary and alternative medicine is assessed with far less frequency (in comparison to CM), but important contribution do exist (for reviews of the literature, see Andrews et al., 2004; Andrews & Boon, 2005; Hollenberg & Bourgeault, 2009; Meyer, 2010). Although quite infrequent, studies that detail the geographic distributions of CAM specialists and/or their clients are available for: chiropractors (Alcantara et al. 2010; Smith & Carber, 2002; Waalen & Mior, 2005), naturopaths (Albert, 2009; Albert & Butar, 2004a, 2004b), chiropractors and naturopaths (Williams, 2000), chiropractors and homeopaths (Brindle & Goodrick, 2001), homeopaths, paranormal healing and manual therapy (Verheij, 1999) and CAM in general (Andrews, 2003; Meyer, 2008, 2010; Millar, 2001). However, an explicit assessment of the geographic tendencies of acupuncture (a specific type of CAM) has not occurred. This Ontario-wide and Toronto-specific study is believed to be the first to blatantly describe the geography of acupuncture activity and assess the pattern of acupuncture offices over space.

3. Methodology

For this analysis, a number of datasets were utilized:

1. The locations and sales estimates of 386, 465 and 431 offices (for the years 2004, 2007 and 2010, respectively) for which acupuncture is the primary medical service;
2. The locations of 4,957 CAM proprietors (for 2007); and
3. The locations of 8,709 CM offices (2007) were added to the CAM total to provide a sample of 'total' health care locations in Ontario.

All of these offices were placed on a street network layer using the 'address locator' geocoding function within the ArcGIS (version 9.3.1) geographic information systems (GIS) software package. With the use of the ArcGIS 'spatial join' feature, these points were agglomerated into Ontario-wide census subdivisions (CSD), which are essentially municipalities, and Toronto-based census tracts (CT) polygons for further analysis. A number of thematic maps and tables were generated to display important geographic patterns. Ethnicity-based 2006 census data were also utilized.

All health care office locations and sales estimates were purchased from InfoCanada (2004, 2007, 2010). The foundation of InfoCanada's databases is phonebooks, but additional records are added with, and the entire collection is verified by, subsidiary sources. While the data-provider estimates an error of up to 10 percent, there is no evidence of systematic bias in the datasets used herein and these samples are believed to accurately reflect the spatial distribution of health care locations in Ontario. Digital base maps (road network files, CSD

and CT) and ethnic origin census variables (Chinese and Southeast Asian) were obtained from Statistics Canada (2006a, 2006b). Figure 1 provides a view of Ontario, the province's four main districts and the largest census subdivisions.

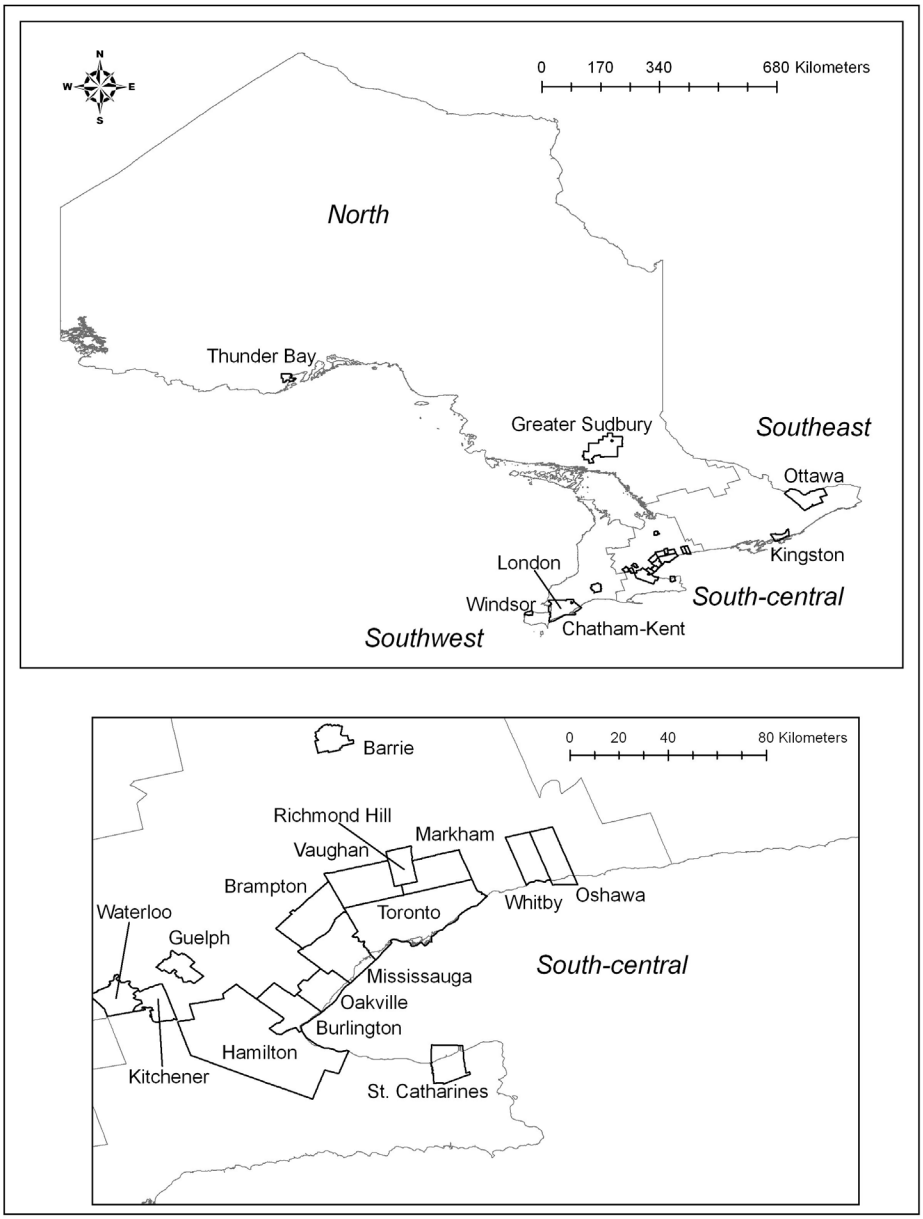

Fig. 1. Census subdivisions in Ontario with a population greater than 100,000 and provincial districts

To understand relative presence or absence, acupuncture office location quotients were calculated for the census subdivisions throughout Ontario. The following computation was used:

> (Number of acupuncture offices in a given CSD / Number of health care offices in a given CSD) / (Number of acupuncture offices in Ontario / Number of health care offices in Ontario)

Location quotient values of 1.2 or greater signify a strong relative concentration or relative specialization (Coffey & Shearmur, 2002; Meyer, 2007) in primary function acupuncture and values of less than 1 are indicative of relative absence. Location quotients were also derived for census tracts within the Toronto census metropolitan area; calculations were derived for acupuncture offices and ethnic origin (Chinese and Southeast Asian). The following approach was used:

> (Number of people of Chinese ethnicity in a given CT / Total number of people by ethnic group in a given CT) / (Number of people of Chinese ethnicity in the Toronto CMA / Number of people by ethnic group in the Toronto CMA)

For the Toronto-specific portion of the analysis, 'significantly clustered acupuncture CSDs' were determined by the Moran's I test for local spatial autocorrelation. Spatial autocorrelation (SAC) can be positive (clustering of phenomena with similar values) or negative (in which neighbouring values are dissimilar) and can be tested for overall global patterns or to determine local hotspots of activity (Boots & Tiefelsdorf, 2000). In this application, census tracts within the Toronto census metropolitan area (CMA) that test statistically significant (at a confidence interval of 95%) for local positive spatial autocorrelation comprise clusters of primary function acupuncture offices with comparatively high levels of sales. In other words, this analysis identifies hotspots of acupuncture service activity within the Greater Toronto area. The local spatial autocorrelation analysis was repeated for all CAM offices and for total health care offices (CAM and CM) for comparison purposes.

A temporal dimension was also included in which acupuncture office locations were compared for 2004 and 2010 within metropolitan Toronto. Spatial-temporal trends were evaluated by calculating mean centers and standard deviation ellipses for the distribution of acupuncture office location points. Moreover, temporal change and spatial clustering observations were compared to agglomerations of people with Chinese and/or Southeast Asian origins.

4. A spatial overview of primary function acupuncture offices in Ontario

As shown in Table 1, both the acupuncture and more general CAM (chiropractic, massage, holistic, naturopathic, homeopathic and acupuncture) samples show 'urban area' and 'southern province' biases; yet important differences in spatial patterns are apparent. Census subdivisions that are part of census metropolitan areas (populations of 100,000 or more), comprise the largest proportion of both samples: 89.2 and 79.5 for PFA and CAM offices respectively. Yet, CSDs within the Toronto CMA alone contain over half of all acupuncture offices which is far higher than the CAM share (35.7%) in Canada's largest metropolitan center. CSDs inside smaller metropolitan areas or within census agglomerations (CAs with populations of 50,000 to 100,000) exhibit much higher CAM percentages than PFA (11.0 versus 6.9). While areas outside of CMAs or CAs, show lower proportions, these numbers are consistently far more modest for the specific case of primary

function acupuncture office activity, in comparison to CAM; and this is true for all categories (strongly, moderately, weakly or not influenced).

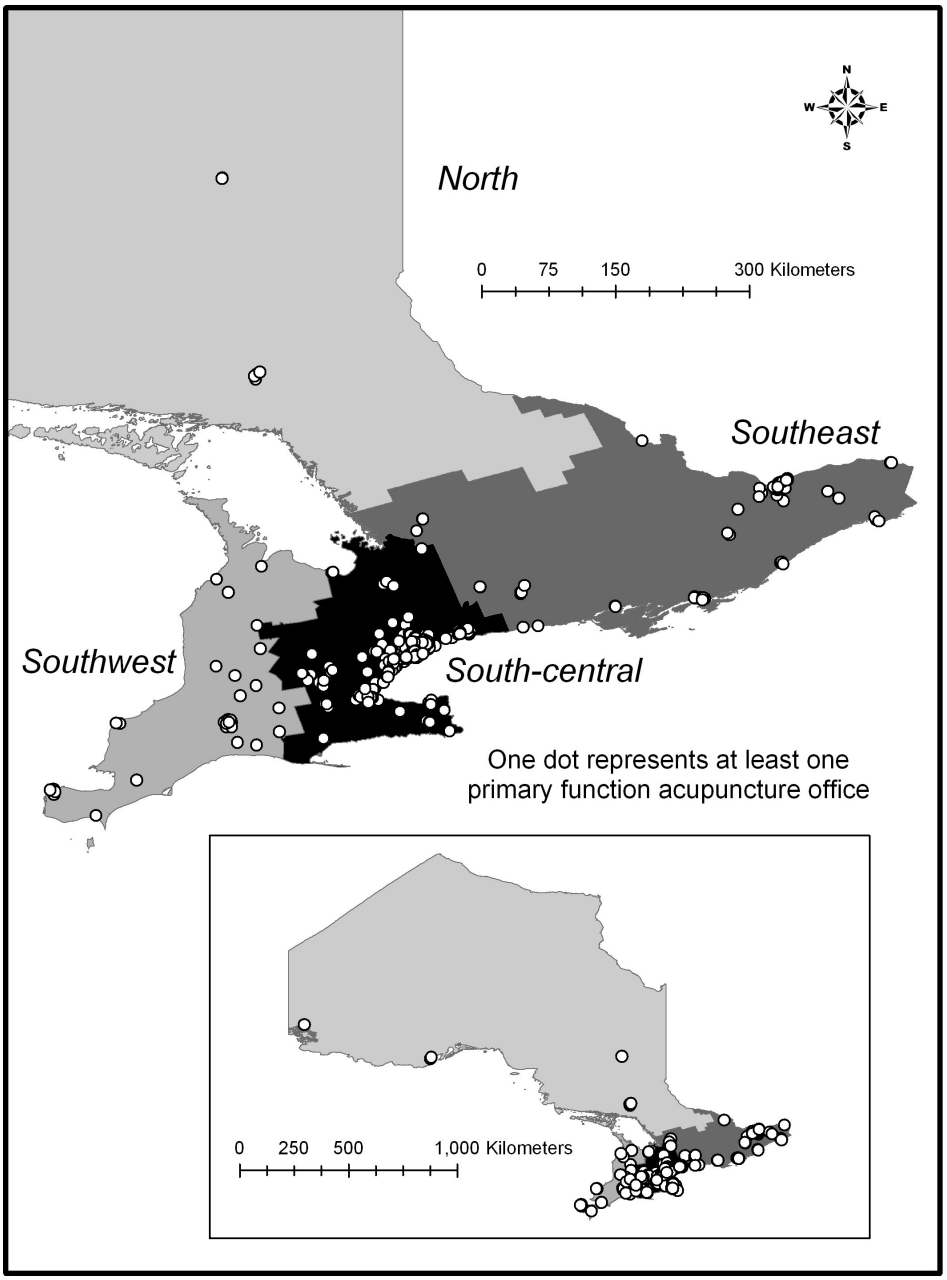

Fig. 2. Location of primary function acupuncture offices in Ontario

Location propensity differences are also evident by provincial district. The south-central part of the province is the most important in terms of amassing sheer numbers of CAM offices, but the specific case of acupuncture is outstanding with almost three-quarters of the province's PFA establishments in this district (which of course includes the Toronto CMA). The PFA sample has a slightly higher proportion in the southeast. This district contains Ottawa, the province's second largest CMA and the nation's capital city, which houses 6.7% of Ontario's PFA offices (and 5.0% of CAM). Conversely, the share of acupuncture office activity, vis-à-vis CAM, is considerably lower in the southwest and northern districts. For specific PFA office locations, see Figure 2.

Census Subdivisions by Statistical Area Classification (SAC) Category and Provincial District	Number of PFA Offices and CAM offices	Percent of Total : PFA and CAM office	Average Census Subdivision PFA Location Quotient
SAC			
Census metropolitan area (CMA):			
Part of Toronto CMA	249 /1945	53.5 /39.3	0.77
Part of other CMAs	166 / 1991	35.7 /40.2	0.31
Part of a census agglomeration (CA)	32 / 544	6.9 / 11.0	0.97
Strongly influenced by CMA or CA	6 / 147	1.3 / 3.0	0.77
Moderately influenced by CMA or CA	10 / 181	2.2 / 3.7	0.52
Weakly influenced by CMA or CA	2 / 142	0.4 / 2.9	0.06
Not influenced by CMA or CA	0 / 5	0.0 / 0.1	0.00
Provincial District			
Southwest	42 / 729	9.0 / 14.7	0.57
South-central	341 /3255	73.3 / 65.7	0.67
Southeast	69 / 658	14.8 / 13.3	0.70
North	13 / 313	2.8 / 6.3	0.07
Ontario (total)	465 /4955	100 / 100	0.51

Table 1. Primary function acupuncture (PFA) and complementary and alternative medicine offices (CAM) in Ontario

The location quotient analysis identifies specific CSDs which are strongly specialized in terms of PFA. Collectively these CSDs display a provincial pattern that provides further evidence that the location choices of acupuncture office practitioners are, in some ways, distinguishable from the more general CAM situation. Meyer (2010) found that CAM specialization in Ontario was wide-spread and prevalent in all four provincial districts and quite evenly distribution across the urban-peripheral continuum (in comparison to conventional medical offices). In contrast, it is very rare for communities to specialize in acupuncture. The last column on Table 1 shows that all of the location quotient averages for the provincial districts or SAC categories are below, and in some cases well below, unity with only the census agglomeration category being close to 1.0. In all, there are only 34 CSDs that scored above 1.20 and achieved 'specialized' status in terms of PFA activity. As shown

on Figure 3, almost half (15) of these CSDs are located in the south-central (with five in the Toronto CMA alone). Both the southeast and southwest had nine specialized CSDs and it is noteworthy that all but one (Kingston) of these 18 CSDs are located outside of a CMA or CA. The north had only one 'acupuncture specialized' CSD. Although small in number, these municipalities are collectively very important: 294 of the 465 PFA offices in Ontario, or 63.2%, are located in these 34 specialized CSDs.

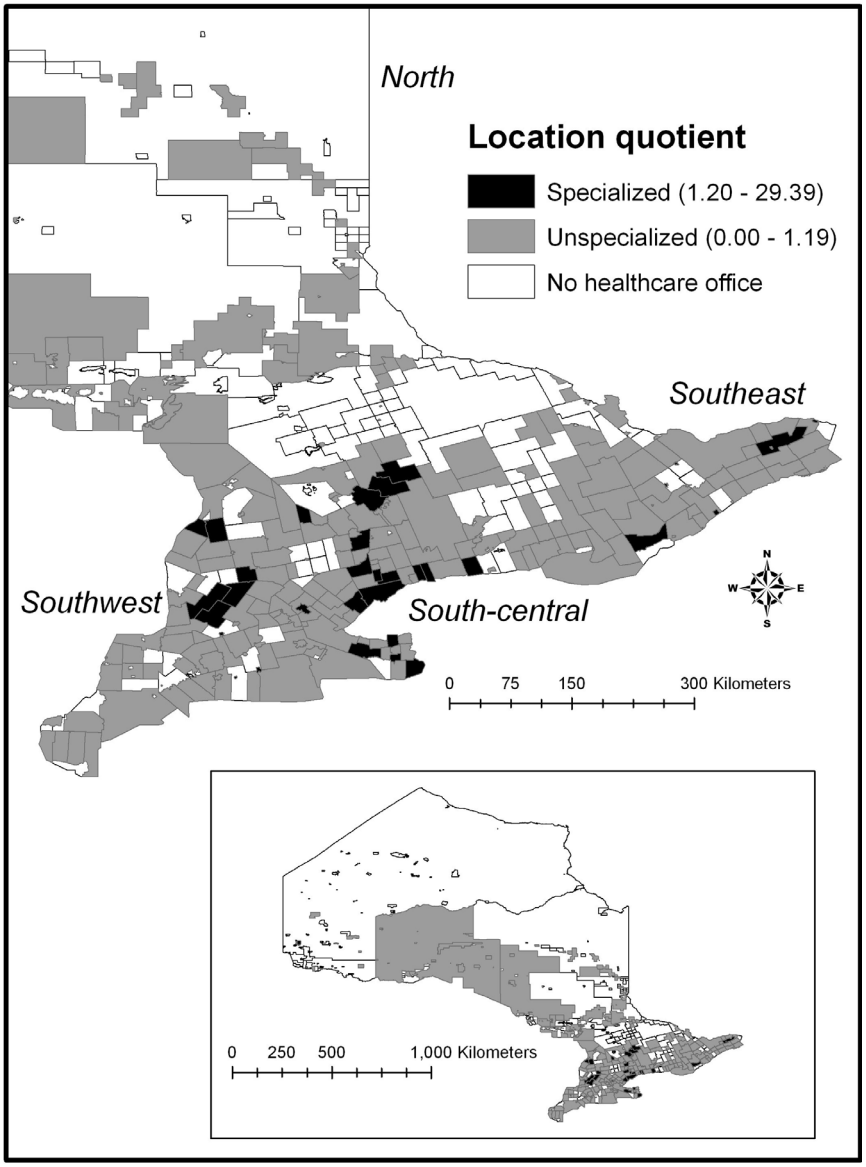

Fig. 3. Primary function acupuncture office by level of census subdivision specialization

In comparison to other health care providers, the specific subset of PFA features smaller operations with average annual sales of about $460,000; which is less than total CAM ($490,000) and much lower than the total health supply ($830,000). This modest size of acupuncture offices remains consistent whether considering the entire PFA sample or the subset of offices in areas of 'acupuncture location quotient specialization'.

Therefore in terms of space, acupuncture office activity shows both commonalities and distinctiveness with the wider complementary and alternative medicine sample. The relatively broad dispersal of CAM in Ontario may be compensating for the lack of CM resources in some less populated and/or underserved areas (Meyer, 2010; Sirois & Purc-Stephenson, 2008) and the occurrences of PFA specializations in the southeast and southwest do occur almost exclusively in more peripheral municipalities. On the other hand, PFA office activity is, both in absolute numbers and relative specialization, more polarized and strongly biased to the southern parts of the province and to the Greater Toronto area in particular. With the small size of acupuncture offices and the almost complete abandonment of northern Ontario not withstanding, the extremely strong attraction of PFAs to Toronto might be the most striking contrast between acupuncture and CAM in terms of location choice.

5. Acupuncture office activity in the Toronto census metropolitan area: clusters, temporal trends and links with ethnic origin

Since over half of Ontario's PFA establishments (249 of 465 in 2007) are located in the greater Toronto area, this metropolis was designated for detailed analysis. Please refer to Figure 4 to locate metropolitan Toronto's municipalities (CSDs). A local spatial autocorrelation analysis (using Moran's I) was performed in the GIS environment to identify local hotspots of office activity. As shown on Figure 5, there are a number of CTs that were statistically significant for positive local SAC, but arguably four agglomerations stand out. Note the cluster of positive local SAC census tracts in the southern extreme (downtown) of the City of Toronto that radiates northward. As well, there are three other more suburban clusters of acupuncture activity: one that extend from the City of Toronto north into Vaughan, another which bridges Toronto with Markham and a smaller agglomeration in Richmond Hill. These clusters could be dubbed Toronto's 'acupuncture hotspot districts'. Notice that many of these clustered zones are 'partitioned' by census tracts of negative local spatial autocorrelation. Local negative SAC is indicative of abrupt change over space and, in this case, contains CTs with very low PFA sales activity just adjacent to some of Toronto's acupuncture hotspots.

Collectively these localized acupuncture clusters (the positive local SAC agglomerations) represent only 80 of Toronto's 1,003 census tract polygons, but contain an impressive 58.6% of the CMA's PFA establishments and almost 60% of sales. These results contrast with CAM and total health care supply (CAM and CM). As also shown on Figure 5, hotspots of total health office activity create a slightly more constricted pattern, compared to the acupuncture office distribution, as these 67 positive local SAC census tracts are located almost exclusively in the City of Toronto. Moreover, these CTs contain roughly 30 percent of the CMA tally in terms of both total number of health care offices and contribution to sales. In contrast, cases of positive local SAC for CAM display a more expansive pattern within the City of Toronto which includes some hotspots in more peripheral communities. Yet, the proportional importance of the CAM establishments in these positive local SAC census tracts echoes the total health care scenario of about 30 percent.

Thus, primary function acupuncture practitioners create a unique and highly polarized geographic pattern of activity in comparison to other health care providers in general. While the tight spatial clustering of acupuncture activity is not unlike that of total health care (CAM and CM), the proportionate size of influence of acupuncture agglomerations is almost double in the hotspots. In addition, the spatial distribution of PFA is very distinct from 'all' types of complementary and alternative medical offices: acupuncture activity is more enclave-like both in geographic distribution and in magnitude of activity (in terms of sales).

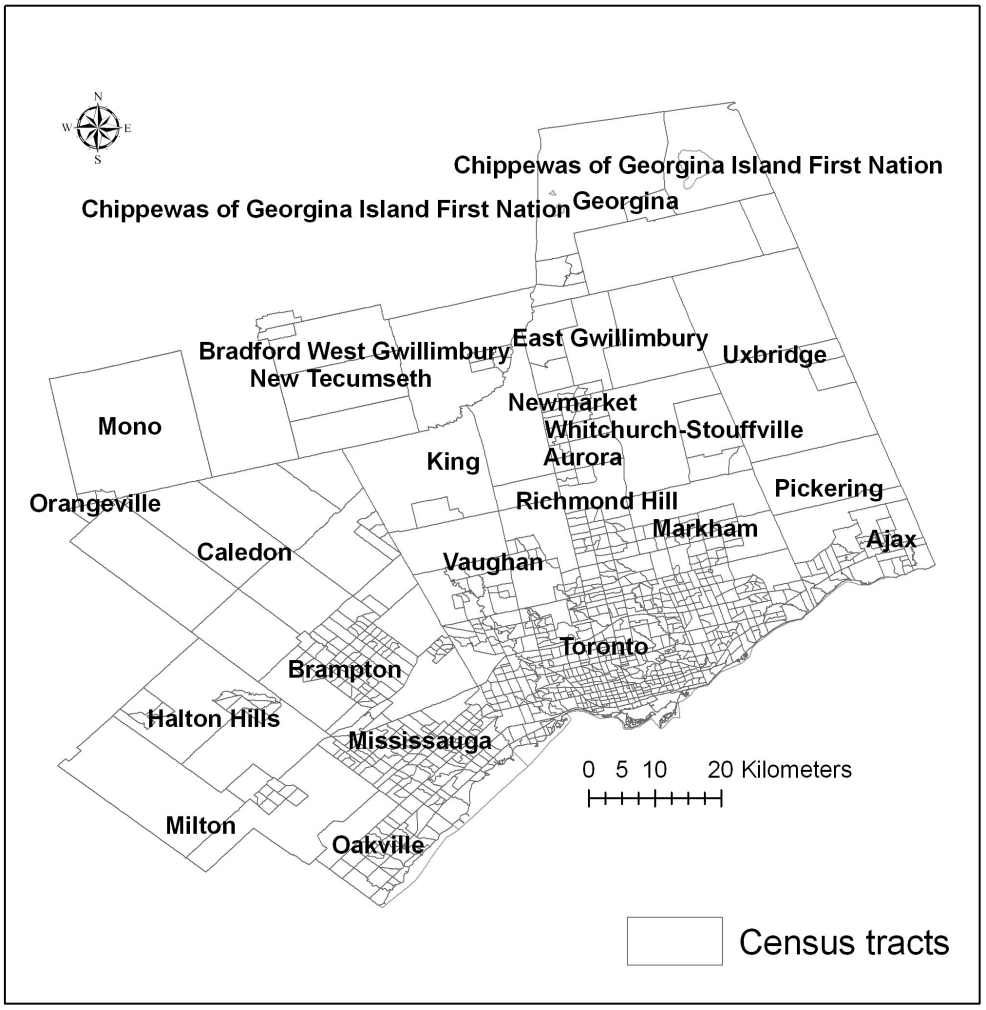

Fig. 4. Municipalities in metropolitan Toronto

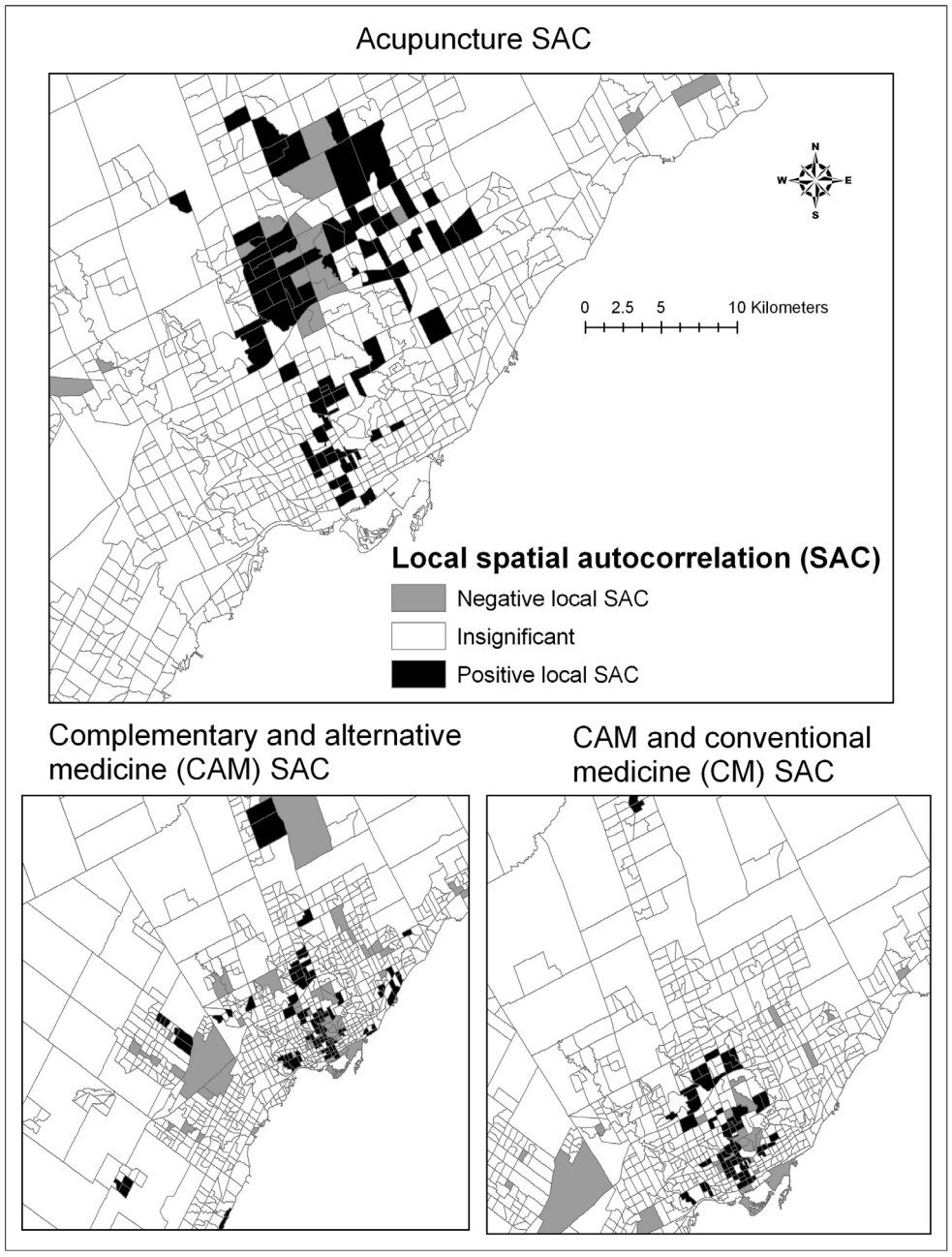

Fig. 5. Statistically significant local spatial autocorrelation census tracts in metropolitan Toronto

The metropolitan Toronto portion of the paper includes a temporal-spatial analysis of primary function acupuncture activity in which the locations of offices were compared from 2004 to 2010. The overall number of offices did not drastically increasing over this time period (204 to 227). As Figure 6 shows, the vast majority of census tracts either: retained the same number of PFA practitioners, gained one office, lost one office or did not have a PFA office in 2004 or 2010. PFA office loss of two or more did occur in a small number of CTs in the City of Toronto (seven) and one in Richmond Hill. Gains of more than two PFA offices also occurred sporadically in the City of Toronto and in Mississauga. By in large, though, the temporal pattern is quite constant. This stability is further illustrated with the mean centers and standard deviation ellipses. One standard deviation will normally contain 68% of the features (PFA offices) and two standard deviations will contain 95%. As Figure 6 shows, there is only a small southwestern shift from 2004 to 2010; that is perhaps indicating modest growth in acupuncture offices in Mississauga. Even with an admittedly short (six year) time frame, it can be said that temporal-spatial shifts are minor and areas in metropolitan Toronto that emphasize PFA tend to be persistent.

Thus, acupuncture activity in the Toronto CMA exhibits clustering tendencies and temporal consistency. This raises another important geographic inquiry: what place-specific criteria are influencing the polarization and persistence of acupuncture establishments? While this is a complex question, there is evidence to suggest a link with PFA and Chinese and Southeast Asian ethnic origins. For census tracts in metropolitan Toronto, location quotients were calculated for both Chinese ethnicity and those of Southeast Asian origins. Yet, given that the Pearson's correlation coefficient of these location quotient values is 0.96 (based on 995 census tracts with ethnic information) and henceforth indicative of almost indistinguishable spatial distributions, only the Chinese ethnic origin results are presented (see Figure 7). Areas of strong relative presence of Chinese populations exist in the City of Toronto's downtown area and in more northern locations of the City of Toronto, Markham and Richmond Hill. These tight clusters correspond with Toronto's 'Chinatown' districts. Visual inspection of Figure 7 with Figure 5 reveals spatial similarities with the acupuncture SAC pattern. Specifically, the acupuncture office clusters indentified by the local spatial autocorrelation analysis appear to correspond closely with neighbourhoods featuring high proportions of Chinese populations.

Table 2 shows the results of more formally assessing this relationship. Chinese ethnic origin location quotients were compared with acupuncture-based positive local SAC and location quotient specialization. Given that the location of acupuncture offices might also be influenced by high Chinese populations in adjacent CTs (and vise versa), buffers were computed around the centroid of local SAC acupuncture CTs and around acupuncture office location CTs with quotients greater than or equal to 1.20. Roughly one-quarter of the 'Chinese' CTs were also classified as acupuncture office hotspots (either by SAC analysis or location quotients). Moreover, over 50% of the 'Chinese' CTs were no further than 500 meters from key acupuncture areas and almost all were within 2 kilometers. This apparently strong correspondence between acupuncture office activity and Chinese ethnicity population is perhaps not surprising. The techniques inherent to acupuncture have historically been associated with traditional Chinese medicine and it would appear that contemporary spatial patterns of PFA offices remain intrinsically linked with Chinese and/or Southeast Asian enclaves in metropolitan Toronto.

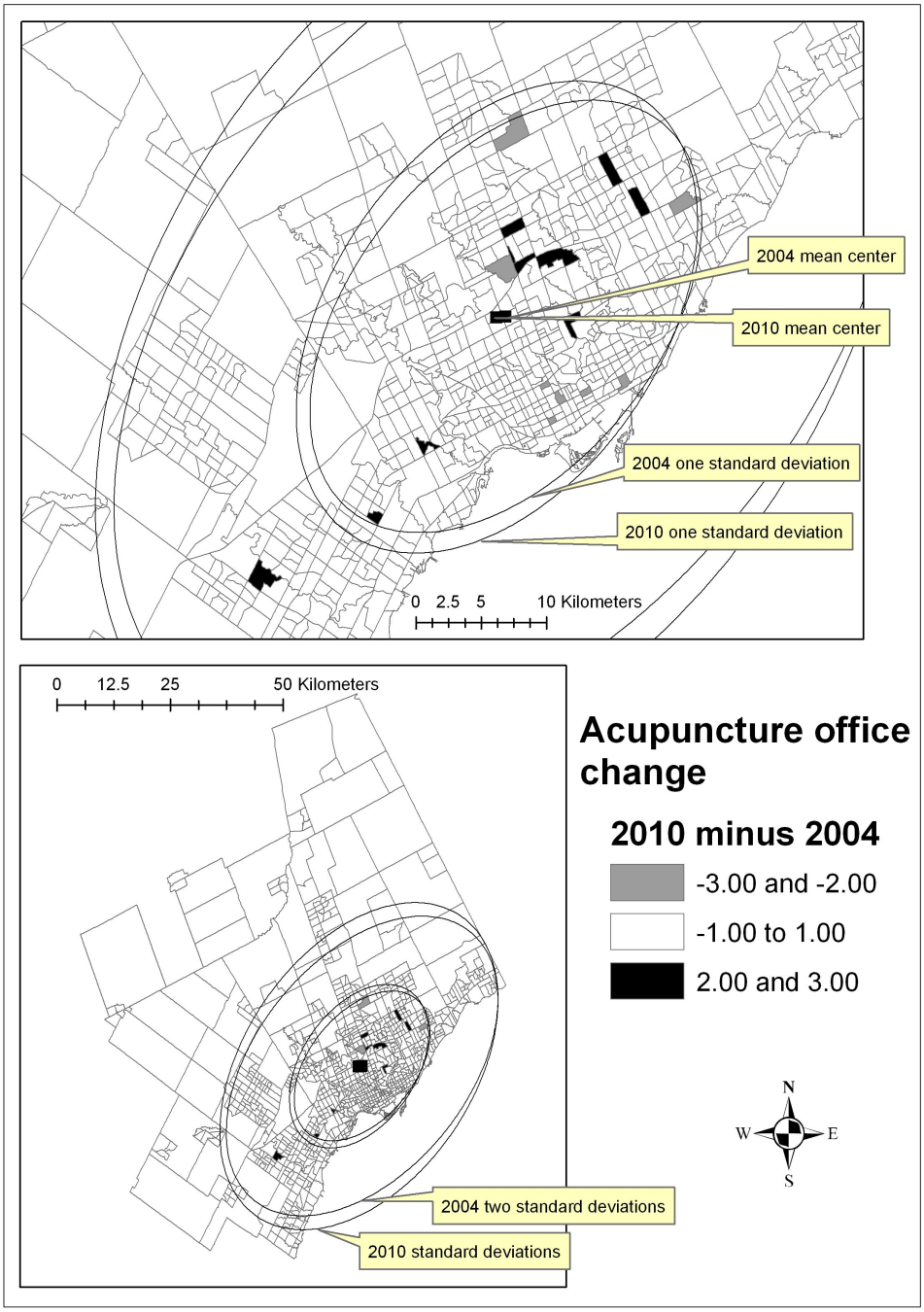

Fig. 6. Temporal changes in primary function acupuncture offices in metropolitan Toronto

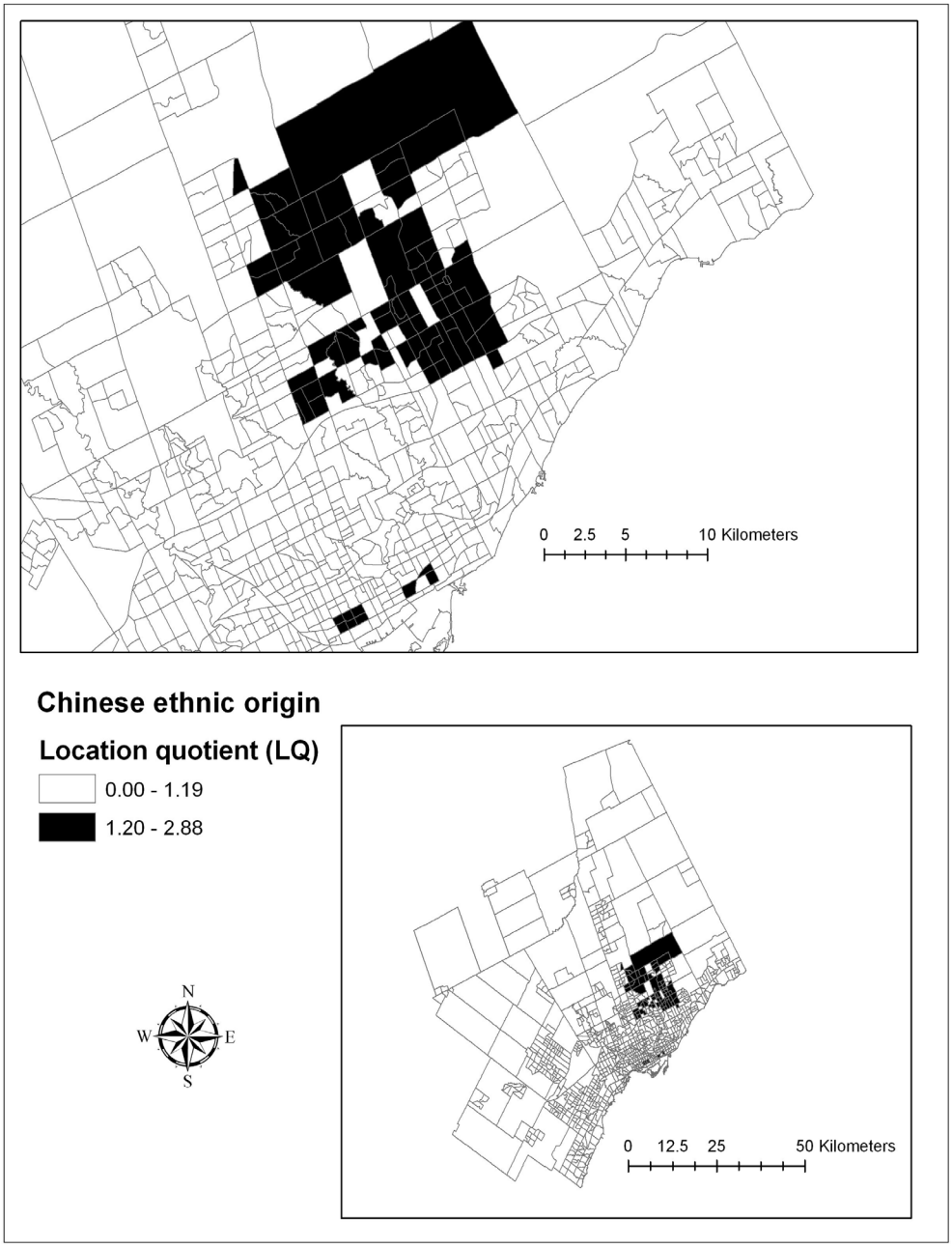

Fig. 7. Chinese ethnicity location quotients in metropolitan Toronto

Chinese ethnicity census tracts:	Number of occurrences
Intersecting significant local spatial autocorrelation CTs (based on acupuncture office sales)	22
Intersecting significant local spatial autocorrelation CTs (based on acupuncture office sales) and within a 0.5 kilometer buffer of a CT centroid	43
Intersecting significant local spatial autocorrelation CTs (based on acupuncture office sales) and within a 1 kilometer buffer of a CT centroid	63
Intersecting significant local spatial autocorrelation CTs (based on acupuncture office sales) and within a 2 kilometer buffer of a CT centroid	78
Intersecting specialized acupuncture CTs with location quotients ≥ 1.20	21
Intersecting specialized acupuncture CTs with location quotients ≥ 1.20 and within a 0.5 kilometer buffer of a CT centroid	45
Intersecting specialized acupuncture CTs with location quotients ≥ 1.20 and within a 1 kilometer buffer of a CT centroid	67
Intersecting specialized acupuncture CTs with location quotients ≥ 1.20 and within a 2 kilometer buffer of a CT centroid	80
Total number of Chinese ethnicity CTs	84

Table 2. Relating 'Chinese ethnicity' (location quotient ≥ 1.20) census tracts to acupuncture local spatial autocorrelation and acupuncture specialization

6. Conclusions

Specialists trained in the discipline of acupuncture not only have the ability to relieve pain and other ailments, but can provide a service that is part of a regimen for maintaining good health. Yet, as with any type of health care service, supply needs to be reasonably proximate to demand. The results from this study illustrate that the spatial distribution of those offering acupuncture treatment is not even and tends to agglomerate in specific portions of the province and indeed within identifiable clusters in metropolitan Toronto. This is certainly true of primary function acupuncture offices. Although given that CAM practitioners in general are more dispersed throughout Ontario and within Toronto, it is likely that some acupuncture services, delivered by chiropractors for example, are fulfilling demand requirements in areas void of PFA offices. Future study could ascertain the extent

of 'secondary function' acupuncture locations and if these establishments are adequately meeting the demand for acupuncture services. Specifically, research could build on the findings of Bishop et al. (2011), who determined that acupuncturists' trustworthiness and competence are important to potential customers, and establish if these and other patient-centered attitudes towards 'primary' and 'secondary' function acupuncture treatment vary over space.

Giordano et al. (2004) call for discourse regarding the mechanisms for which acupuncture therapies, and more widely Chinese traditional medicine and complementary and alternative health care approaches, could become more integrated into a jurisdiction's public health network. They indicate that much of this responsibility lies with the academic community (doctoral, post-doctoral, facility research and the like) to nurture a 'core of specialists' who would offer advice on how Chinese traditional medicine and other CAM methods could be better incorporated with conventional medicine and in the overall delivery of health care. The authors also state that Chinese traditional medicine is by nature substantially different from allopathic medicine and, thus, this will be a challenge. Tang (2006) goes further and wonders if acupuncture specialists actually *should* become more integrated with CM and risk becoming 'biomedicalised' and losing its alternative status. Regardless of where one stands on amalgamative possibilities, it is clear that in order to improve the delivery of health care a fundamental understanding of where the demand and supply for these services are located over space is needed. If only conventional medical locations are considered (hospitals and family doctors for example), then a jurisdiction is grossly undercounting resources. A crucial step in health care policy starts with wider recognition for the need to inventory 'total' health care supply. When the dialog from the academic community or policy makers begins to include *where* CM *and* CAM resources are located over space and if these resources are *where* they need to be, then motivation for students to pursue CAM professions becomes increasingly validated, researchers' interests to analyze spatial trends are sharpened and a jurisdiction's stock of health care supply is more accurately portrayed. As such, integrative thinking should go at least to the point of enumerating the various types of CM and CAM in a given place, even if traditional Chinese medicine and other alternative approaches never reach the point of family doctors routinely referring patients for acupuncture treatment, for example.

Given that the quality and quantity of health care resources, funding and overall patient accessibility to medical supply will differ from country to country, province to province and city to city, research on *each* jurisdiction's inventory of health care supply is desirable; as, admittedly, many aspects will inevitably be case-specific. Yet, this is not to say that universal characteristics are not retrievable. If acupuncture and other CAM stations are clustered in identifiable locations within regions and cities, then research needs to continue to uncover the place-specific criteria driving these distributions. While patient-centered narratives and biographies regarding the *use* for certain types of CAM (or CAM collectively) are being accumulated by the research community, the *nature of places* that attract or repel CAM business owners (and overall supply levels) requires much more consideration. This study spatially links high Chinese ethnicity proportions with acupuncture hotspots in Toronto and this particular outcome could also be true in other parts of the world. Even so, this research only begins to uncovering possible explanations for acupuncture office supply concentrations (in Toronto or elsewhere).

Curtis & Riva (2010) state that many health geographers are concerned with the health-centered properties of certain places; an idea often associated with 'therapeutic landscapes'. They also recognize that therapeutic landscapes, or places of healing, might not be experienced the same by everyone. One might hypothesize that the high proportions of acupuncture offices found in close proximity of Chinese ethnicity enclaves (in Toronto or perhaps elsewhere) might be because the acupuncture 'experience' delivered by the offices in these areas coincide with familiar and favorable places of healing for those of Chinese or Southeast Asian backgrounds. Do people of non-Southeast Asian ancestry view these acupuncture hotspots in the same way, or are they just as likely to seek acupuncture services in other areas and/or through a naturopath, chiropractor or registered massage therapist? Williams (2002) claims that it is important for geographers in particular to explore the experiences of patients/individuals as care from formal spaces (such as hospitals and doctor's offices) are shifted to more informal spaces like the home. As such, are acupuncture spaces in Chinese enclaves more secure and 'homelike' as a place of healing? Do the acupuncture specialists themselves, either deliberately or inherently, create a healing environment that appeals to those of Southeast Asian descent in the enclaves? The possible link between acupuncture, ethnicity and places of healing needs to be more completely analyzed.

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13

Development of Scientific Publications on Acupuncture

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1. Introduction

During the last couple of decades, the use of acupuncture, in order to treat and relieve different kinds of symptoms and disorders, has increased dramatically in Western societies (e.g., Eisenberg et al., 1993; Harris & Rees 2000; Thomas et al. 2001; Vincent & Furnham, 1996). Together with some other integrative and complementary therapies[1], such as osteopathy and chiropractic, acupuncture has evolved from being a rather unusual treatment to a relatively accepted one, and has been integrated in public health sectors. Today, it is often possible to get acupuncture from biomedical professionals, such as doctors and midwives, or visit trained acupuncturists as part of conventional health care, which is covered by general social security systems.

Previous studies indicate a rapid increase in research of integrative and complementary medicine in general (Danell & Danell, 2009; Fu et al., 2011) and on acupuncture (Han & Ho, 2011). There are also indications of a growth in evidence-based research, in the form of clinical trials, in sub-fields such as acupuncture and musculoskeletal manipulations (Danell & Danell, 2009), and increased publications in mainstream biomedical journals (Barnes et al., 1996; Fontantarosa, 2001). There has also been notable institutional and financial support for research on integrative and complementary medicine through, for example, the establishment of the National Center for Complementary and Alternative Medicine (a branch of the US National Institute of Health) and associations such as the International Society for Complementary Medicine Research.

However, the establishment of integrative and complementary medicine in biomedical contexts is far from uncontroversial. In most Western countries there are both symbolic and institutional divisions between biomedicine on one hand, and alternative/complementary/integrative medicine on the other (e.g., Jütte, 2001). One crucial

[1] In this chapter, we will use the concept "integrative and complementary medicine" in order to describe practices that are not currently an integral part of Western biomedicine, since this is the concept used as subject category in SCI Expanded. However, there are several other concepts—such as "complementary and alternative medicine", "holistic medicine", and "non-conventional medicine"—that are used in a similar way (for a more detailed discussion on concepts see, Cant and Sharma (1999), Easthope (2003), and Gorski (1996)). In general, complementary and integrative therapies are used in addition to conventional biomedicine, while alternative counterparts are used instead of conventional treatments.

aspect is that most of these traditions, at least to some extent, rely on ideas and procedures that are either in conflict with or not fully explained in relation to biomedical norms. It is far from self-evident that research on integrative and complementary therapies is accepted in biomedical research teams, published in high standard biomedical academic journals, or funded by public research foundations—even if the therapies have gained a relatively high degree of public acceptance and integration in the public health care system. One of the most crucial strategies from an integrative or complementary perspective is to engage in peer-reviewed research and, ultimately, to perform high quality clinical randomized trials (Adams, 2002).

The purpose of this chapter is to investigate the development of scientific publications, defined as documents in the Web of Science database, on acupuncture during the time period 1990-2010. What is the general publication trend over the time period? What kinds of documents are published? Are documents on acupuncture found in general biomedical journals or in specialized ones (either on integrative or complementary medicine or other biomedical sub-fields)? To what extent, if any, are the publications found in prestigious or high ranked biomedical journals? Where are highly cited documents published? Is there an increase of clinical trials on acupuncture? Where is the research conducted with regard to countries or larger regions?

2. Data and method

The data for this chapter is retrieved from SCI Expanded in the Web of Science database. Some parts of the analysis, as will be outlined below, is complemented by information from the PubMed database and Journals Citations Report.

To identify documents on acupuncture, we use the keywords acupunct*, acupoint*, electroacupunct*, and electro-acupunct* to search titles, abstracts, and keywords in SCI Expanded. The time period is restricted to the years 1990 to 2010, and the material is analyzed with the help of bibliometric methods.

3. Results

3.1 General publication trend and types of documents

During the time period 1990-2010, 5870 documents were published on acupuncture, according to the SCI Expanded. Overall, the publication trend can be divided into two periods (Figure 1). The first period covers the 1990s, where there was a yearly growth between 83 and 201 documents. The second period starts in the early 2000s, and is characterized by a more rapid increase of published items. During the last two years, 2009 and 2010, more than 600 documents on acupuncture were published each year.

For the whole period, the great majority of the documents (80 percent) are classified as Articles. About ten percent are classified as Reviews, eight percent as Letters, and less than one percent as Notes. In absolute numbers, articles and reviews have increased the most during the period (Figure 2). There are no notes at all after 1996.

However, if looking at the share of published items, the pattern is more stable (Figure 3). The share of articles varies between 77 and 85 percent, and there is no clear trend over time. In the case of reviews it is possible to identify an increase in the share of published documents, with a development from two percent at the beginning of the time period to 15 percent at the end. This increase, both in absolute numbers and in the share of all

publications on acupuncture, can very well be a sign of the growing interest in research on acupuncture and the scientific need of overviews on particular aspects of the research—for example on the use of acupuncture in the treatment of specific disorders. It may also be a sign that acupuncture is established as a scientific sub-field.

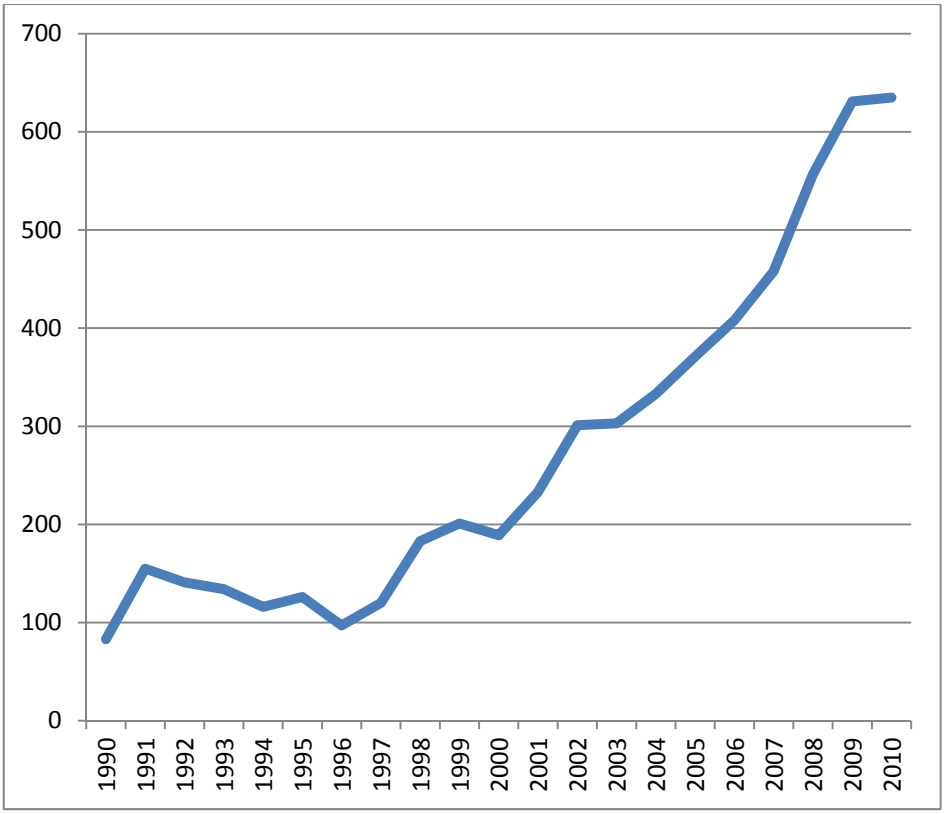

Fig. 1. Number of documents on acupuncture. 1990-2010.

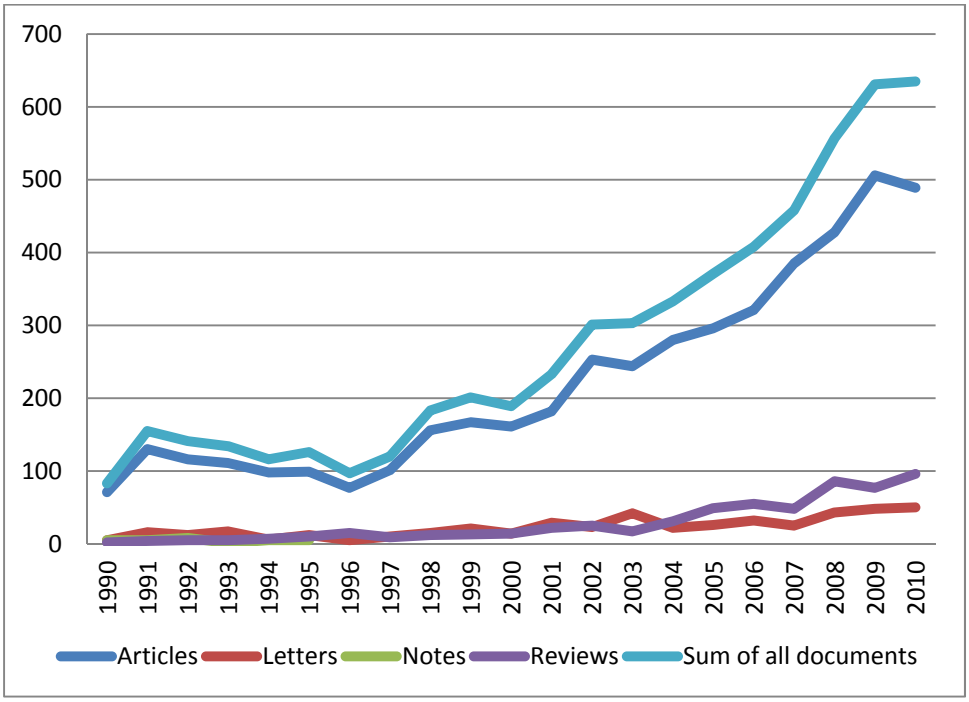

Fig. 2. Number of different types of documents on acupuncture. 1990-2010.

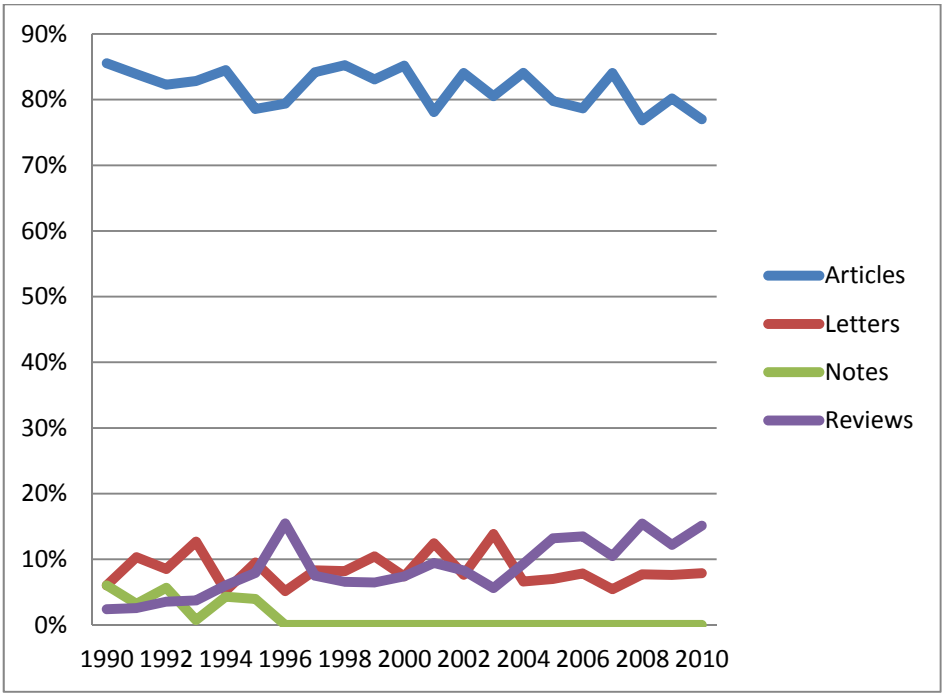

Fig. 3. Share of different types of documents on acupuncture. 1990-2010

3.2 Academic journals

An interesting aspect of the publication pattern is in what kind of journals the documents are published. Are documents on acupuncture found in general biomedical journals or in specialized ones (either on integrative or complementary medicine, or other biomedical sub-fields)? To what extent, if any, are these publications found in prestigious or high ranked biomedical journals? Where are highly cited studies published?

If we just take a look at the subject categories[2] of the journals for the whole time period, we can see that the most frequent category is Integrative and Complementary Medicine, which includes 24 percent of the documents on acupuncture. In this broad category, we find general journals (such as the *Journal of Alternative and Complementary Medicine* and *Complementary Therapies in Medicine*), those specialized on acupuncture (such as *Acupuncture in Medicine* and *Acupuncture & Electro-therapeutics Research*), and other sub-fields (such as homeopathy). Other frequent subject categories among the journals are Clinical Neurology (nine percent), Medicine General & Internal (nine percent), Neurosciences (seven percent), and Anesthesiology (seven percent).

To refine the results, and get a clearer picture of the development over time, we have re-classified the journals according to three broad categories: Integrative and Complementary Medicine[3], Biomedicine[4] and Other[5].

[2] According to SCI Expanded.

[3] Defined as journals of which one subject category is Integrative and Complementary Medicine.

From this re-classification (Figure 4), we can see that a majority of the documents for the whole time period are published in journals classified as Biomedicine. We can also see that the publication trend within all three categories is rather similar, especially concerning a rapid growth in the 2000s.

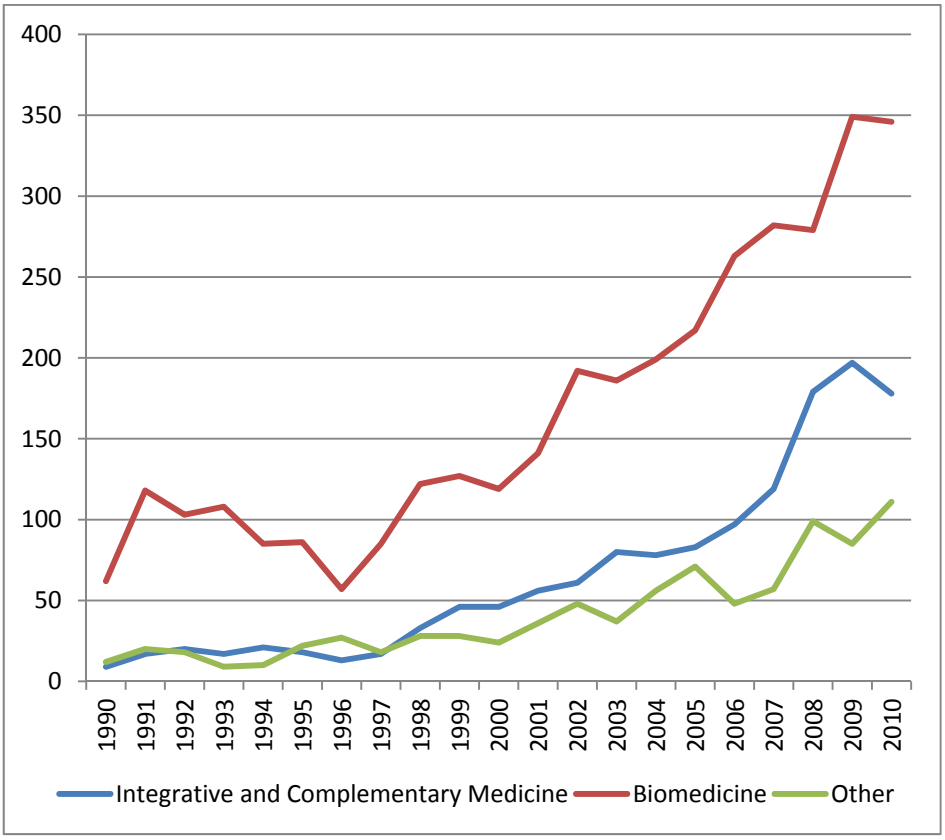

Fig. 4. Number of documents published in the journal categories Integrative and Complementary Medicine, Biomedicine and Other. 1990-2010.

[4] Defined as all other medical subject categories (for example, surgery, oncology, nursing, allergy, rehabilitation).

[5] Defined as all other subject categories (for example, material science and textile, sport science, statistics).

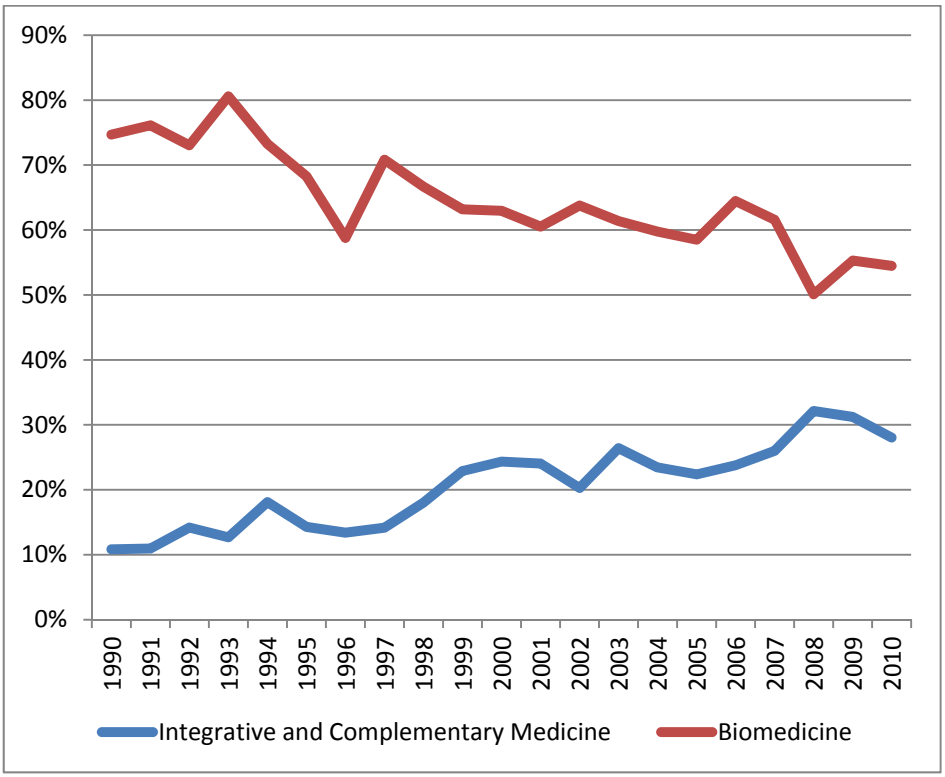

Fig. 5. Share of documents on acupuncture published in the journal categories Integrative and Complementary Medicine and Biomedicine. 1990-2010.

However, if we look at the shares of the categories in relation to all of the documents on acupuncture, we can identify another pattern (Figure 5). Publications in the category Integrative and Complementary Medicine have increased from about ten percent in 1990 to almost 30 percent in 2010. In the category Biomedicine the development shows almost the opposite. In 1990, about 75 percent of the documents were published within this broad category. In 2010, the share decreased to about 55 percent. The relatively low numbers, and small share, of documents published in the category Integrative and Complementary Medicine at the beginning of the time period can probably be explained, as indicated above, by the fact that the category has expanded during the last couple of decades, both by the establishment of new journals and by the inclusion of already existing journals in the database (cf Danell & Danell, 2009).

To measure to what extent the publications on acupuncture are published in prestigious biomedical journals, we have combined two measures. First, we have matched the documents with Abridged Index Medicus/Core Clinical Journals[6] in PubMed, which is an index that includes both general biomedical journals and specialized ones (in areas such as anaesthesia, cancer, gynecology, neurology, and surgery). Second, we have matched the documents with the top 20 journals, according to the general impact factor, in the category Medicine General & Internal in the Journal Citations Report. To some extent these two measures overlap, but in general the last one is more narrowly defined.
Of the 5870 documents about ten percent are published in Core Clinical Journals and about 4 percent in the top 20 category (Table 1). We can also see that these classifications overlap to some extent. Of the 588 documents published in Core Clinical Journals 137 are also found in the top 20 category. These are items published in the *American Journal of Medicine*, *Annals of Internal Medicine*, *Archives of Internal Medicine*, *British Medical Journal*, *Lancet*, and *MAYO Clinic Proceeding*.

	Other journals	Top 20	Sum of documents
Core Clinical Journals	451	137	588
Other journals	5160	122	5282
Sum of documents	**5611**	**259**	**5870**

Table 1. Classification of documents due to the categories Core Clinical Journals, Top 20 journals and Other journals.

If one looks at the absolute numbers of publications within the categories Core Clinical Journals and the top 20 journals, there is a clear increase over the time period (Figure 6). In 1990, it was only 4 and 2 published items in each category, respectively. At the end of the period, there were 32 and 17 items, respectively. The peak was in 2007, when 50 documents were published in Core Clinical Journals.
However, if we compare the publications within these to two categories with the general publication trend, another pattern becomes visible. In Figure 7, we show the share of published items in Core Clinical Journals and top 20 journals in relation to all of the documents on acupuncture for each year. Even if one should not overestimate variations between specific years, because of the low numbers, there is a peak in publications in both categories in the middle of the time period. It is also possible to identify a decrease in both categories during the last couple of years.
Of the 20 most cited documents eight are found in Core Clinical Journals and six in the top 20 category (four of the documents are found in both categories). Only one of these documents (Thomas et al., 2001) is published in a journal that focuses on integrative and alternative medicine (*Complementary Therapies in Medicine*). The rest are found in general journals (such as *Proceedings of the National Academy of Sciences of the United States of America*) or in specialized ones (such as *Pain*, *Journal of Clinical Oncology*, and *European Journal of Pain*). An interesting aspect of the highly cited documents on acupuncture is that only two of them are classified as clinical trials. Most of them include different kinds of reviews, recommendations or surveys.

[6] The latest version is from August 2010 and includes 119 journals.

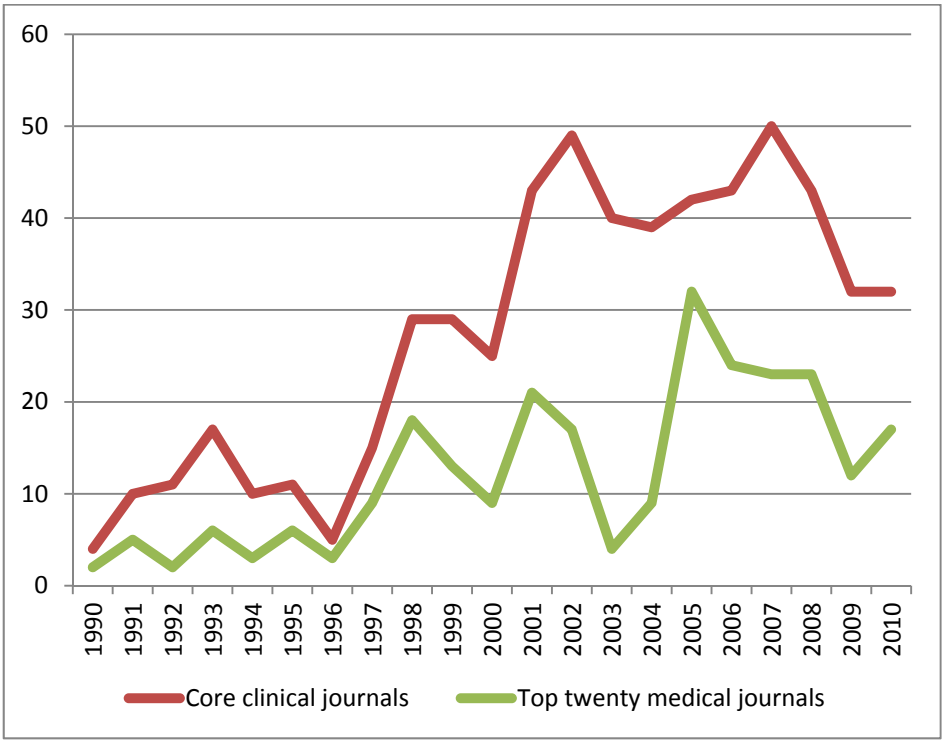

Fig. 6. Number of documents published in Core Clinical Journals and Top 20 journals. 1990-2010.

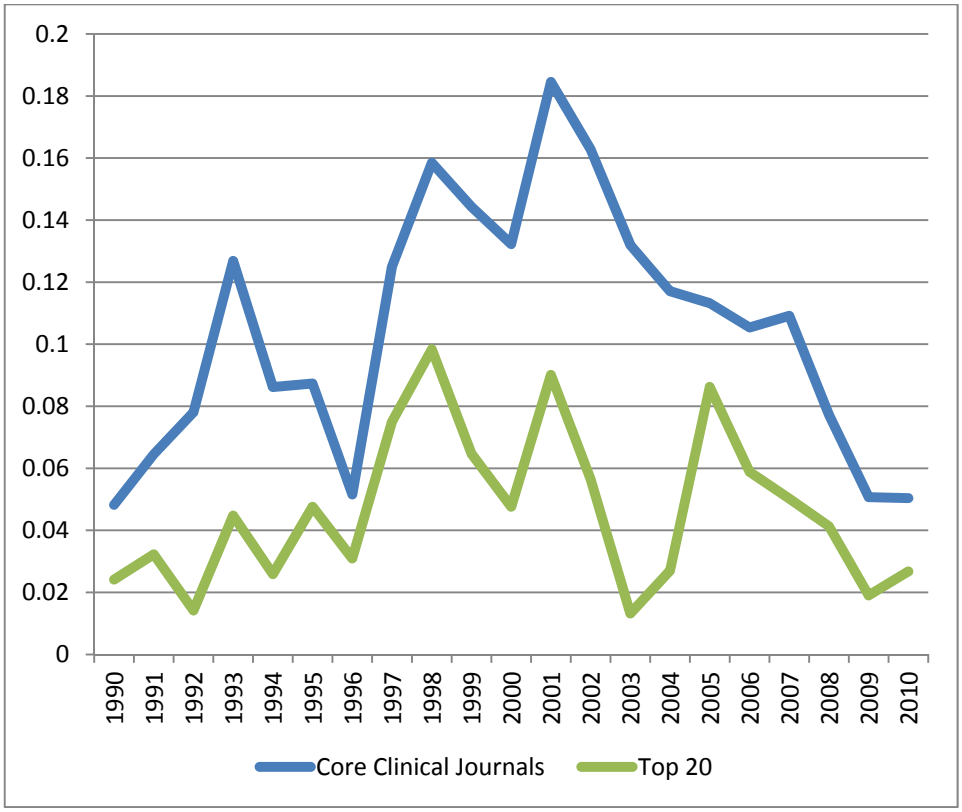

Fig. 7. Share of documents published in Core Clinical Journals and Top 20 journals in relation to all documents on acupuncture. 1990-2010.

3.3 Clinical trials

As mentioned in the introduction, one of the most crucial strategies to achieve legitimacy in biomedical contexts is to engage in evidence-based research, especially in the form of high quality clinical trials. Since there is no information on whether a document includes a clinical trial or not in SCI Expanded, we have matched the documents with the PubMed database according to full title. From this search procedure we found about 800 documents classified as clinical trials. Since this kind of classification is seldom perfect, we have also searched the titles of the 5870 documents for the expression "clinical trial". From this procedure we added 20 more documents, which can be classified as some kind of clinical trial. In total, we found 814 clinical trials on acupuncture during the time period 1990-2010.

If we start with the absolute numbers, we can see that there were very few clinical trials published on acupuncture in the early 1990s (Figure 8a). In 1990, there were only four, and at the end of the decade there were 26. At the end of the 1990s, there was a small but steady increase, a development that has been accentuated in the last 10 years. The years 2009 and 2010 specifically stand out with 117 and 116 clinical trials on acupuncture, respectively. Most of these studies are not classified according to phases. Only four are classified

according to phase I (Singh et al., 2008; Mazetto et al., 2007; Kim et al., 2004; Zeltzer et al., 2002), and another four according to phase II (Beer et al., 2010; Lansdown et al., 2009; Vickers et al., 2004; Wong et al., 2003).

A relevant question is if this growth is larger than the general increase of published items on acupuncture? If we compare the share of documents classified as clinical trials with all other documents, we can identify a clear increase in the first category (Figure 8b). In the early 1990s, between two and five percent of the yearly publications on acupuncture were classified as clinical trials. At the end of the period the share is 18-19 percent.

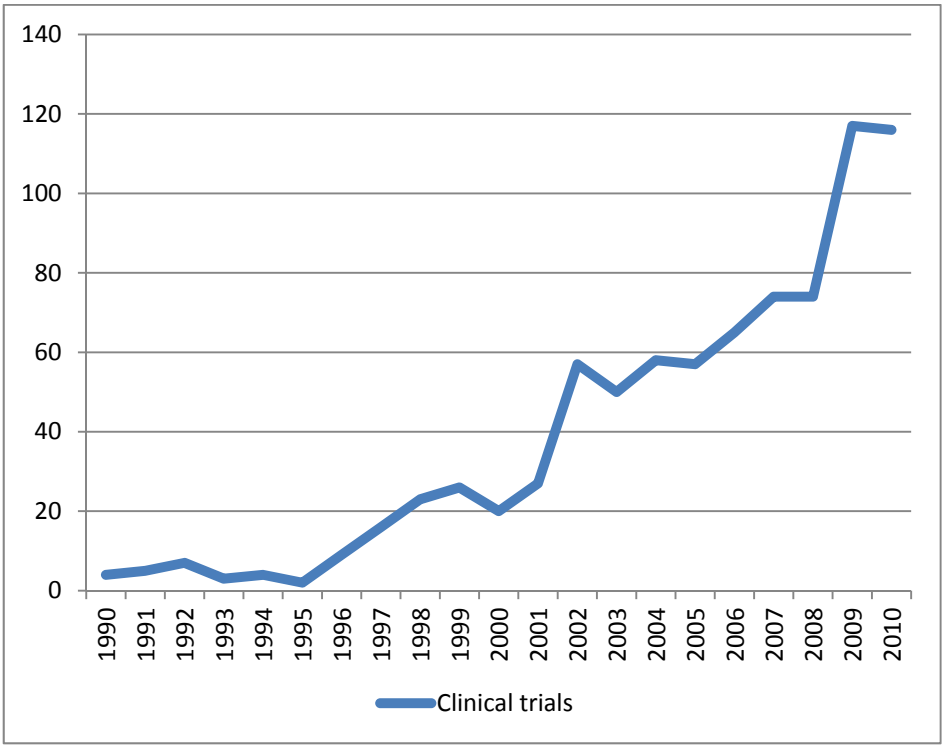

Fig. 8a. Number of clinical trials on acupuncture. 1990-2010.

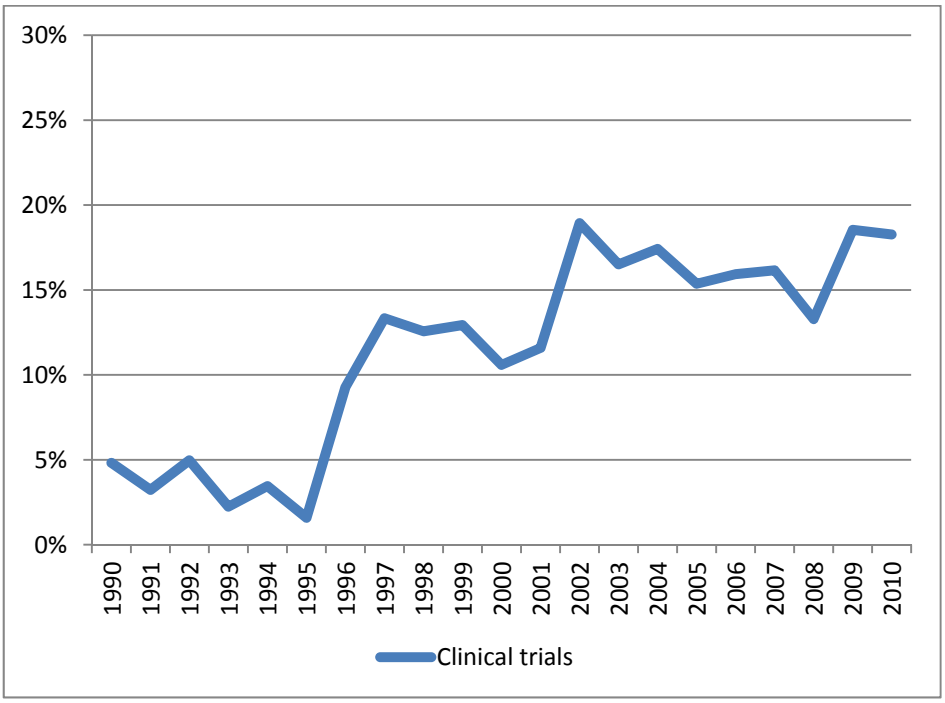

Fig. 8b. Share of clinical trials in relation to all documents on acupuncture. 1990-2010.

3.4 Countries and regions

In order to grasp where the research is conducted, we have fractionalized the addresses of the authors of the documents. This means that if there are two authors on one article, one from the US and one from Japan, each country will get 0.5 in value for the publication.

If we start with an overview for the whole time period, the greatest share of publication is from the US (25 percent), followed by the People's Republic of China (14 percent), the United Kingdom (nine percent), Germany (seven percent), and South Korea (seven percent). The US is also dominating in the publication of clinical trials on acupuncture with 167 for the whole time period, which can be compared with 97 from China, 53 from the United Kingdom, 69 from Germany, and 53 from South Korea. However, if we add the development over time, another pattern also becomes visible (Figure 9). The increase of publications set off earlier in the US, in the late 1990s, than in China and South Korea. During the last two years, there has also been a shift in that China now has more publications on acupuncture each year than the US.

To obtain a complementary view of the development over the time period, we have re-classified all countries included in the material according to four larger regions: Asia, the European Union, North America, and Other countries (Figure 10). From this it is clear that the increase in the publication activity in Asia has been rapid, especially during the last couple of years. Furthermore, this region is now dominating. From this categorization, the development in North America and in the European Union is more similar than what is obvious when looking at individual countries.

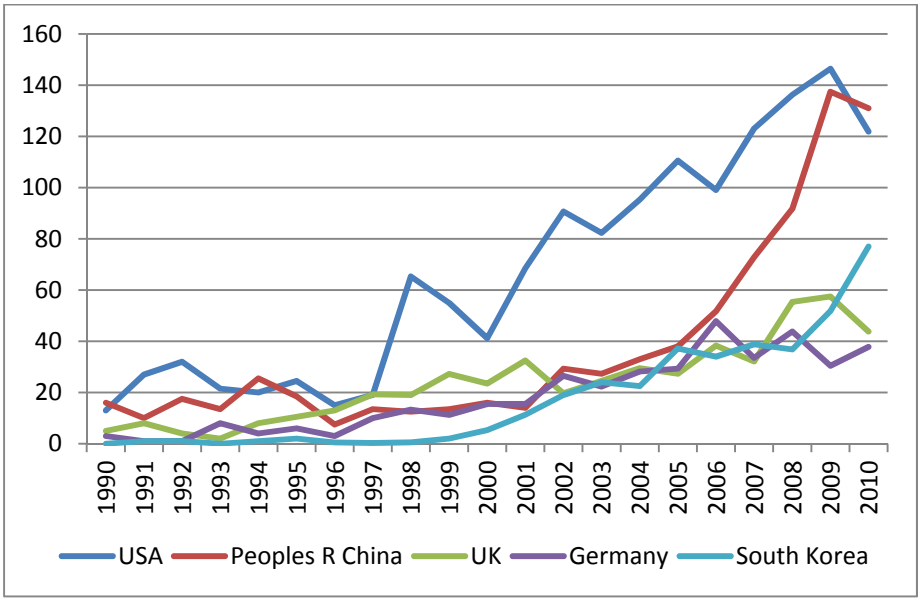

Fig. 9. Number of publications on acupuncture from the US, China, the United Kingdom, Germany, and South Korea. 1990-2010.

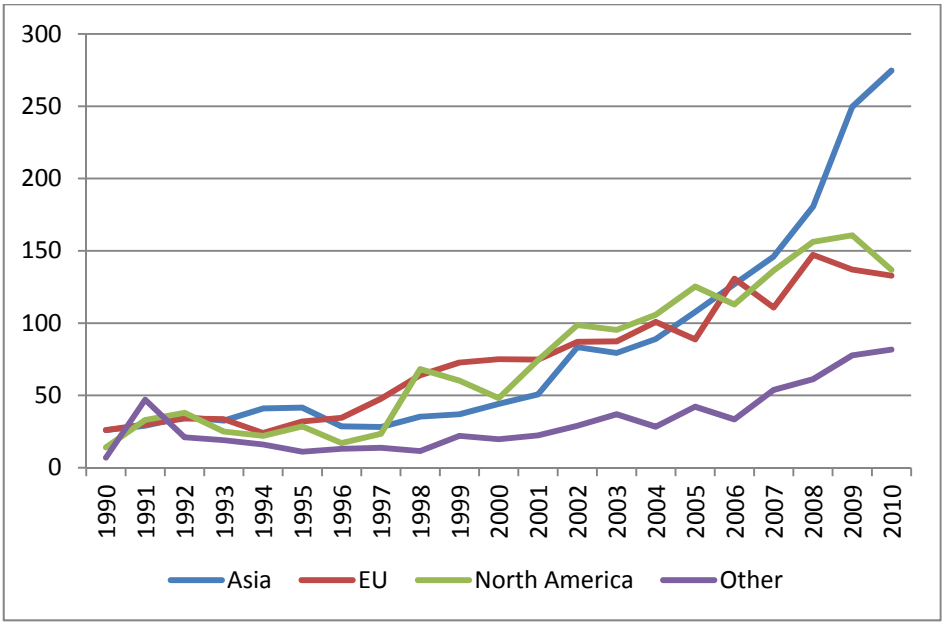

Fig. 10. Number of publications in Asia, the European Union, North America, and Other countries. 1990-2010.

4. Conclusion

To conclude, we can identify a dramatic increase in published documents on acupuncture, especially during the 2000s. The majority of the documents are classified as Articles, even if Reviews have increased relatively more over time. This development can very well be a consequence of the fact that research on acupuncture is becoming more established as a medical sub-field, and is in a scientific need of overviews and reviews (for example, on the use of acupuncture in treatment on specific disorders). A majority of the documents on acupuncture are found in journals that can be classified as biomedical (general or specialized). However, over time it has become more common with publications in journals classified as Integrative and complementary medicine. As mentioned above, it is reasonable to relate this development to the fact that the category of Integrative and complementary journals has expanded during the couple of last decades. About 10 percent of the documents are published in what is defined as prestigious and high ranked journals with regard to Abridged Index Medicus/Core Clinical Journals and top 20, according to the general impact factor, in the category Medicine General & Internal in Journal Citations Report.

An interesting part of the results is that there is a peak of publications in prestigious and high ranked journals in the middle of the time period, and a decrease in the total share at the end. Many of the most cited documents are also found in the categories of prestigious and highly ranked journals. When it comes to clinical trials on acupuncture it is also possible to identify a clear increase. The share of clinical trials in relation to all publications on acupuncture has evolved from two to five percent at the beginning of the period to 18-19 percent at the end. Most of the publications on acupuncture are from the US and China with

25 and 14 percent for the whole time period, respectively. An interesting result is that there has been a shift between those two countries in terms of general publication activity, and that most publications during the last two years are from China. There is also a notable increase in the publication activity in South Korea. This pattern is also supported by the analysis of the regions. During the last couple of years the growth has been most notable in Asia.

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