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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 (NO_x-) 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 CO₂ instrument to measure the transcutaneous CO₂ emission at 13 points along the pericardium meridian line, found that the distribution of transcutaneous CO₂ 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).

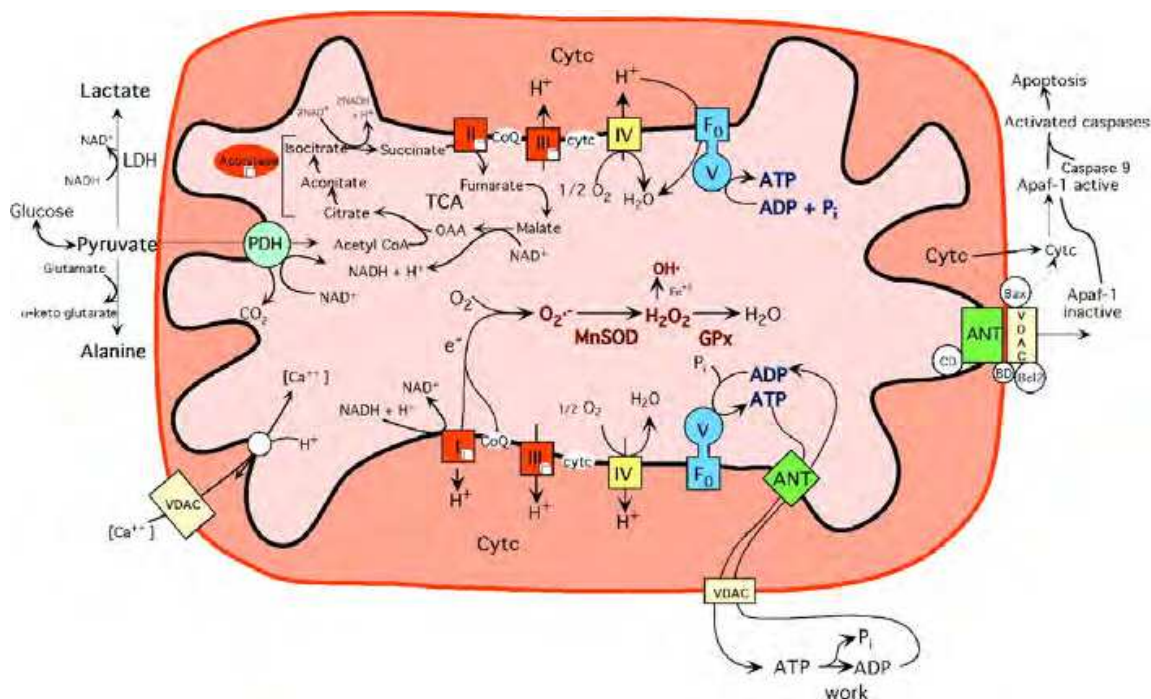


Fig. 1. Superoxide production in mitochondrial, cited from (Raha & Robinson, 2000)

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 CoQH₂/CoQ ratios and the local O₂ concentration.

Superoxide in mammalian 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 μ g 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 .

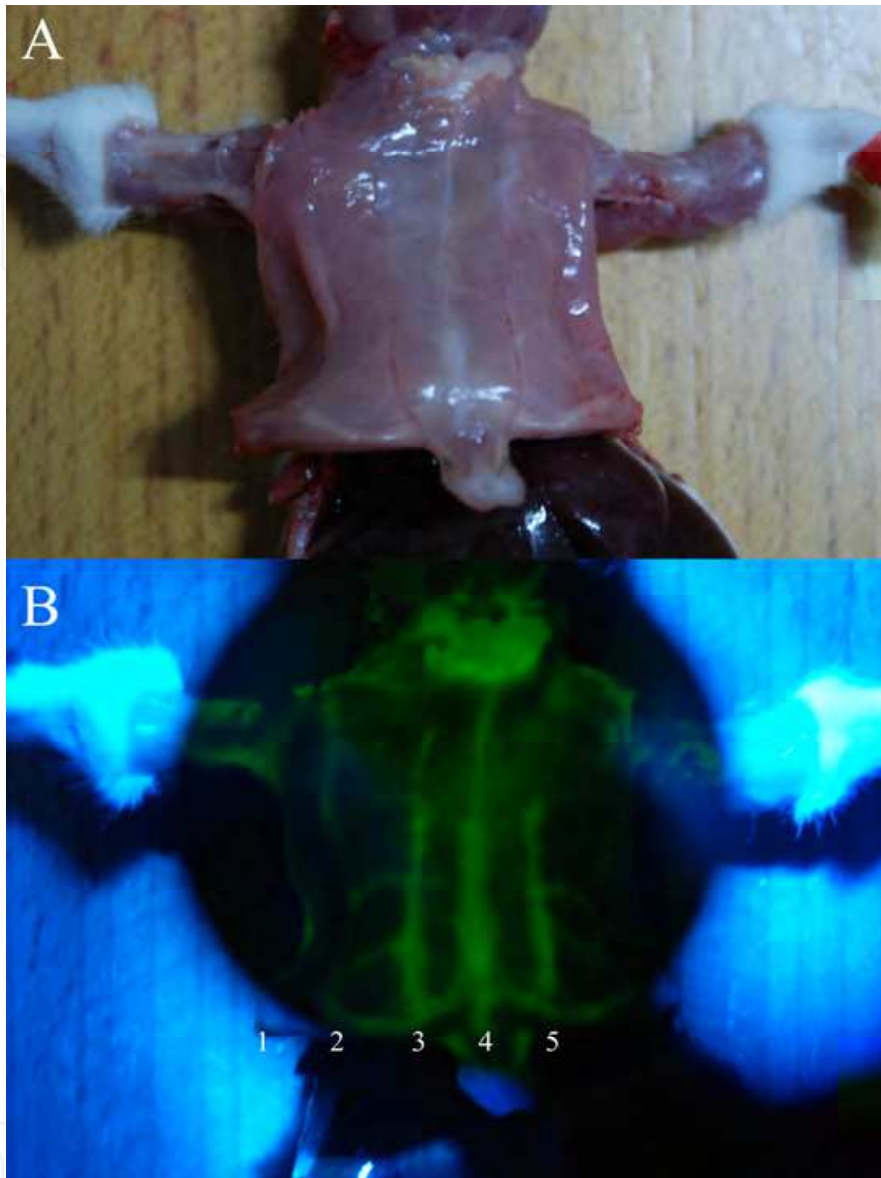


Fig. 3. A) Image of the frontal abdominal interior wall of a 6 weeks old male SD rat whose abdominal wall was incised 30 minutes after the injection of 1 ml of DCFH-DA solution (10 mg in 1 ml dimethyl sulfoxide) through the tail vein. B) Fluorescence image of the frontal abdominal interior wall of the 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.

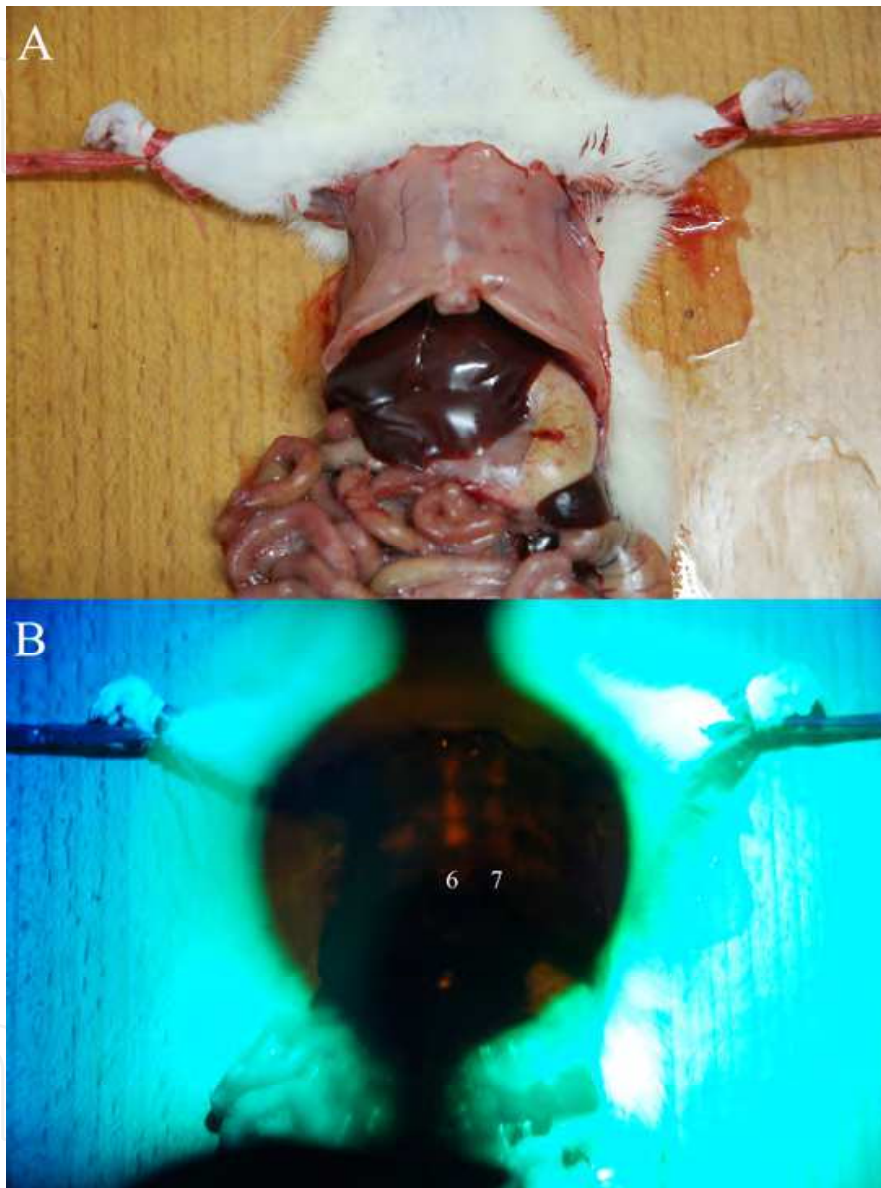


Fig. 4. A) Image of the frontal abdominal interior wall of a 6 weeks old male SD rat whose abdominal wall was applied with MitoSOX™ Red reagent (100 μ g in 1 ml dimethyl sulfoxide) by direct smearing with living rats without any other prior treatments. B) Fluorescence image of the frontal abdominal interior 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)

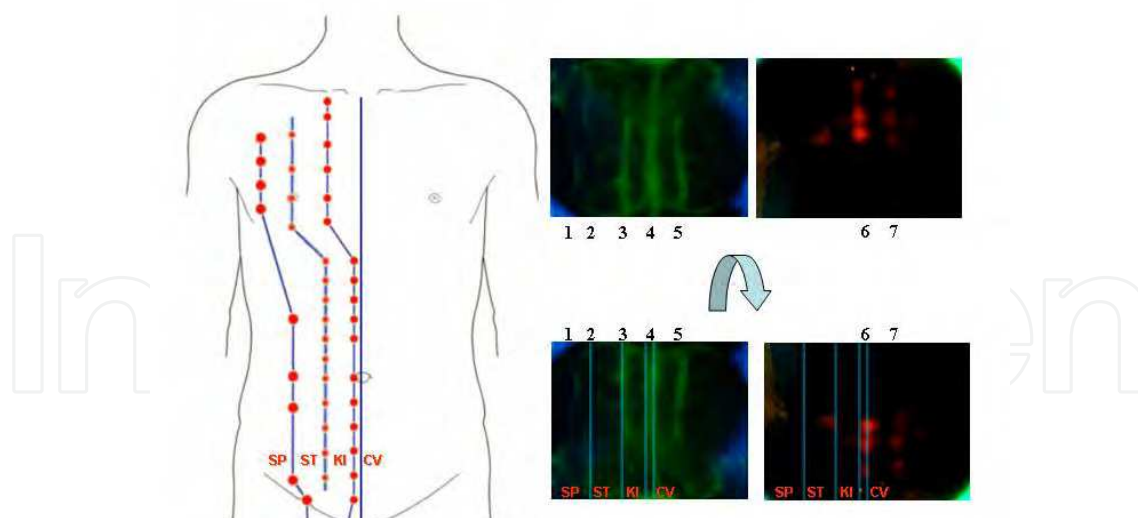


Fig. 5. Illustrations for location of human acupuncture meridian and the superimposition with ROS distribution patterns in Fig. 3B and Fig. 4B. SP, spleen meridian; ST, stomach meridian; KI, kidney meridian; CV, conception vessel, cited from (Guo, et al., 2000)

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/m²) or obese (BMI >30 kg/m²) 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, DH5 α) 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 mm² 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 height², kg/m²) 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	<i>P</i> 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 drugs (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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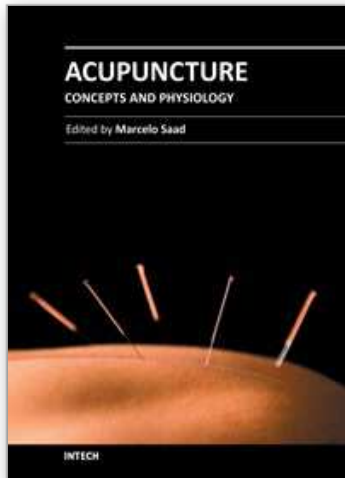
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Acupuncture and related techniques are useful tools for treating a spectrum of diseases. However, there are still many areas of controversy surrounding it. We hope this book can contribute to guide the advance of this ancient medical art. In the present 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 but the main objective is to share elements to make acupuncture more and better understood at health systems worldwide.

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