

Original Article

The Preliminary Study on the Changes of Mammary Tissue Oxygenation During *Zusanli*(ST₃₆) Acupuncture Monitored by Diffuse Optical Imaging

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국문초록

Diffuse Optical Imaging으로 측정된 족삼리(ST₃₆) 자침이 유방조직 산소공급 변화에 대한 기초 연구

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목적 : 족삼리(ST₃₆) 자침이 유방 조직에 미치는 혈액학적 변화를 측정하기 위하여 diffuse optical imaging 기법을 사용하였다.

방법 : 실험에 사용한 쥐는 자침을 하지 않은 대조군 7마리, 자침을 한 실험군 7마리로 총 2개의 군으로 나누었다. 몸무게 170g 정도의 건강한 암컷 쥐는 100% 산소와 1.5% isoflurane을 혼합한 것을 이용하여 마취 시켰다. 자침은 경골 조면에서 2mm 외측에 위치한 지점인 족삼리(ST₃₆)에 20분간 시행하였다. 자침을 시작할 때와 자침 후 10분에 각각 침을 5회 회전하였다. Diffuse optical imaging system을 이용하여 자침하는 동안의 산화혈색소(OHb), 탈산소혈색소(RHb), 총 혈색소(THb)와 조직 산소 포화도(StO₂)의 변화를 측정했다.

결과 : 실험 결과 족삼리(ST₃₆) 자침을 시행한 실험군에서 대조군에 비하여 유의성이 있는 OHb, RHb, THb의 증가와 유의성이 없는 StO₂의 감소가 나타났다.

결론 : 이러한 결과 족삼리(ST₃₆) 자침이 혈류를 증가시키고 동시에 세포 대사 활동을 증가 시키는 것을 알 수 있었다. 또한 diffuse optical imaging 기법으로 족삼리(ST₃₆) 자침 시의 유방 조직의 산소공급과 혈류량의 변화를 확인할 수 있었고, 이는 비침습적으로 자침의 효과를 측정하는 데 활용될 수 있을 것으로 생각된다.

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핵심 단어 : Acupuncture, ST₃₆ point, mammary tissues, diffuse optical imaging, tissue oxygenation

I. Introduction

Acupuncture has been used thousands of years in East Asia to treat a variety of diseases and symptoms. It also becomes accepted as a complementary and alternative medicine in Western medicine recently. The theory behind acupuncture treatment depends on oriental philosophy rather than biological or physiological facts. Therefore, there have been many studies to understand the mechanism of acupuncture in scientific ways. For instances, Langvin et al. proposed that acupuncture works by transmitting mechanical signals to connective tissue cells^{1,2)}. As a way to understand the mechanism of acupuncture, non invasive optical imaging systems such as a laser doppler flow meter or a near infrared spectroscopy have been popularly utilized to monitor physiological changes such as blood flow and tissue oxygenation associated with acupuncture treatment³⁻⁵⁾.

Diffuse optical imaging(DOI) or spectroscopy(DOS), another name of near infrared spectroscopy, has been applied to study various sites of the body such as brain^{6,7)} and muscle^{8,9)} by providing information of oxyhemoglobin(OHb), deoxyhemoglobin(RHb), total hemoglobin(THb), and tissue oxygen saturation(StO₂). It also has been very active using DOI or DOS in breast cancer studies to detect tumors or to monitor the therapy effects¹⁰⁻¹³⁾ since tumors have typically more blood volume and also they are either more oxygenated or poorly oxygenated than normal tissues depending on the state of tumors which can be measured by DOI/DOS non invasively.

Breasts and surrounding areas are full of meridians and points, including the stomach meridian.

According to the visceral manifestation and meridian theory, mammary tissue is associated with stomach meridian. *Zusanli*(ST₃₆) is the sea point where the meridian qi goes deep into the body of the stomach meridian. This point has a tonifying function, and it is an important point for health maintenance and disorder of abdomen. Recent studies have shown that electro-acupuncture stimulation at ST₃₆ may regulate nerve-endocrine-immune network by influencing the production and expression of neurotransmitters, hormones and cytokines¹⁴⁻¹⁸⁾. Combined with other acupuncture points, ST₃₆ acupuncture showed the improvement of fatigue after chemotherapy¹⁹⁾ and the reduction of gastrointestinal reaction during the platinum involved chemotherapy²⁰⁾.

The oxygenation in tumors has a great role in cancer treatments. It has been known that hypoxic tumor cells are nearly 3 times more resistant to radiation therapy²¹⁾ and some types of chemotherapy agents^{22,23)}. The efficacy of photodynamic therapy is also known to be dependent of tumor oxygenation level^{24,25)}. As a result, many methods have been tried to improve tumor oxygenation such as hyperoxic gas inhalation alone²⁶⁾ or with nicotinamide²⁷⁾, hyperbaric oxygen chamber^{28,29)}, administration of recombinant human erythropoietin which elevates hemoglobin levels and hematocrit^{30,31)}.

Therefore, it will be beneficial to improve the efficacy of cancer therapy if acupuncture can modulate oxygenation and blood flow in tumors. To find out its possibility, we have tried ST₃₆ acupuncture on healthy female rats and monitored the changes of blood volume and oxygenation in mammary tissues using a non invasive diffuse optical imaging system.

II. Materials and Methods

1. Animals

Adult female Fisher 344 rats (~170g) were used for this study. They were divided into two groups which are a control group(n=7) did not have acupuncture application and a test group(n=7) received acupuncture on *Zusanli*(ST₃₆) for 20min. One day prior to acupuncture stimulation, low abdominal area including two pairs of caudal mammae were shaved using an electric hair clipper and the left hairs were removed by applying a depilatory cream(Nair lotion, Church & Dwight CO, Inc. Princeton, NJ).

Anesthesia of animals was first induced by applying 5% isoflurane with 100% oxygen in an

Table 1. Changes of Chromophores Concentration 20min Post Acupuncture from ST₃₆ Acupunctured Group and Control Group

	ST ₃₆	Control
OHb(mM)	1.99±0.80	0.99±0.56
RHb(mM)	1.65±0.21	0.26±0.35
THb(mM)	3.64±0.88	1.11±0.63
StO ₂ (%)	-0.77±0.29	-0.06±0.30

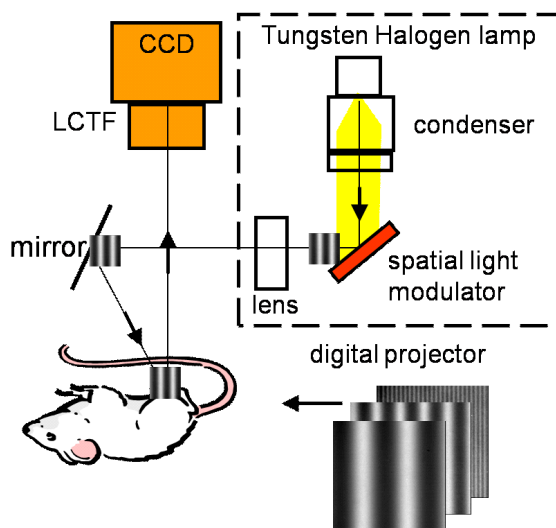


Fig. 1. A schematic setup of modulated imaging system

CCD : charge-coupled device.
LCTF : liquid crystal tunable filter.

induction chamber and maintained using 1.5~2.5% isoflurane mixed with 100% oxygen during hair removal, and image acquisition before and after acupuncture treatment. A schematic setup for the imaging is shown in Fig. 1. During image acquisition, a rat breathed anesthesia gas through a nosecone and the waste gas is filtered by an activated charcoal filter(f/Air, A.M. Bickford Inc., NY). Arterial blood oxygen saturation values and heart rate were monitored by a pulse oximeter (8600V, Nonin Medical Inc., MN) probe placed on the tail.

2. Acupuncture

We have used sterilized acupuncture needles(No. 3 × 0.5mm, Korim Seo Won, Republic of Korea) for acupuncture stimulation on rats. Acupuncture was applied to the point 2mm lateral to the anterior tubercle of the tibia(point *Zusanli*, ST₃₆) for 20min. Stimulations were given at the time 0 and 10min post a needle insertion by twisting the needles 5times.

3. Modulated imaging

Modulated imaging system is a new way of collecting images of tissue optical properties and has been developed at the Beckman laser institute and medical clinic at the University of California at Irvine. The detailed description of system can be found in previous report³²⁾. Briefly, the MI instrument uses patterned illumination and camera-based detection to obtain quantitative subsurface images of the optical properties of biological tissues over a wide field-of-view. A schematic setup is shown in Fig. 1. Imaging at multiple wavelengths(between 650~980nm) provides quantitative measures of the in-vivo concentrations of OHb and RHb. In this study, we obtained images at every 3min during acupuncture treatment and the spectral resolution was 10nm from 650nm to 980nm with the spatial frequency of 0 and 0.073/mm.

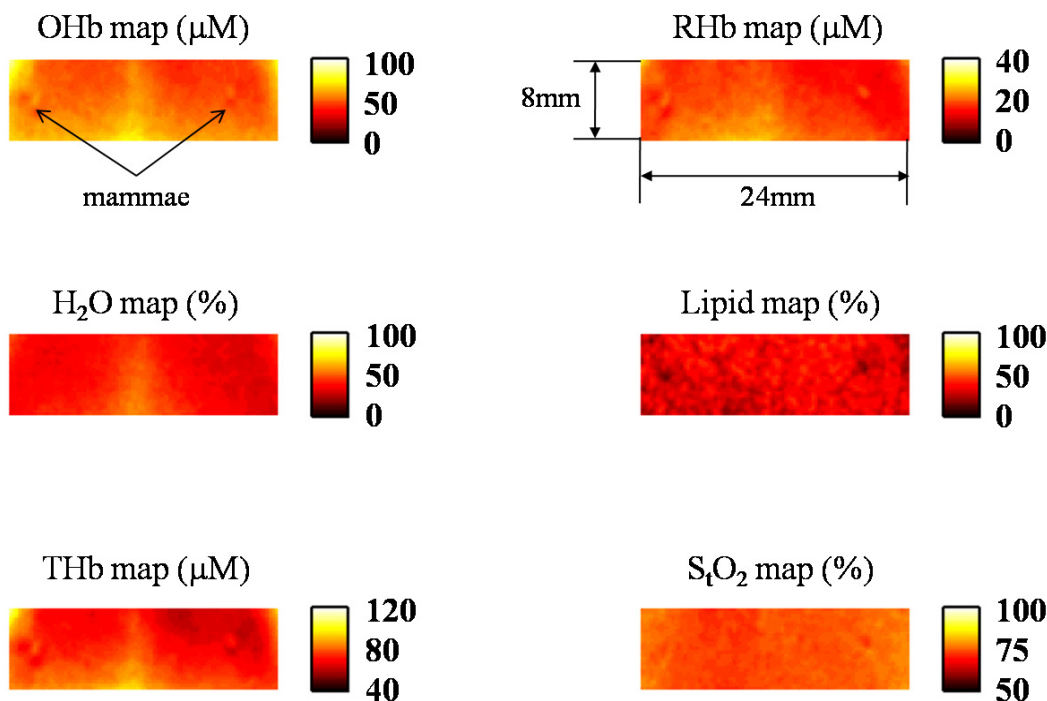


Fig. 2. A representative map of each chromophores concentration

4. Data Processing

Once images at different spatial frequency are obtained, they are demodulated to obtain tissue optical properties of absorption and scattering at each wavelength. By doing so, we can obtain the absorption and scattering spectra of measured tissue from 650 to 980nm. The absorption spectra of each pixel is then fitted to estimate the concentration of chromophores in tissues, mainly OHb and RHb, water, and lipid. THb which can represent the blood volume is obtained by adding OHb and RHb concentration, and StO_2 is calculated by dividing OHb with THb concentration. Fig. 2 shows a representative map of each chromophore from the animal that was used in this study.

III. Results

Changes of OHb, RHb, THb, and StO_2 values during 20min of acupuncture treatment are compared

between control group and acupuncture treated group shown in Fig. 3. Acupuncture treatment on ST_{36} caused a rapid increase of OHb then made a plateau 10min after needle insertion while control group that did not receive acupuncture treatment showed slight increase of OHb as shown in Fig. 3a. A significant difference of OHb change between control and acupuncture group was found between 9 and 12min after initial acupuncture needle insertion, but no significant OHb difference between the control and test group was found at the end of acupuncture stimulation .

While we did not observe a significant difference of OHb at the end of treatment between control and ST_{36} acupuncture treated group, we found a significant difference of RHb changes between control and acupuncture treated group. RHb from control group tends to slightly increase during 20min of acupuncture, but acupuncture treated group shows an more rapid exponential increase of RHb as shown in Fig. 3b. The significance in RHb difference could be found starting from 9min post needle insertion.

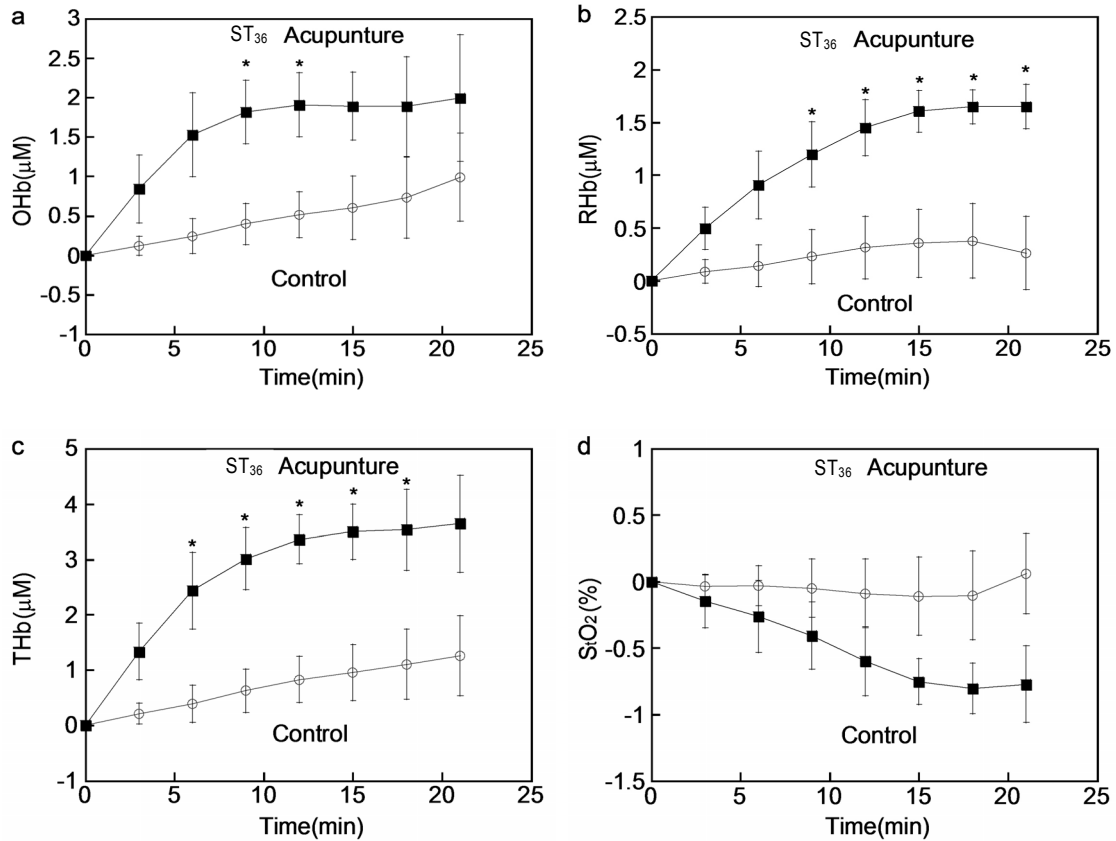


Fig. 3. Averaged changes of oxyhemoglobin(OHb) concentration from control group(non acupuncture treatment, n=7) and test group(ST₃₆ acupuncture treated group, n=7)

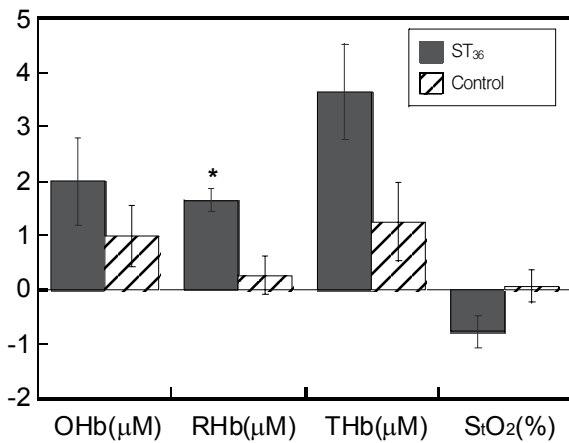


Fig. 4. Comparison of each chromophore concentration changes during 20min of acupuncture treatment between control group(non acupuncture treatment, n=7) and test group(ST₃₆ acupuncture treated group, n=7)

* : $p < 0.05$ compared to control group.

Total hemoglobin concentration in mammary tissues from acupuncture treated group also showed exponential increase during 20min of acupuncture treatment while control group showed a slight increase in THb(Fig. 3c). Since THb is obtained by adding OHb and RHb, the initial increase was steeper than RHb increase, and we could see the significant difference of THb values between control and acupuncture treated group from 6 min time point. However, we could not see the significant difference between two groups at 21min time point due to the large deviation of OHb. Tissue oxygen saturation changes during 20min of acupuncture are shown in Fig. 3d. Control group StO₂ did not show a great change from the baseline and acupuncture treated group showed about 1% decrease of StO₂ from the baseline although there is no statistically significant difference between two groups.

Changes of OHb, RHb, THb, and StO₂ at 20min

post initial acupuncture treatment from control and ST₃₆ acupuncture treated group are summarized in Table 1 and Fig. 4 as shown below. Again it shows that 20min of ST₃₆ acupuncture treatment caused increase of OHb, RHb, and THb compared to those from control group. However, only RHb was significantly increased in ST₃₆ treated group compared to that from control group and no significant difference of StO₂ between two groups were found.

IV. Discussion and Conclusion

Acupuncture is one of the oriental medicines and has been used to treat a variety of diseases and for over two thousands of years in east asia. However, acupuncture has not been accepted as another way of medicine in western society until recent years mostly due to scientifically unknown its mechanism. The acupuncture theory is based on oriental philosophy that emphasizes the harmony between yin and yang, and smoothness of energy flow(qi) through the body. The disease is believed to come from the disruption of qi flow and inharmony between yin and yang. Therefore, acupuncture is believed to work by stimulating meridians to harmonize yin and yang and to correct the disruption of qi flow³³⁾. This theory lacks of biological or physiological facts and thus it was hard to be accepted in western society.

Many studies have been performed to understand how the acupuncture treatment works. It has been recently understood that acupuncture causes the increase of neurotransmitters level in plasma and brain tissue. Those increased level of neurotransmitters such as endomorphin-1, beta endorphin, enkephalin, serotonin, and dopamine cause analgesia, sedation, and recovery in motor functions³⁴⁻³⁶⁾. Furthermore, acupuncture also has immunomodulator effect on the immune system and lipolytic effect in metabolism^{37,38)}. Due to these effects, the use of acupuncture is gradually increasing worldwide in the treatment of pain syndrome illnesses, gastrointestinal

disorders, psychological illnesses, disorder of motor function, and metabolic diseases³⁹⁾.

Measurements of blood flow and blood oxygenation changes during acupuncture treatment have been a popular way of monitoring physiological changes in the body. A laser doppler flow meter or a diffuse optical spectroscopy has become a common tool to monitor blood flow or oxygenation changes in tissues non-invasively during acupuncture treatment⁴⁰⁻⁴³⁾. If the changes of blood flow, volume, and oxygenation are associated with acupuncture effects, LDF or DOS/DOI can be a very useful tool to monitor the effects of acupuncture and furthermore, it may enable us to quantify the level of acupuncture stimulation on specific sites.

Our results showed that acupuncture on ST₃₆ point caused an increase of OHb, RHb, and THb compared to those from control group. There can be two main sources of OHb increase. First, an increase of arterial blood supply to the tissues will increase the OHb concentration in tissues. Secondly, reduction of cellular activity will decrease the oxygen consumption resulting in an increase of OHb concentration in tissues. Since the ST₃₆ acupuncture increased both OHb and RHb, the increase of OHb is mainly due to an increase of arterial blood supply to the tissues. We can also conclude that ST₃₆ acupuncture increased the tissue metabolism from the fact that RHb increased during the treatment. Tissue blood volume represented by THb has been also increased during ST₃₆ acupuncture treatment. However, StO₂ which represent tissue oxygenation did not change much from the baseline during ST₃₆ acupuncture. All the results tell us that ST₃₆ acupuncture induced increase of blood volume in tissues along with boosting the tissue metabolism.

Since tumor vascular structure is irregular and leaky, blood oxygenation or volume in tumors are different from normal tissues. Therefore, the hemodynamic effects from acupuncture will be different in tumors from non tumors, and this may be used as a contrast to detect tumors by using a DOI/DOS. It will be interesting to see the results of ST₃₆ acupuncture from tumor bearing rats since

tumors may show the different responses to acupuncture due to their abnormal vascular structure and high cellular metabolism, which can be a good candidate as a contrast to detect tumors.

In summary, we have applied acupuncture stimulation at ST₃₆ points on healthy rats to observe any changes in mammary tissue oxygenation or blood volume using a diffuse optical imaging system. The results showed that acupuncture on ST₃₆ points caused increase of OHb, RHb and THb, but did not cause significant changes in StO₂. These preliminary results showed that diffuse optical imaging technique can detect the changes of tissue oxygenation and blood volume during acupuncture treatment which can be very useful to monitor the acupuncture treatment effects non-invasively. Furthermore, it may enable us to quantify the level of acupuncture effects on specific sites.

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