Original Article

The Hemodynamic Effects of Acupuncture on *Taechung*(LR_3) on Mammary Tissues of Rat Monitored by Diffuse Optical Imaging

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국문초록

Diffuse Optical Imaging으로 측정한 태충(LR_3) 자침이 쥐의 유방 조직에 미치는 혈역학적 변화

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목적 : 태충(LR_3)에 자침을 하는 것이 쥐의 유방 조직에 미치는 혈역학적 변화를 측정하기 위하여 diffuse optical imaging 기법을 사용하였다.

방법 : 실험에 사용한 쥐는 자침을 하지 않은 대조군 7마리, 태충에 자침을 한 실험군 8마리로 총 2개의 군으로 나누었다. 몸무게 170g 정도의 건강한 암컷 쥐는 100% 산소와 1.5% isoflurane을 혼합한 것을 이용하여 마취시켰다. 자침은 양 발등의 첫째와 둘째 중족골 사이의 지점인 태충(LR_3)에 20분간 시행하였다. Beckman Laser Institute and Medical Clinic에서 개발된 modulated imaging system을 이용하여 자침하기 전과 자침하고 있는 동안에 산화혈색소(OHb), 탈산소혈색소(RHb), 총 혈색소(THb)와 조직 산소 포화도(StO_2)의 영상을 얻었다.

결과 : 실험 결과 태충(LR_3)에 자침을 한 실험군에서는 대조군에 비하여 유의성이 있는 OHb · THb의 감소와 유의성이 없는 RHb의 증가가 나타났다.

결론 : Diffuse optical imaging 기법으로 자침 중에 조직 산소공급과 혈류량의 변화를 확인할 수 있었고, 이는 비침습적으로 자침의 효과를 측정하는 데 활용할 수 있다고 생각한다. 또한 자침으로 인한 유방조직의

* This study was supported by the Laser Microbeam and Medical Program(LAMMP) NIH grant P41 RR01192
· Acceptance : 2010. 10. 29. · Adjustment : 2010. 12. 10. · Adoption : 2010. 12. 11.
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혈역학적 조절은 암 검진뿐만 아니라 암 치료에도 다른 치료와 병행하여 활용할 수 있으리라 생각한다.

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

Ⅰ. Introduction

Taechung(LR_3) is Great Surge, shu stream point and yuan source point on the liver channel, and earth point on wood meridian. It locates on the dorsum of the foot, between the first and second metatarsal bones, approximately 2 cun superior to the web margin. Acupuncture on LR_3 spreads liver *Qi*, subdues liver *Yang*, nourishes liver blood, clears the head and eyes, regulates menstruation and the lower burner, nourishes liver *Yin*, and expels wind[1]. For the application of acupuncture on LR_3 in gynecology, LR_3 can be used to treat diseases from breast and reproductive organs[2].

There are many reports that show the changes of blood flow and tissue oxygenation in brain[3-5] or periphery muscle and skin during acupuncture treatment[6].

However, there is little study on monitoring the hemodynamic effects of acupuncture on mammary tissues. Since tumor oxygenation is so important in cancer therapies, we came to have an idea of applying acupuncture to modulate the tumor oxygenation, and have tested our idea on the healthy rats as shown in this study.

Ⅱ. Materials and Methods

1. Animals

We have used female Fisher rats weighing around 170g for this study and divided into two groups. Control group(n=7) did not have acupuncture application and test group(n=8) received acupuncture on *Taechung*(LR_3) points for 20min. One day prior to acupuncture stimulation, low abdominal area including two pairs of caudal mammae was shaved using an electric hair clipper and the left hair was 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 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. Fig. 1. shows the animal setup during image acquisition. Rat breathed anesthesia gas through a nosecone and the waste gas is filtered by an activated charcoal filter(f/Air, AM. Bickford Inc, NY). Arterial blood oxygen saturation and heart rate were monitored by a pulse oximeter (8,600V, Nonin Medical Inc, MN) probe placed on the tail.

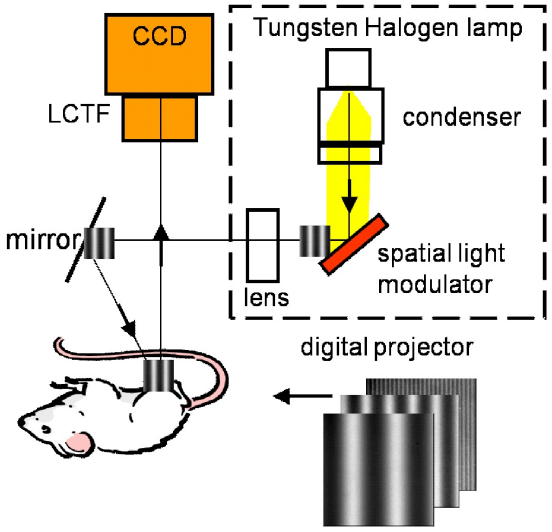

Fig. 1. A schematic setup of modulated imaging system
CCD : charge-coupled device.
LCTF : liquid crystal tunable filter.

2. Acupuncture

We have used sterilized acupuncture needles (0.25 × 30mm, Haenglim Seowon, South Korea) for acupuncture stimulation on rats. Acupuncture was applied to the dorsum of both feet between the first and second metatarsal bones(point *Taechung*, LR_3) for 20min. Stimulation was given at the time 0 and 10min post a needle insertion by twisting the needles 5 times.

3. Modulated imaging

Modulated imaging(MI) system was developed at the Beckman laser institute and medical clinic at the University of California at Irvine and the detailed description of system can be found in previous report[7]. 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 650~980 nm) provides quantitative measures of the *in-vivo* concentrations of oxyhemoglobin and deoxyhemoglobin. 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 0.073mm.

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 650nm to 980nm. The absorption spectra of each pixel are then fitted to estimate the concentration of chromophores in tissues, mainly oxyhemoglobin(OHb), deoxyhemoglobin(RHb), water and lipid. Total hemoglobin(THb) which can represent the blood volume can be obtained by adding OHb and RHb concentration, and tissue oxygen saturation (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

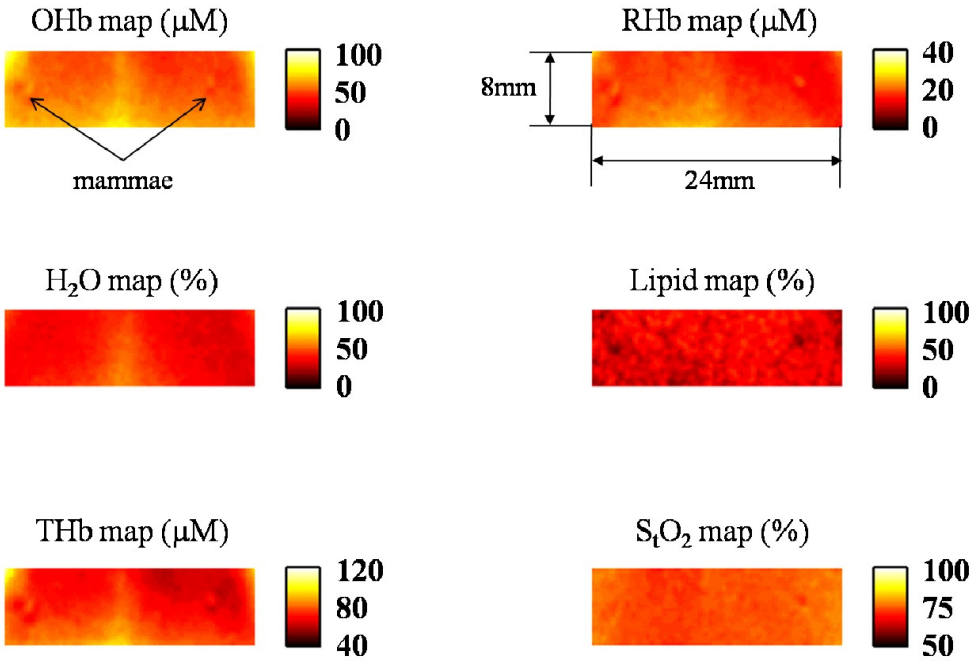

Fig. 2. A representative map of each chromophores concentration
Field of view : 24 × 8mm.

in this study.

Ⅲ. Results

Changes of OHb, RHb, THb and StO_2 values during 20min of acupuncture treatment are compared between control and test group shown in Fig. 3. Acupuncture treatment on both sides of LR_3 point caused a gradual decrease of OHb while control group that did not receive acupuncture treatment showed a slight increase of OHb as shown in Fig. 3-a.

Changes of RHb from control group tends to slightly increase during 20min of imaging while test group shows an initial decrease of RHb, but then recovered to the baseline and even rise a little bit higher than the baseline level afterwards as shown in Fig. 3-b.

Since test group showed decrease of OHb with minimal change of RHb, THb also showed gradual decrease while control group showed a slight increase in THb as shown in Fig. 3-c.

StO_2 changes are shown in Fig. 3-d. StO_2 of Control group did not show a great change from the baseline, and test group showed about 1% decrease of StO_2 from the baseline.

Changes of OHb, RHb, THb and StO_2 at 20min post initial acupuncture treatment from control and test group are summarized in Table 1 and Fig. 4 as shown below.

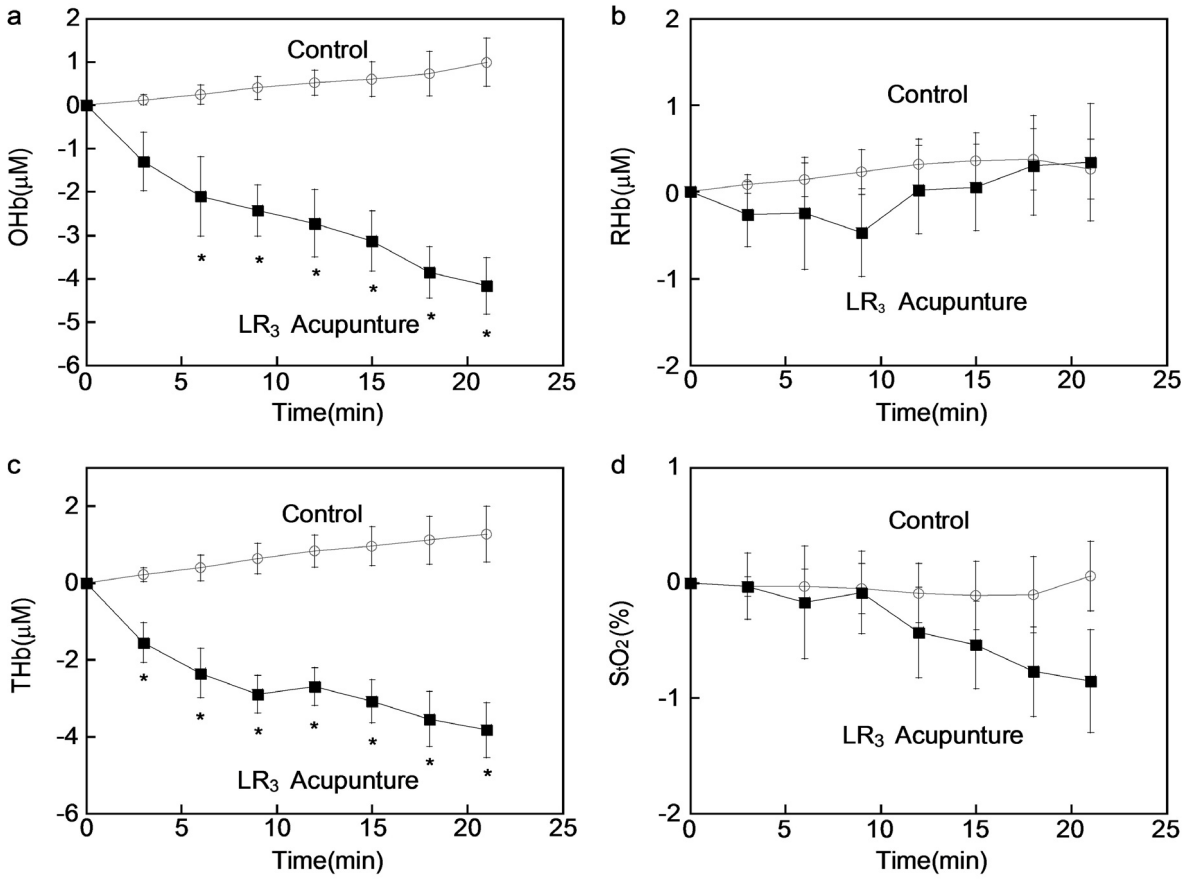

Fig. 3. Averaged changes of oxyhemoglobin(OHb), deoxyhemoglobin(RHb), total hemoglobin(THb) concentration, and percentage of tissue oxygen saturation(StO_2) from control group(non acupuncture treatment, n=7) and test group(acupuncture on LR_3 treated group, n=8)

Values are given as mean±SEM(bar). * : $p<0.05$ vs control group at each time point.

Table 1. Changes of Chromophores Concentration During 20min of Acupuncture from Control Group (Non Acupuncture Treatment, n=7) and Test Group (Acupuncture on LR_3 Treated Group, n=8).

	LR_3	Control
OHb(mM)	-4.17±0.65*	0.99±0.56
RHb(mM)	0.34±0.67	0.26±0.35
THb(mM)	-3.83±0.71*	1.11±0.63
StO_2(%)	-0.85±0.45	-0.06±0.30

Values are given as mean±SEM.
* : $p<0.05$ compared to control group.

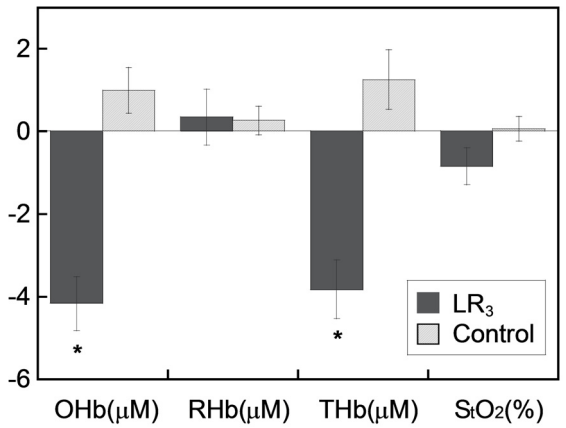

Fig. 4. Comparison of each chromophore concentration changes during 20min of acupuncture treatment between control group(non acupuncture treatment, n=7) and test group(acupuncture on LR_3 treated group, n=8)
* : $p<0.05$ compared to control group.

Ⅳ. Discussion and Conclusion

Acupuncture has become accepted as an alternative medicine in western medicine recently. However, it is still not clear how acupuncture really works even though there are some hypotheses based on experiments. For instances, Langevin et al. proposed that acupuncture works by transmitting mechanical signals to connective tissue cells[8,9]. Other hypotheses can be also found from review articles[10,11]. As an effort to understand the mechanism of acupuncture, researchers have tried to monitor physiological changes such as blood flow and tissue oxygenation associated with acupuncture treatment by using non invasive methods like a laser Doppler flow meter or a near infrared spectroscopy[12-14].

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[15,16] and muscle[17,18] by providing information of OHb, RHb, THb and StO_2. DOI/DOS also has been very actively applied in breast cancer studies to detect tumors or to monitor the therapy effects 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[19-22].

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 therapy[23] and some types of chemotherapy agents[24,25]. The efficacy of photodynamic therapy is also known to be dependent of tumor oxygenation level[26,27]. As a result, many methods have been tried to improve tumor oxygenation such as hyperoxic gas inhalation alone[28] or with nicotinamide[29], hyperbaric oxygen chamber[30,31], administration of recombinant human erythropoietin which elevates hemoglobin levels and hematocrit[32,33]. Therefore, it will be beneficial to improve the efficacy of cancer therapy if acupuncture can modulate oxygenation and/or blood flow in tumors. 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.

Acupuncture on LR_3 has shown improvements in multiple symptoms by combining with other acupuncture points. For instances, it has been shown that acupuncture on LR_3 and GB_{34} points resulted its neuroprotective effects on Parkinson disease rats[34], and acupuncture on LR_3 combined with GV_{20}, BL_{60}, SI_3 points decreased pelvic and low-

back pain during late pregnancy[35]. Acupuncture on SP_6 and LR_3 has shown the effectiveness on relieving the vasomotor symptoms such as hot flushes and night sweat from the cancer patients who are under hormone replacement therapy[36].

In Fig. 3-a, it starts to show the significant difference of OHb change between control and acupuncture group from 6min post initial acupuncture. In Fig. 3-b, due to the large deviation of data, we did not find any significant difference between two groups during 20min of acupuncture treatment. In Fig. 3-c, we can see the significant difference of THb values between control and test group starting from 3min time point. In Fig. 3-d, there is not a statistically significant difference between two groups. In Table 1 and Fig. 4 it shows that acupuncture treatment on LR_3 caused decrease of OHb and THb while it did not cause significant changes of RHb and StO_2 compared to the data from control group.

Acupuncture on LR_3 points caused a very little decrease of tissue oxygenation(1% drop of StO_2) during treatment even though there were significant decreases of OHb and THb compared to the data from control group. Therefore, LR_3 point may not be a good candidate to modulate tumor oxygenation. However, it did decrease the blood volume (THb) mostly by reducing arterial blood volume (OHb) in tissues. It will be interesting to see the results of acupuncture on LR_3 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 LR_3 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 LR_3 points caused decreases of both OHb and THb, but did not cause significant changes in RHb and StO_2. 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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