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RESEARCH ARTICLE

Heterogeneity of Skin Oxygen Density Distribution: Relation to Location of Acupuncture Points

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Abstract

To investigate possible functions of acupuncture, oxygen (O_2) levels were measured at two different acupuncture points (APs) [Hegu and Laogong] and at the corresponding non-APs (3–5 cm away from the APs) in real time using a high sensitive electrochemical O_2 microsensor. The sensor had a small planar sensing platinum disk (diameter = 25 μm) and therefore was able to monitor the O_2 levels at the localized APs. Significantly higher O_2 levels ($p < 0.05$) were observed at both APs ($n = 5$, without exceptions) in comparison with the non-APs. Sufficient sensor sensitivity to distinguish the O_2 level differences between APs and non-APs was achieved. This research provides useful information on possible functions of APs and meridians.

1. Introduction

Acupuncture has been used for longer than 2500 years in traditional Eastern medicine. Nowadays, it is widely used as an alternative and complementary therapeutic treatment even in Western cultures [1]. Fourteen known main meridians, relating to internal organs, pass through and connect acupuncture points (APs) on the skin. Previous research indicated that acupuncture increases blood flow [2], and that APs and meridians show high electrical conductance [3,4]. Recently, modern techniques, such as positron emission tomography imaging [5] and functional magnetic resonance imaging [5,6], have been used to study the neuronal activity changes in the brain during acupuncture treatments. However, the mechanisms of action of acupuncture at the various meridians are not yet clearly understood.

In acupuncture practice, *Qi*, the vital energy, is considered to flow throughout the whole body via meridians which interconnect APs. Recently, it was reported that nitric oxide synthase expression was higher at skin APs and meridians compared with other areas [7]. Nitric oxide is known to be an important signaling molecule in vasodilatation directly connected to blood flow and volume [8], thus increasing delivery of oxygen (O_2) to tissue is necessary for energy metabolism. These separate observations indicate that APs are possibly associated with body O_2 supply.

In this paper, we demonstrate the real-time quantitative measurements of O_2 levels on two different APs and on the corresponding non-APs using a highly sensitive electrochemical O_2 microsensor. The sensor had the small planar sensing disk (diameter = 25 μm). Therefore, it was able to

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monitor the localized O_2 levels at the confined APs with effective dimensions were known to be small.

2. Materials and Methods

2.1. Electrochemical O_2 microsensor

High sensitive electrochemical O_2 microsensors were fabricated using a method similar to that previously described for nitric oxide microsensors [9]. The Clark-type O_2 sensor was composed of a platinized disk cathode (Pt diameter = 25 μm; Good Fellow, Cambridge, UK) and a coiled Ag/AgCl anode (diameter = 127 μm; A-M Systems Seguim, WA, USA) immersed in 30 mM NaCl and 0.3 mM HCl internal solution, which were covered with a PTFE gas-permeable membrane (thickness < 19 μm, porosity 50%, pore size 0.05 μm; W. L. Gore & Associates, Elkton, MD, USA). Currents between the cathode and the anode were recorded as a function of time using a CHI1000A electrochemical analyzer (CH Instruments Inc., Austin, TX, USA) while a potential (–0.6 V *vs*. the anode) to induce an O_2 reduction reaction was applied to the cathode. The measured currents were linearly proportional to O_2 levels in samples. The sensors were calibrated before and after O_2 measurements using an O_2 standard solution which was prepared by bubbling deoxygenated phosphate-buffered saline (PBS) solutions (pH 7.4; Fisher Scientific, Rochester, NY, USA) with O_2 gas (Dong-A Gas Co, Seoul, Korea). The sensors exhibited a sensitivity of 286.8 ± 58.0 pA/mmHg to O_2 ($n = 7$). The sensors maintained the sensitivity within < 0.5% variation before and after the O_2 measurements on skin, confirming the stability. In addition, sensitivity of the sensor was also validated to vary within < 0.5% for 10°C temperature change (25–35°C).

2.2. O_2 measurements on skin

For the measurements of O_2 levels, a drop of PBS (pH = 7.4) solution was applied to the skin area of interest. Then the planar O_2 sensor was immersed in the PBS solution and carefully positioned above the skin AP maintaining the separation ~1 mm while the sensor currents were recorded as shown in Figure 1A. Once the measured currents, proportional to the O_2 levels, was stabilized, the sensor was moved horizontally to the corresponding non-APs where the sensor currents were measured until stable currents were obtained. The whole procedure was repeated three times.

Sensor currents responding to O_2 levels were measured at two different APs [large intestine 4 (LI 4, Hegu), pericardium 8 (PC 8, Laogong)] and the corresponding non-APs, or non-APs, (3–5 cm away from the APs) using the prepared O_2 microsensors. The positions analyzed are shown in Figure 1B. The measurements were performed at room temperature on five healthy volunteers (average age = 23 years) in calm and restful conditions. None of the subjects had previously inserted acupuncture needles at the skin locations investigated. The measured sensor currents were converted to the corresponding O_2 levels using prior calibration data.

2.3. Data analysis

For each subject, the O_2 levels obtained by three repetitive measurements at the same skin locations were averaged and the standard deviation was also calculated independently. This averaged data was normalized (See "Results"). For each subject, the normalized data obtained at the same skin locations were also averaged and the data at APs and non-APs were compared using a two tailed t test. A p value of < 0.05 was considered to be statistically significant.

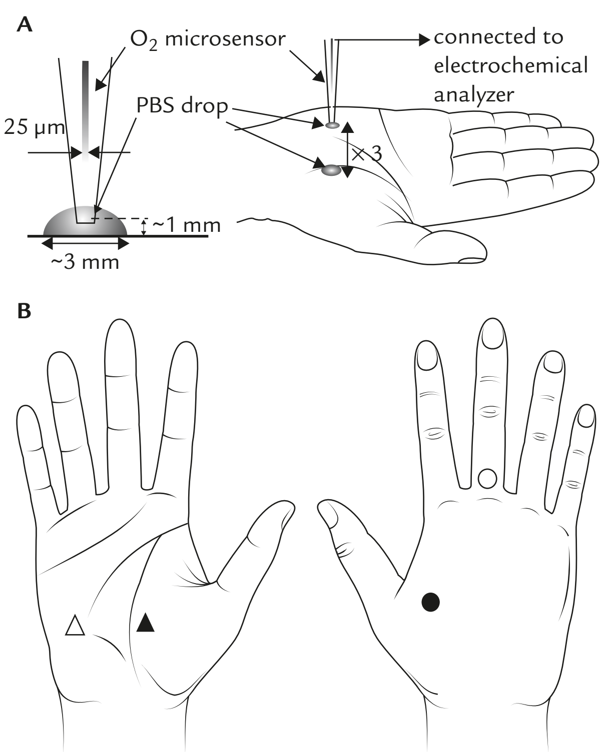

Figure 1 (A) Schematic representation of the positioning of an oxygen (O_2) microsensor for the measurement of O_2 levels over hand skin. (B) The positions of analyzed points on the hand skin: LI 4 (▲) and the corresponding non-acupuncture point (△), PC 8 (●) and the corresponding non-acupuncture point (○). PBS = phosphate-buffered saline.

3. Results

The sensor was positioned near the skin surface of the LI 4 point to monitor O_2 levels. After measurement for a certain time to obtain stable signals, the sensor was moved horizontally to the non-APs and positioned near the skin surface while maintaining the sensor vertical, away from the skin in a similar manner to that used to measure the APs.

Figure 2 shows typical partial O_2 pressure-time curves obtained. The partial O_2 pressures (pO_2) were calculated from the measured sensor currents using the sensor calibration data prior to the measurements. For all five volunteers, without exception, high O_2 densities were observed at the LI 4 and PC 8 APs compared with the non-APs. The measured pO_2 were 146.9±23.7 mmHg (*vs.* 141.8±22.7 mmHg at the corresponding non-AP) and 169.7±28.5 mmHg (*vs.* 164.4±27.4 mmHg at the corresponding non-APs) at LI 4 and at PC 8, respectively. A wide range of background O_2 levels (with large standard deviation) near the skin were observed, depending on the subject. The variation may be due to the individual physiological condition of the volunteers. Thus the measured pO_2 were normalized for statistical analyses and comparison. In fact, the normalized pO_2 was obtained as follows:

$$pO_{2\text{normalized}} = pO_{2\text{average}} / pO_{2\text{non-APs}}$$

where $pO_{2\text{normalized}}$ is the normalized partial oxygen pressure, $pO_{2\text{average}}$ is the average of the partial oxygen pressures measured, $pO_{2\text{non-APs}}$ is the average of the partial oxygen pressures measured at non-APs.

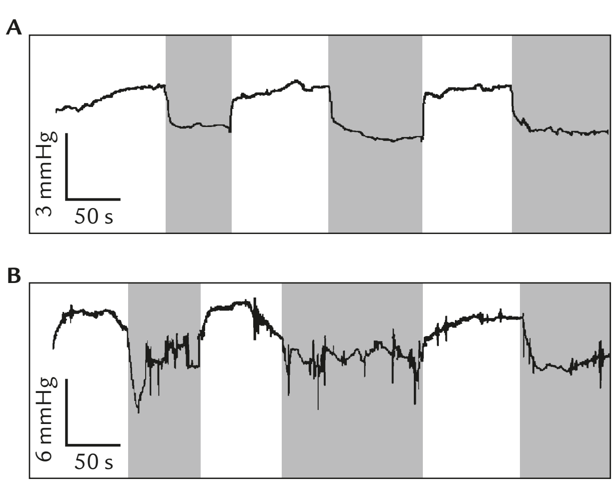

Figure 2 Representative measured partial oxygen pressures as a function of time when the sensor was positioned over (A) LI 4 and the corresponding non-acupuncture point (AP) and (B) PC8 and the corresponding non-APs. Gray-colored regions represent the measurements made over non-APs.

Thus $pO_{2\text{normalized}}$ at non-APs was always 1. The calculated $pO_{2\text{normalized}}$ values at the APs (LI 4 and PC 8) compared with $pO_{2\text{normalized}}$ at non-APs are shown in Figure 3. Each $pO_{2\text{normalized}}$ value was obtained by averaging three repetitive measurements at the corresponding same location for each subject. The average values of the $pO_{2\text{normalized}}$ for five subjects were 1.036±0.016 and 1.032±0.012 at LI 4 and PC 8, respectively.

The normal distribution of the obtained data was confirmed by a normal probability plot. Using a two-tailed *t* test (with a significance level of $p<0.05$), the measured O_2 levels at LI 4 or PC 8 points were significantly different ($p=0.009$ and $p=0.002$, respectively) compared with the corresponding non-APs.

4. Discussion

According to the experimental results, APs were clearly distinguished from non-APs in terms of higher O_2 densities around the APs. It should be noted that the measured O_2 levels were dependent on the distance between the sensor end plane and skin. Indeed, higher O_2 levels were observed when the sensor-skin separation was small. Therefore, the measurements of O_2 levels were carried out while maintaining the sensor-skin vertical distances as similar as possible at both APs and non-APs. Even with care, there could be slight differences in the distance between skin and sensor when the sensor was moved and repositioned over different locations. However, the observed O_2 level variations depending on the vertical distances (at the same locations) were smaller than those between APs and non-APs: ≤1.75 mmHg changes in $pO_{2\text{average}}$ between 0.5–1.5 mm separations versus 4.7–8.8 mmHg difference in $pO_{2\text{average}}$ between APs and non-APs. Therefore, the observed higher $pO_{2\text{average}}$ at the APs can be considered meaningful.

Although the $pO_{2\text{average}}$ differences between the APs and the non-APs were small (ca. 2–5% in $pO_{2\text{normalized}}$; i.e. ca. 4.7–8.8 mmHg in absolute pO_2), they were sufficiently large to be differentiated with our high sensitive O_2 microsensor. Recently, Zhang et al reported that higher transcutaneous carbon dioxide emissions were observed on 12 points along the Pericardium meridian line compared with control points beside the meridian line [10]. Our observation of higher O_2 levels at the APs than at the non-APs correlates with Zhang's report and may provide a crucial component of the biological/physiological functions of the APs.

The observed high contents of O_2 at the APs may reflect the relatively reduced O_2 uptake from the atmosphere through the APs in comparison to the

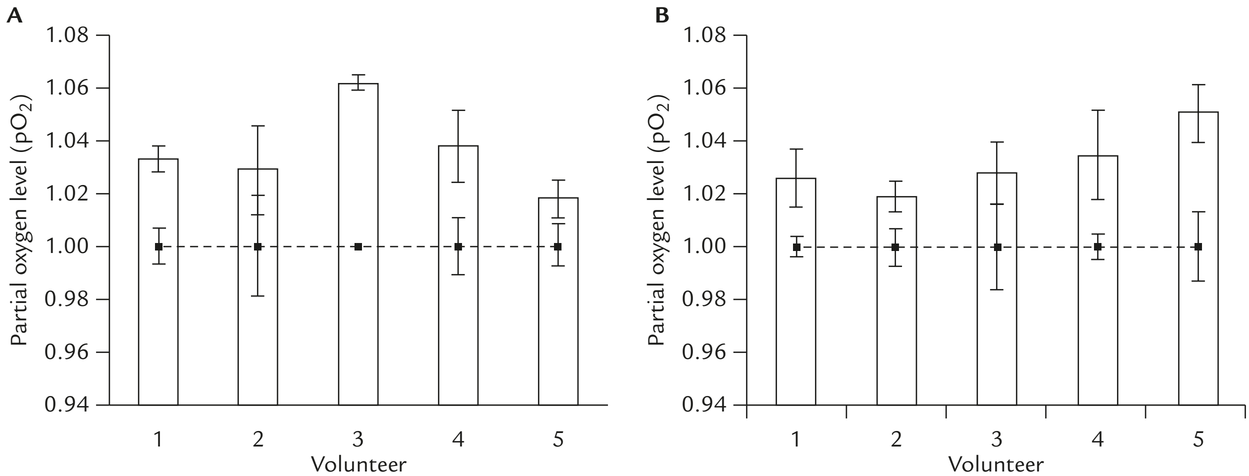

Figure 3 Normalized oxygen levels measured at (A) LI 4 and (B) PC 8 points for five subjects. Dashed lines represent normalized oxygen levels measured at the corresponding non-acupuncture points.

non-APs. Stücker et al suggested that the O_2 supply to skin is a balance between O_2 transported by blood and uptake from the atmosphere [11] and also demonstrated that the transcutaneous O_2 flux is increased by interruption of blood flow to skin on the volar forearm [12]. Therefore, the higher O_2 levels measured at the APs may imply that the O_2 supply by capillary O_2 transport is greater at the APs than at the non-APs. Possibly large blood vessels or primo-nodes (Bonghan corpuscle)/vessels, which were proposed to be corresponded to APs by Dr B.H. Kim [13] and recently rediscovered by a group from Seoul National University [14], could be present directly underneath the skin APs. To confirm this conjecture, further research (e.g. anatomical studies) is required.

Our current investigation may provide scientific evidences for the physical existence and physiological functions of APs in which traditional Eastern medicine has believed for many years.

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