Specificity of auricular acupoints in reflecting changes of qi and blood measured by diffuse reflectance spectroscopy

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OBJECTIVE: This study aimed to assess the specificity of auricular acupoints in responding to changes of qi and blood which, in traditional Chinese medicine, are considered as the fundamental substances in the human body for sustaining normal vital activity.

METHODS: A miniature fiber optic system was used to invasively measure the diffuse reflectance spectra of three auricular acupoints, namely, Uterus (TF2), Gan (CO12) and Neifenmi (CO18), at different stages of menstruation, when the female body exhibits regular changes of qi and blood. The spectra of different acupoints were compared to find their difference in responding changes of qi and blood, especially the reflectivity of absorption peaks of hemoglobin.

RESULTS: The reflectivity of the same auricular acupoint during menstruation is higher than that before and after menstruation, and this trend is more obvious for the Uterus point compared with the points Gan and Neifenmi. The average reflectivity of the Uterus point during menstruation was significantly higher than that before or after menstruation (P<0.01). The D-values during and after menstruation of the Uterus point were greater than those of the points Gan and Neifenmni at 544.06 and 577.47 nm, respectively (P<0.05).

CONCLUSION: The diffuse reflectance light of auricular acupoints changes as qi and blood fluctuates, and there is relative specificity among different auricular acupoints in reflecting changes of qi and blood. The Uterus point may be the most sensitive auricular acupoint in reflecting uterus function and subsequent changes of qi and blood.

KEYWORDS: otopoint; uterus SV; spectrum analysis; reflectance spectrum; menstruation; qi-blood
Auricular acupuncture, in which acupuncture-like stimulation is applied to acupoints located on the auricles\(^{[1]}\), is one of the most common acupuncture approaches. This form of acupuncture has the advantages of being effective, easy to handle, inexpensive and with almost no side effects. Auricular acupuncture has been widely used to treat pain\(^{[2]}\), anxiety\(^{[3]}\), and drug dependence\(^{[4]}\). Although auricular acupuncture was developed in the West in the 1950s by Paul Nogier, who held that all internal organs are represented in the ear, it has been used in traditional Chinese acupuncture for thousands of years according to the Huangdi Neijing (Huangdi’s Canon of Internal Medicine). This text asserted that the ears are not a separate sense organ but closely connected with zang-fu organs as well as meridians. One of the many problems with auricular acupuncture is that many maps exist with little agreement regarding acupoint location\(^{[5]}\). According to the theory of traditional Chinese medicine, acupoints have specificity in therapeutic effects, biophysical characteristics, pathological responses, and morphological structure\(^{[6]}\); thus the specific acupoints are carefully selected as the stimulated acupoints for treatment based on syndrome differentiation or the detected acupoints for clinical diagnosis. However, there remains basic controversy over the specificity of auricular acupoints. Currently, physical methods have been used for exploring the acupoints’ specificity in vivo, such as electrical resistance\(^{[7]}\) and infrared radiation\(^{[8]}\). The common conclusion is that accompanying the physiological or pathological changes, the physical variables in acupoints may alter, which may provide objective characterization for acupoint’s specificity. Therefore, in this study, in order to explore the specificity of auricular acupoints and establish a new method for investigation of acupoint, a set of spectroscopic measurements are used to detect diffuse light in three auricular acupoints which could be the specific acupoints for reflecting changes in qi and blood during menstrual period in females. The relationship between diffuse reflectance spectra of auricular acupoints and qi and blood changes during menstrual period is discussed.

1 Subjects and methods

1.1 Subjects This study was conducted in the Laboratory of Photonics Chinese Medicine, South China Normal University. In total 32 female volunteers aged 20 to 27 years (23.5 ± 2.4) were recruited from different colleges of South China Normal University. On admission, all the participants received a physical examination by a physician unrelated to the study. Participants had to meet the following criteria, including (1) healthy with no disease history of heart, liver, kidney, incretion, and so on; (2) regular menstrual rhythm with a 28- to 30-d menstrual cycle, fluctuations of no more than 5 d, a 3- to 5-d menstrual period, and normal menstrual flow; (3) no history of use of hormone medication in last 3 months; (4) and no cutaneous trauma in the auricles. Exclusion criteria included menstrual disorder, hormone drug use, having medical conditions requiring treatment, and of an age less than 20 or more than 40. All participants gave written informed consent, and the protocol was approved by a relevant ethics committee.

1.2 Instruments The experimental system for detecting diffuse reflectance spectrum of acupoints employed a miniature fiber optic spectrometer with a 3648-element linear charge-coupled device (Ocean Optics, USB 4000-VIS-NIR, asymmetric cross Czerny-Turner design). Illumination was provided by a 6.5 W LS-1 tungsten-halogen white light source. A bifurcated optical fiber (R400-7-VIS/NIR) was used to guide light to the acupoints and collect scattered light from the skin, which has six 400 μm diameter fibers for light delivery and a single core 400 μm diameter fiber for light collection, with the six illumination fibers placed around the central collection fiber in a circular manner. The measurement range of the system is 345 to 1 037 nm with a spectral resolution of 0.5 nm.

Related Articles

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approximately 1.5 nm and signal to noise ratio (SNR) of approximately 300 : 1. All spectra were referenced to a calibration spectrum measured on WS-1 diffuse reflectance standard (LabSphere, USA) and saved using the SpectraSuite software, as Figure 1 shows. As the diameter of the fiber probe is approximately 5 mm, far less than the diameter of the acupoints, the probe can contact the auricular acupoint light tightly to ensure that the weak optical signal from auricular acupoints can be recorded.

1.3 Localization of auricular acupoints Localization of auricular acupoints was determined by a certified and experienced acupuncturist. Three auricular acupoints, the Uterus (CO12), Gan (CO18) and Neifenni (TF2) were selected to study the relative specificity of the auricular acupoints in presenting the changes of zang-fu organs and the state of qi and blood. The selection of the specific auricular acupoints was based on principles described previously[10], because these auricular acupoints may present specific changes in menstrual period and were most often used in the treatment of gynecopathy and endocrine diseases, such as dysmenorrhea and menstrual disorder.

1.4 Recording methods The first measurement was conducted on 1 to 3 d before menstruation, which was determined according to previous menstrual cycles. The second measurement was conducted on the second day during menstruation period, and the last on the second day after menstruation. All measurements were conducted at 9:00 to 12:00 AM for eliminating time effects.

Before measurement, the participants lay in a prone position for 15 min to adapt to the dark room with room temperature at (22 ± 3) °C and 45%~65% relative humidity, and the auricular acupoints were wiped slightly with 75% alcohol. After the alcohol evaporated, the fiber probe was put on the auricular acupoints vertically with no gaps. The parameters were set as follows: integration time 26 000 ps, scans to average 10, boxcar width 4. All spectra were referenced to a dark noise spectrum and to a calibration spectrum measured on a diffuse reflectance standard. Spectra were collected in the 345 to 1 037 nm range with a system spectral resolution of approximately 1.5 nm. The shape of reflectance spectrum and reflectivity (%) indicated the reflective intensity and were the main analytical index. Considering the great noise in both ends, the authors analyzed the spectrum from 400 to 900 nm only, and mainly compared the reflectivity at three troughs, 544.06, 555.17 and 577.47 nm, respectively, which come into being with the light being absorbed mainly by oxyhemoglobin and deoxygenated hemoglobin.

1.5 Statistical analysis Reflectivity was presented as mean ± standard deviation. Statistical analysis was performed with SPSS (Version 13.0, SPSS Inc., USA). One-way analysis of variance (ANOVA) was used for comparisons between auricular acupoints, further comparison between two auricular acupoints were analyzed by LSD-t test. Repeated measures ANOVA was used for comparison of diffuse reflectance in the same acupoints at different time. P values less than 0.05 were considered as statistically significant.

2 Results

2.1 Shape of reflectance spectrum from auricular acupoint Thirty-two volunteers were enrolled in this study, and data of eight participants were discarded due to inappropriate parameters of the spectrometers or incomplete records. As different auricular acupoints have the same spectral shape, only the reflectance spectra of Gan were given as an example. As shown in Figures 2A and 2B, the auricular acupoints of different individuals displayed the same reflectance spectra shape of the auricular acupoints, with the troughs appearing at 420, 546, 555 and 577 nm, respectively, just as other literature has reported[11]. The reflectivity of the same auricular acupoint fluctuated with qi and blood changes, and the reflectivity during menstruation is the highest at all the three stages, as shown in Figure 2C. On the other hand, different auricular acupoints have the same position of wave crests and wave troughs with different reflectivity, as shown in Figure 2D.

Figure 1 Schematic plot of the instruments used in this study
2.2 Comparison of the same auricular acupoint’s reflectivity at different time points. To determine if there is specificity among the three auricular acupoints in reflecting qi and blood fluctuation caused by menstruation, the reflectivity of 544.06, 555.17 and 577.47 nm from the same acupoints at three different time points were compared, respectively, as shown in Table 1. The reflectivity of Gān during menstruation was higher than that of before and after menstruation without significant differences (P > 0.05). The reflectivity of Neiifenmī during menstruation was higher than that of before and after menstruation, also without significant differences (P > 0.05). The reflectivity of the Uterus during menstruation was significantly higher than that of before and after menstruation (P < 0.01).

2.3 Comparison of difference value of reflectivity among auricular acupoints. To investigate whether the difference in value of reflectance intensity fluctuating with time shows significant difference among auricular acupoints, two difference values, D-B and D-A, were calculated by subtracting the value of reflectance during menstruation (D) from the value of before menstruation (B) or after menstruation (A), and then D-B and D-A values were compared among the three auricular acupoints. As Figure 3 shows, the D-A value of Uterus was greater than those of Gān and Neiifenmī, but there was no significant difference (P > 0.05). Figure 4 indicates that the D-B value of Uterus was greater than those of Gān and Neiifenmī at 544.06 nm, 577.47 nm (P < 0.05), and 555.17 nm (P = 0.062). In summary, with changes of qi and blood caused by menstruation, the changes of reflectivity in the Uterus point are the most remarkable among the three auricular acupoints.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>n</th>
<th>Reflectivity of Gān</th>
<th>Reflectivity of Neiifenmī</th>
<th>Reflectivity of Uterus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>During</td>
<td>After</td>
</tr>
<tr>
<td>544.06</td>
<td>24</td>
<td>36.85±7.44</td>
<td>46.01±7.56</td>
<td>36.02±8.52</td>
</tr>
<tr>
<td>555.17</td>
<td>24</td>
<td>37.66±7.29</td>
<td>41.10±7.33</td>
<td>36.79±9.44</td>
</tr>
<tr>
<td>577.47</td>
<td>24</td>
<td>34.80±7.13</td>
<td>37.83±8.76</td>
<td>37.04±9.62</td>
</tr>
</tbody>
</table>

** P < 0.01, or during menstruation.
3 Discussion

Both Chinese auricular acupuncture and Nogier auriculotherapy hold that auricular acupoints have specific relation to viscera and organs, which is the theoretical basis of selecting acupoints in clinical practice. Therefore, specificity of auricular acupoints is always a research hotspot, and many new methods have been used for exploring the specificity. Diffuse reflectance in acupoint carries abundant photon information, which may reflect an acupoint’s function or structure. For this reason, diffuse reflectance spectroscopy was used for researching on acupoint’s specificity\cite{12,13}. In the present study, the authors detected three auricular acupoints’ diffuse reflectance spectra, and assessed the specificity of auricular points in responding to qi and blood which are considered as the fundamental substances in the human body for sustaining normal vital activity according to the basic theory of traditional Chinese medicine. We found that different auricular acupoints have the same shape of diffuse reflectance spectra, and the absorption peaks are similar. This results indicate that the overall shape of diffuse reflectance spectra in auricular points cannot demonstrate the changes of qi and blood, which are consistent with previous research on acupoint’s reflectance spectroscopy\cite{11} and infrared spectroscopy\cite{14}. In this study, the auricula was illuminated with white light source, which would be absorbed mainly by hemoglobin with the oxygenation and deoxygenation of blood when contacting with biological tissue\cite{15}. Accordingly, the troughs of reflectance spectroscopy are nearby at 544, 577 and 555 nm, respectively. For this reason, the reflectance intensity at these three wavelengths may be closely related with qi and blood.

Interestingly, we found that with qi and blood presenting regular wax or wane changes caused by
menstruation, the acupoint’s diffuse reflectance showed increase or decrease, in which the reflectance intensity of the same auricular acupoints during menstruation was higher than those before and after menstruation, and this trend is most obvious for the Uterus point at all wavelengths. The cause of these effects may be due to the reduction of blood volume during menstruation, corresponding to the hemoglobin decline, so reflectance in auricular acupoints will increase in proportion with the diminution of absorption. Further analysis indicates that the differential value of reflectance intensity fluctuating with time was significantly different among the three auricular acupoints ($P < 0.05$), and the changes of diffuse reflectance in the Uterus point were the most remarkable among the three auricular acupoints. According to the principle of auricular acupuncture, Gan, Neifenmi and Uterus are the specific acupoints corresponding to the uterus and changes of qi and blood caused by menstruation. These results lead to the assertion that the Uterus point is the most sensitive auricular acupoint in reflecting the changes of qi and blood and uterus function, and the Uterus point may be the preferred auricular acupoint in treating menstrual or uterine diseases. Although this research cannot realize precise quantification of qi and blood, as a noninvasive, convenient and efficient method, diffuse reflectance spectroscopy shows potential in future research on acupoints and meridians.

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5 Competing interests

The authors declare that they have no competing interests.

REFERENCES


从漫反射光谱探讨耳穴反映气血变化的特异性

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目的：观察耳穴在月经前后人体气血盈亏变化过程中的反射光谱特征及其差异性，探讨耳穴反映人体气血变化的相对特异性。

方法：采用线阵电荷耦合元件探测器的光纤光谱仪无损获取子宫（TF2）、肝（CO12）、内分泌（CO18）3 个耳穴在月经前后不同时段的可见光反射光谱，并对特定波长处的反射率进行比较。

结果：随著人体机体气血盛衰的变化，3 个耳穴的漫反射光谱表现出规律性的变化，即月经期的漫反射率高于月经前和月经后，这种趋势以 TF2 表现更为显著；在血红蛋白吸收峰附近 544.06 nm、555.17 nm 及 577.47 nm 波长处，TF2 在月经期的反射强度显著高于月经前及月经后（P<0.01）；TF2 反射强度变化的差值大于 CO12 及 CO18，尤其以 TF2 月经期与月经后的差值显著高于 CO12 及 CO18 月经期与月经后的差值（P<0.05）。

结论：耳穴漫反射光的变化与机体气血盛衰有关，其在反映机体气血盛衰方面存在相对特异性，子官为反映胞宫气血变化最灵敏的耳穴。

关键词：耳穴，子宫（穴），光谱分析，反射光谱，月经，气血