Image formation of brain function in patients suffering from knee osteoarthritis treated with moxibustion

Hongwu Xie, Fangming Xu, Rixin Chen, Tianyou Luo, Mingren Chen, Weidong Fang, Fajin Lü, Fei Wu, Yune Song, Jun Xiong

OBJECTIVES: Functionalmagnetic resonance imaging (fMRI) technology was used to study changes to the resting state blood flow in the brains of patients with knee osteoarthritis (KOA) before and after treatment with moxibustion at the acupoint of the left Dubi (ST 35) and to probe the cerebral mechanism underlying the effect of moxibustion.

METHODS: The resting state brain function of 30 patients with left KOA was scanned with fMRI before and after treatment with moxibustion. The analytic methods of fractional amplitude of low frequency fluctuation (fALFF) and regional homogeneity (ReHo) were used to observe changes in resting state brain function.

RESULTS: The fALFF values of the right cerebrum, extra-nucleus, left cerebellum, left cerebrum and white matter of patients after moxibustion treatment were higher than before treatment, and the fALFF values of the precentral gyrus, frontal lobe and occipital lobe were lower than before treatment ($P<0.05$, $K \geq 85$). The ReHo values of the thalamus, extra-nucleus and parietal lobe of patients were much higher than those before moxibustion treatment, and the ReHo values of the right cerebrum, left cerebrum and frontal lobe were lower than before treatment ($P<0.05$, $K \geq 85$).

CONCLUSION: The influence of moxibustion on obvious changes in brain regions basically conforms to the way that pain and warmth is transmitted in the body, and the activation of sensitive systems in the body may be objective evidence of channel transmission. The regulation of brain function by moxibustion is not in a single brain region but rather in a network of many brain regions.
ttries. The effectiveness of acupuncture and moxibus-
tion has been recognized in clinical practice around the
world. Acupuncture is used more widely while use of
moxibustion has gradually withered. Over many years, research by scholars at home and abroad into the me-
chanism of acupuncture and moxibustion has been main-
ly limited to acupuncture. Moxibustion has unique ad-

teas in treating chronic diseases and refractory dis-
eases, in preventing diseases and in healthcare. In re-
cent years, with the discovery of the characteristics of
moxibustion, it has seen increasing use.
In this study, knee osteoarthritis (KOA), a disease that
can be effectively treated with moxibustion, was select-
ed as the site for research. We used the analytic meth-
ods of fractional amplitude of low frequency fluctua-
tion (fALFF) and regional homogeneity (ReHo) with
functional magnetic resonance imaging (fMRI) to
study changes in resting brain function before and after
moxibustion at the left Dubi (ST 35) and to probe the
cerebral mechanism of moxibustion.

METHODS

Target for research
Between March and December 2010, 65 patients with
left KOA at the Department of Orthopedics and the
Department of TCM combined with Western Medi-
cine in the First Hospital affiliated to Chongqing Medi-
cal University were divided into a moxibustion group
and a control group. A lit moxa-cigar was circled,
bird-pecked and moved back and forth for 30 s each
about 5 cm above the skin of the left Dubi point.
Warm moxibustion was performed in the rest time.
Specific steps were as follows: circling moxibustion
was performed for 30 s to warm the local Qi and blood,
bird-pecking moxibustion was done for 30 min to
strengthen sensitization, back and forth moving moxi-
bustion was performed along a channel for 30 min to
stimulate channel-Qi, and warming moxibustion was
used to start transmission and open channels and col-
laterals. Patients with penetrating heat, expanding heat,
transmitting heat, far-site heat with no local heat (or
slight heat), deep-site heat with no superficial heat (or
light heat) or other non-heat sensations (such as ach-
ing, distending, pressing and heavy sensations) at the
Dubi (ST 35) were assigned to the moxibustion group.
Otherwise, patients were assigned to the control group.
Thirty patients in the moxibustion group conformed to
the corresponding diagnostic standard. The research
plan of this experiment was approved by the ethics
committee of Chongqing Medical University, and all
subjects or their families provided informed consent.
There was no statistical difference (P>0.05) between
the two groups in terms of sex, age, or illness course.

Diagnostic standard
The diagnostic standard stipulated in 1995 by the
American Rheumatic Association (ARA) was used ac-
cording to clinical symptoms and signs of the patients
as well as imaging and other auxiliary tests.
Clinical and radiologic standards: 1) There is pain in
the knee at most times over nearly one month. 2) Roentgenogram shows the formation of osteophytes.
3) Joint liquid test indicates osteoarthritis. 4) Age ≥40
years. 5) Morning rigidity ≤30 min. 6) There is the
sound of bony friction. Patients with 1)+2) or 1)+3)+
5)+6) or 1)+4)+5)+6) can be diagnosed as suffering
from KOA.
Inclusive standard: 1) Patients with bilateral KOA,
whether male or female aged 45-65 years, conform to
the above-mentioned diagnostic standard. 2) Patients
have no disease in the nervous system and no history
of mental disease. 3) Patients sign an agreement indicat-
ing knowledge of the condition of scientific research in-
to KOA.
Exclusive standard: 1) Patients have myofascial strain,
cervical spondylolysis, protrusion of intervertebral
disc, postherpetic neuralgia, scapulohumeral periarth-
ritis and other chronic pain. 2) Patients have pathologi-
cal changes in the brain. 3) Patients have hypertension,
diabetes or other chronic diseases. 4) Patients have
cognitive dysfunction with a score in the simple scale of
mental state less than 28. 5) Patients are unsuitable for
MRI (including patients with the syndrome of confin-
ing themselves indoors). 6) Patients refuse to sign an
agreement indicating knowledge of the condition of sci-
cientific research into KOA.

Methods for research
Design of research: a 3.0 T MRI device (Signa, GE
Healthcare, Waukesha, WI, USA) was used to carry
out the resting state fMRI scan before and after moxi-
bustion in the First Hospital affiliated to Chongqing
Medical University. A specific head coil was used for
the scan. The heads of the imaging subjects were fixed
with foam material to decrease motion, and rubber ear-
plugs were provided to protect hearing. The patient
was asked to be familiar with the environment and to
keep calm while lying on their back with eyes closed
while maintaining a conscious state, decreasing the mo-
tion of the head and body. The patient was also asked
to try and stop thinking as much as possible. At the
end of the scan, the patient was asked if he or she had
fallen asleep. Patients that gave an affirmative or vague
answer were excluded from further study.
Order of resting fMRI scan: 1) Gradient echo order
3D-T1 W1 is used for structural image: Time of re-
peat / time of echo (TR/TE)=12 ms/4.2 ms FA=15°,
249 layers, layer thickness/layer gap=1.2 mm/0 mm,
field of view (FOV)=240 mm×240 mm, matrix=64×64,
resolution=1 mm. 2) Echo-planar imaging (EPI)
order is used for the resting functional imaging MR:
TR/TE=2000 ms/40 ms, FA=90°, 33 layers, layer
thickness/layer gap=5 mm/L mm, voxel size c=4×4×4,
FOV=240 mm×240 mm, matrix=64×64, scanning
time=7 min 10 s.
Data processing

Image pre-processing: DPARSF (Data Processing Assistant for Resting-State fMRI, by Yan et al, http://www.restfmri.net) was used to complete image pre-processing with the following steps: DICOM data format was switched, the TRs of the first 10 time points in the resting state data were removed, head motion was corrected for, and data of displacement >1 mm and/or rotation >1° were deleted. Image calibration was as follows: 4 mm full width at half-maximum (FWHM) is used to make the space slide, filtration was 0.01 Hz< f<0.08 Hz, linear drift was removed, fALFF was calculated and ReHo analysis was carried out.

Statistical analysis

FALFF calculation: statistical parametric mapping 8 (SPM8, http://www.fil.ion.ucl.ac.uk/spm) software was used to carry out a paired t-test on the mfALFF image before and after moxibustion. Rest Slice Viewer (http: www.fil.ion.ucl.ac.uk/spm) was used to carry out multiple calibrations and to obtain an image. Brain regions showing a statistical difference were identified by defining a threshold for each cluster >85 voxels and a threshold of a voxel <0.05 (calibrated). The 3-dimensional (3D) picture of the whole brain was obtained with microgl (http://www.restfmri.net). XJView8 (http://www.restfmri.net) software was used to see the anatomic positions of the brain regions that corresponded to Montreal Neurological Institute. In the paired t-test, the threshold value of a cluster with more voxels was calibrated to obtain an image. Brain regions showing a statistical difference were identified by defining a threshold for each cluster >85 voxels and a threshold of a voxel <0.05 (calibrated). The 3-dimensional (3D) picture of the whole brain was obtained with microgl (http://www.restfmri.net). XJView8 (http://www.restfmri.net) software was used to see the anatomic positions of the brain regions that corresponded to Montreal Neurological Institute.

ReHo analysis: the Resting-State fMRI Data Analysis Tool kit software (REST, Song et al, http://www.restfmri.net, Beijing Normal University) was used to process the data on brain function. The identity in time order of every voxel and its surrounding 26 voxels of the whole brain was calculated to obtain Kendall’s coefficient of concordance (KCC) for every voxel. The KCC value of every voxel of the whole brain divided by the mean value of KCC values over all voxels yields a standardized ReHo image. Finally, the ReHo image was smoothly processed by convolution with a Gauss nucleus of FWHM=4 mm to ensure that image data had a random Gauss field to meet the statistical hypothesis of SPM and to enhance the signal-noise ratio.

SPM8 was then used to statistically analyze the ReHo image with a paired t-test. The threshold value of a single voxel was P<0.05. The minimum cluster with more than 80 continuous voxels was calibrated to obtain brain regions with a statistical difference between the data of the two groups.

RESULTS

Results of routine skull MRI

A small laminated abnormal signal can be seen in the lateral ventricle of some patients with a wider cerebral fissure in some brain regions, indicating that patients have mild degeneration of white matter and mild encephalatrophy. However, resting state fMRI data met the conditions for processing.

Figure 1 and Table 1 show the brain regions (by paired t-test) that had a changed (increased or decreased) fALFF after moxibustion (P<0.05). Figure 2 and Table 2 show the ReHo comparison (by paired t-test) before and after moxibustion.

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Voxels</th>
<th>Peak MNI coordinates</th>
<th>Peak intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X</td>
<td>Y</td>
</tr>
<tr>
<td>Right cerebrum</td>
<td>191</td>
<td>24</td>
<td>-36</td>
</tr>
<tr>
<td>Extra-nucleus</td>
<td>241</td>
<td>-12</td>
<td>-6</td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>150</td>
<td>-12</td>
<td>-66</td>
</tr>
<tr>
<td>Left cerebrum</td>
<td>269</td>
<td>-60</td>
<td>-3</td>
</tr>
<tr>
<td>White matter</td>
<td>85</td>
<td>-15</td>
<td>-39</td>
</tr>
<tr>
<td>Precentral gyrus</td>
<td>113</td>
<td>39</td>
<td>-21</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>279</td>
<td>-21</td>
<td>-21</td>
</tr>
<tr>
<td>Occipital Lobe</td>
<td>310</td>
<td>18</td>
<td>72</td>
</tr>
</tbody>
</table>

Notes: fALFF: frequency fluctuation; MNI: Montreal Neurological Institute. AlphaSim calibration.

Table 2 Changes in ReHo value before and after moxibustion

<table>
<thead>
<tr>
<th>Clusters of voxels</th>
<th>Brain regions</th>
<th>Peak Intensity</th>
<th>Peak MNI coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>Thalamus</td>
<td>6.1624</td>
<td>-3</td>
</tr>
<tr>
<td>99</td>
<td>Extra-nucleus</td>
<td>5.2246</td>
<td>54</td>
</tr>
<tr>
<td>139</td>
<td>Parietal lobe</td>
<td>3.7505</td>
<td>33</td>
</tr>
<tr>
<td>96</td>
<td>Right cerebrum</td>
<td>-4.1649</td>
<td>33</td>
</tr>
<tr>
<td>103</td>
<td>Left cerebrum</td>
<td>-4.4573</td>
<td>-15</td>
</tr>
<tr>
<td>1012</td>
<td>Frontal lobe</td>
<td>-6.1189</td>
<td>-18</td>
</tr>
</tbody>
</table>

Notes: Relto: regional homogeneity; MNI: Montreal Neurological Institute. In the paired t-test, the threshold value of a voxel was P<0.05, and the threshold value of a cluster with 85 continuous voxels was K≥85.

DISCUSSION

In TCM, there is no formal term for KOA, which belongs to the category of arthralgia syndrome or rheumatism involving the bone. Differing from heat therapy as applied in Western Medicine, moxibustion can exact a curative effect on KOA through the promotion of the flow of Qi which warms the channel and eliminates arthralgia syndromes by dispelling cold. Moxibustion is a new therapy that can be individualized in clinical
practice and dynamically stimulates local points to give play to the transmitting effect of channels and collaterals and regulate the function of internal organs and the body. Moxibustion has the effect of integrating heat therapy, light therapy, medication and point stimulation. In this experiment, fMRI technology was used to observe changes in resting brain function before and after moxibustion at the left Dubi (ST 35) to treat KOA. We have found that the fALFF values of the right cerebrum, extra-nucleus, left cerebellum, left cerebrum and white matter region after moxibustion were much higher than before moxibustion, and that the fALFF values of the precentral gyrus, frontal lobe and occipital lobe were lower than before moxibustion (P<0.05, K≥85). The ReHo values of the thalamus, extra-nucleus and parietal lobe were much higher than those before moxibustion, and the ReHo values of the right cerebrum, left cerebrum and frontal lobe were much lower than those before moxibustion. (P<0.05, K≥85). The frontal lobe, the largest of the four cerebral lobes, is responsible for thinking and planning, takes part in the integration of information from the interior and exterior environment, regulates emotion, and draws memory of

Figure 1 Change in fALFF before and after moxibustion
T: temperature; fALFF: fractional amplitude of low frequency fluctuation.
All the subjects in the experiment were older people whose cognitive function had probably declined and their KOA was mainly manifested as pain. These main factors may influence the reduction in the value of fALFF and ReHo of the frontal lobe. Because moxibustion was only performed at the left Dubi (ST 35), at the beginning, a strong reaction took place mainly in the hemisphere of the right cerebrum, and with the continuation of moxibustion, the reaction became increasingly stronger in the hemisphere of the left cerebrum. The result of fMRI analysis shows that the strongest reaction occurred in the cerebrum: the fALFF values of the left cerebrum and right cerebrum were much higher than those before moxibustion, and the ReHo values were much lower. The cerebrum is a superb nerve center to control motion, produce sensation and realize higher brain function. A strong reaction of the cerebrum to moxibustion may be a process of reflecting stimulation and integrating information. Obviously, the activated cerebellum mainly controls and coordinates information on fine motion and sensation. Much higher ReHo value of the extra nucleus may be related to the early sensation of patients to moxibustion. The activation of white matter regions comprising nerve fi-

Figure 2 ReHo comparison (by paired t-test) before and after moxibustion
T: temperature; ReHo: regional homogeneity.
bers may be related to pain in KOA patients. After moxibustion, the region may obviously inhibit pain and raise pain thresholds to noticeably alleviate the pain of patients. An obvious reduction in the fALFF value of the precentral gyrus is closely related to peripheral nerves. The remarkable changes seen in these brain regions indicate that the cerebral mechanism of moxibustion basically conforms to the way of transmitting pain in the body. The activation of the sensory system and relevant brain regions (such as the thalamus) may be objective evidence of the transmittance of channels and collaterals. However, the essence of channels and collaterals has not been completely made clear so far, and the mechanism for the functions of the nervous system may be nothing but a part of the system of channels and collaterals. Cerebral functions are regulated by moxibustion not in a single brain region but over a large network of many brain regions.

REFERENCES