Magnetic Resonance Imaging and Acupuncture: A Feasibility Study on the Migration of Tracers after Injection at Acupoints of Small Animals

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Abstract

Objectives: Acupuncture meridians in traditional Oriental medicine are known to be channels connecting specific points in the surface of the body to corresponding internal organs. We investigated the permeation and the transport of magnetic resonance imaging (MRI) contrast agent and tracer after injection at acupoints of small animals, such as rats and mice.

Methods: A geometric and systematic arrangement of acupuncture points on human skin surfaces has been depicted in traditional Oriental medicine, and the positions of the acupoints of small animals were determined by the application of a proportion on the animals corresponding to the morphological structures in humans. After injecting the materials at various acupoints, the agent migration behaviors inside the body were monitored by MRI. The distributions of the injected materials were reconstructed in 3-dimensional images for a more intuitive presentation.

Results: The widely-used gadolinium-compound contrast agent was not useful. Rather, a recently developed fluorine compound was effective for imaging the migration of the agent after injection into the acupoints BL18, 20, and 23.

Conclusions: The final distributions of the agent from each injection point corresponded to the respective organs of the acupoints. The results suggested different migration paths and destinations for pharmacopuncture drugs.

1. Introduction

In traditional Oriental medicine, acupuncture points (or acupoints) and acupuncture meridians are known to constitute channels connecting the body surface to internal visceral organs. The meridians are distributed longitudinally along the body and connect with specific organs, such as the liver, spleen, and...
kidney. Acupuncture has been used in treatments for diverse diseases and has been recognized in the West as a useful and effective procedure in complementary medicine, especially in the treatment of pain [1]. For many years, a number of researchers have attempted to scientifically investigate the field of acupuncture and to establish acupoints and meridians as anatomical entities [2–5]. In the neurophysiologic perspective, for example, they have investigated correlations between acupoint stimuli and the activities on corresponding brain cortices by functional magnetic resonance imaging (MRI) [6–8].

One of the early attempts to study the meridian system as a circulating network of ductules and corpusscles was done by adopting techniques of radiosimetry and radioautography with injections of the radioactive tracer $^{32}$P in the skin of the thigh and of the abdomen of a rabbit [2]. More recently, based on the high conductivity of isotopic tracers along deep skin acupoints and meridians, the migration of a radioactive tracer, technetium-99m was studied after injection at acupoints of human subjects [9]. The preferential pathways taken by the radiotracer were found to coincide with the acupuncture meridians as described in traditional medicine, and those pathways were distinguishable from either lymphatic or vascular routes. These observational views were, however, localized around small regions of some acupoints and were superficial with two-dimensional images. The much more detailed field of view obtained from three-dimensional images is required for a more meaningful study of the overall network.

In this study, we focused on investigating the pathways taken by tracers injected at the acupoints of small animals. Tracers were monitored by MRI over the animals’ trunks. In the field of modern biotechnology, MRI is an advanced medical tool available to researchers or physicians for sectional imaging of the inner body. In our case, two kinds of injection materials were used: a gadolinium-based compound, which is widely used in medicine as an MRI contrast-enhancing agent, and a fluorine compound, which allowed clear differentiation of the tracer from the background of the body. Combined fluorine-19 and proton MRI measurements are carried out in broad areas of biotechnology for many purposes, such as tracking immunotherapeutic cells or guiding the design of improved tumor-oxygenating agents [10,11]. For a more intuitive understanding here, the distributions of injected materials were reconstructed using three-dimensional image-processing software. The experimental imaging data taken from dozens of animals are presented here and the feasibility and limitations of the present methods for the study of acupuncture and the meridian system discussed.

2. Methods

2.1. MRI instruments

All imaging was performed on a 4.7-Tesla MRI system (Bruker BioSpec, Germany), which was designed for experiments with small rodents, such as rats and mice. Its horizontal bore size was about 400mm and there were two resonators for simultaneous detection of protons ($^1$H) and fluorine ($^{19}$F) with resonant frequencies of 200 and 188MHz, respectively. Protons were taken as $T_2$ images with spin echo, and fluorine as $T_2^*$ images with gradient echo. The spatial resolution of a sectioned proton image was around 200 $\mu$m, or that of a sectioned fluorine image around 400 $\mu$m, and the depth resolution 1 $\mu$m in all cases. In other words, all the image information inside the depth was summed into a single image. The field of view was properly adjusted to cover the whole area of the body trunk as much as possible with the injection point at the image center. The animal-keeping holders were also specially designed for experiments with rats and mice and were loaded at the center of the MRI instrument. The cardiac rates of each animal were tracked and synchronized with the MRI to eliminate unnecessary blurring effects caused by movement.

2.2. Small animals: rats and hairless mice

Male Rats (Wistar, body weight ∼200g) were purchased from Jung-Ang Laboratory Animal Company, with thirteen rats used. The animals were housed at room temperature, constant relative humidity, with a 12:12 hour light:dark cycle, and fed food and water ad libitum. A total of 23 hairless, female mice (HR-1, 6-week old; purchased from the Han-Lim Lab. Animal Co., Korea) were maintained for 2 weeks under the same conditions. The procedure involving the animals and their care were in full compliance with the institutional guidelines of Seoul National University and current international laws and policies (Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996). The mice were anesthetized using respiration equipment and the anesthetized mice placed on the observation stage of the MRI after the contrast agents or tracer compounds had been injected.

2.3. MRI contrast agent: gadolinium compound

Magnevist (Schering, Germany), a gadolinium-based MRI contrast agent, was used for the rat experiments at 0.5mmol adjusted with saline solution and injection volumes at 200 $\mu$L. This contrast agent is known to produce magnetic effects and has been
widely used to enhance the visualization of blood vessels, internal organs, and other non-bony tissues by MRI.

2.4. MRI tracer: fluorine compound

Perfluoro-15 crown-5 ether (PFCE; chemical formula (CF₂CF₂O)₅; Exfluor, USA) was purchased and used for the mouse experiments. The injection volumes of 99% liquid PFCE were varied from 20–100 μL depending on the purpose of the experiment, without any dilution or emulsion. The chemical is insoluble in water, and its density is 1.78 g/mL at 20°C.

2.5. Injection points and methods

Four different kinds of injection points were chosen: the LSP (lumbosacral point) on the dorsal midline at the lumbosacral junction, the Bladder Meridian BL18 (Gan Shu, transport point to the liver) lateral to the caudal border of the spinous process of the tenth thoracic vertebra along the longitudinal line of the costal tubercle; the BL20 (Pi Shu, transport point to the spleen) on the twelfth thoracic vertebra; the BL23 (Shen Shu, transport point to the kidney) on the second lumbar vertebra (Figure 1). With the first three types being pairs of points, the selected seven points were identical with those of the transpositional acupoint system in the mouse and rat model proposed by Yin et al [12]. The LSP in the Governing Vessel meridian was the same as

![Figure 1 Injection points on the rats and mice. Points marked on X-ray images.](image)

![Figure 2 Time course of Gd-based contrast agent with five rats. Injection points are the LSP (200 μL), indicated with arrows; rectangular dotted boxes, areas where injected contrast agent had diffused.](image)
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the GV20L of Yin et al. Careful injections were made using insulin syringes and needles (31G, BD Ultra-Fine II, USA) in a direction perpendicular to the skin surface to the depth of the superficial fascia. The duration of the injection for each animal depended slightly on the volume of the injected material, but generally the time was less than 10 seconds for one shot. The injection speed for one shot was more or less the same for every animal at about 10 μL/s.

3. Results

The first experiments involved five rats being injected with the gadolinium compound at their LSP, with all injections made within a few minutes and in the same way for each rat, and then MRI images taken over time. The control image (Figure 2, far left), shows the sagittal section of the first rat, without any injection of the gadolinium compound, loaded into the magnetic resonance chamber. Three hours later, the second rat with gadolinium injected at the LSP was loaded and the MR image obtained, which showed that the injected material had spread around the injection point (Figure 2, dotted box). From successive images recorded at 4, 5, and 6 hours, it was apparent that the maximum intensity over a large area was achieved at 4 hours and decreased thereafter. The fading intensity meant that there was a spreading of the injected material into the entire body and, clearly, the injected material acted as an image contrast enhancing agent and brightened the region. As these slides were single-section images, it was not possible to obtain information on the spread of the material beyond the single section and discerning any fine structures along which material may have flowed was also difficult.

The effects of individual differences in the positions or sizes of the animals were avoided by using a single rat to trace the distribution of the injected material and special computer software to reconstruct 3-D images by combining the slides of successive sections (12 slides corresponding to a 12 mm depth). The results shown in Figure 3 were drawn as volume-rendered images with iso-intensity surfaces and brown and gray are for low and high intensity levels in a relative scale, respectively.

Figure 3 Time course of the Gd-based contrast agent with a single rat, represented by three-dimensional reconstructed images of isointensity surfaces. Fine structures (arrows) of 1 mm seen as flow path along spine 5 minutes after injection; gray and rust colors indicate high and low intensity levels in a relative scale, respectively.
in this region represents the presence of the gado- 
linium compound as a contrast-enhancer. After 1 
hour, the injected materials had spread beyond of 
the fields of view in the sectioned images. This 
method was more informative than the previous 
one but, in practice, distinguishing the injected 
materials from the background proved difficult. 

One strategy for elucidating more fine structures 
of the flow channels was to use tracers more suit-
able for the monitoring machine. For the present 
MRI system, one such tracer was a fluorine com-
 pound which made it possible to clearly distinguish 
the injected material from the background by dou-
bling the time spent for the image scan (Figure 4, 
a typical image). The protons and fluorine could 
be represented by red and green pixels, respectively, 
and a 3-D reconstructed image was generated along 
with 2-D section images. Injections were made at 
the other acupoints (BL18, BL20, and BL23) in 
the same manner with groups of hairless mice, and 
the areas around the injection points monitored using 
the transverse (axial) section images. Figure 5 shows 
typical images of two mice each from injections of 
PFCE at (A) BL20 and BL23 and (B) BL18 bilaterally. 
In mouse 1 (Figure 5A) the image showed the arrival 
of the agent near the stomach (BL20) and near the 
kidney (BL23); in mouse 2, the agent arrival near the 
liver after bilateral injection at BL18 (Figure 5B). 
A detailed description for the final distributions of 
the injected material in each subject is presented 
in Table. The time lapse after the injections was 
also noted to observe its effect on the flow inside 
the body and it was found that the two primary 
pathways followed by the tracer were the spine 
and/or the inner surface of the skin muscle toward 
the ventral side.

4. Discussion

The purpose of the present work was to trace the 
paths from acupoints to internal organs using a 
MRI-based technique. For this purpose, we injected 
two different contrast agents at acupoints LSP, BL18,
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Table Description of the observed distribution of PFCE injected into hairless mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Subject</th>
<th>Time after injection (hr)</th>
<th>Injection points</th>
<th>Observed distribution of PFCE (analyzed with 18 sectioned images in transverse plane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>BL23(L), BL23(R)</td>
<td>On dorsal part of left kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Around vertebrae</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2.0</td>
<td>BL23(L), BL23(R)</td>
<td>On left and right kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Along caudal vena cava</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>18.0</td>
<td>BL23(L), BL23(R)</td>
<td>Around dorsal part of left kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Below vertebral column (ventral side)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>18.5</td>
<td>BL23(L), BL23(R)</td>
<td>Following aorta and covering left lung</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.1</td>
<td>BL18(L), BL20(R)</td>
<td>On skin just around injection points</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Around left lung</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1.0</td>
<td>BL23(L)</td>
<td>Along lumbar vertebrae and around vertebral column</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2.0</td>
<td>BL20(L), BL23(R)</td>
<td>Up to right kidney</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Below vertebral column</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3.0</td>
<td>BL18(L), BL23(R)</td>
<td>Along the left BL line</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Upper part of right kidney</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>20.0</td>
<td>BL20(L), BL23(R)</td>
<td>Along left and right BL lines toward cranial side and up to liver</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>20.5</td>
<td>BL18(L), BL18(R)</td>
<td>Spreading over muscles of body wall and up to spleen</td>
</tr>
</tbody>
</table>

Amount of injected PFCE was 50 μL at various acupoints; MRI images taken at indicated times; PFCE detection areas not assessed over the whole animal trunk; for each mouse, 18 axially sectioned images of transverse planes along the trunk were obtained so that the field of view for the observations covered a distance of 18 mm with an entire sectioned image around the injection points.
BL20, and BL23 and found that the water-soluble gadolinium compound did not provide the desired resolution because it diffused into the muscle, such that it could not be distinguished from the background as time elapsed. On the other hand, the lipid-soluble fluorine compound PFCE produced a distinctive contrast, but the related volume rendering for the 3-D reconstruction image of the tracer was rather coarse and of low resolution; thus the spatial resolution was poor. Overall tracing the channels from the acupoints by MRI was not possible due to the poor spatial resolution of the technique.

A hopeful result from this feasibility study was that migration of the PECE to the organs was more or less consistent with Traditional Oriental Medicine. BL18, 20, and 23 are the control points of the liver, spleen, and kidney, respectively, according to acupuncture theory. In the present results, we found reasonable evidence for the migration of PFCE to the target organs. Non-acupoints were not similarly tested as controls because of the poor spatial resolution of the current method. Nevertheless, without control experiments we still showed different migration paths for different acupuncture points in the same Bladder Meridian.

The current experiments have significant implications for pharmacopuncture. The migration of the drug depended strongly on the tracer’s water- or lipid-solubility and the acupoints of the injections were important factors for determining the destined organs. This also has significance for Western medicine because of its potential for a new modality of drug administration.

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References