Serial evaluation of axonal function in patients with brain death by using anisotropic diffusion-weighted magnetic resonance imaging

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Object. The purposes of this study were to evaluate the serial changes in diffusion anisotropy of the brain, probably reflecting axonal function in brain-dead patients, and thus to explore the possibility of quantitatively estimating the risk of brain death.

Methods. Ten patients suffering from stroke with or without impending brain death and 10 healthy volunteers were studied using three-dimensional anisotropy contrast (3DAC) magnetic resonance (MR) axonography with the aid of a 1.5-tesla MR imaging system. To detect changes in the diffusion anisotropy of neural bundles, the corticospinal tract was evaluated.

Diffusion anisotropy of short axonal fibers decreased immediately after apparent brain death. Whereas the trichromatic coefficients of the corticospinal tract greatly diminished between 6 and 12 hours after apparent brain death, the coefficients of the corpus callosum and the optic radiation decreased in less time, that is, between 1 and 6 hours. The coefficients of these three bundles turned isotropic between 24 and 44 hours after apparent brain death.

Conclusions. Results of 3DAC MR axonography revealed that diffusion anisotropy of neural bundles diminished between 1 and 12 hours after the onset of apparent brain death, probably depending on the length of the bundles, and disappeared between 24 and 44 hours after the onset of brain death, which might reflect dynamic changes of axonal structure and indirectly herald axonal dysfunction. These findings seem to be greatly helpful in establishing an appropriate method to estimate the risk of brain death quantitatively and in forming the basis of future definitions of brain death.

Abbreviations used in this paper: ABR = auditory brainstem response; ATP = adenosine triphosphate; DW = diffusion-weighted; EEG = electroencephalography; MR = magnetic resonance; 3DAC = three-dimensional anisotropy contrast magnetic resonance axonography • axoplasmic transport

The diagnosis of brain death has grown in importance in connection with organ donation. There is no single method, only criteria,1,14,15 with which to estimate quantitatively and objectively the risk of brain death, namely irreversible cessation of entire brain function. Cerebral angiography, single-photon emission computerized tomography, and MR imaging are used to complement these criteria or to confirm brain death. To establish an appropriate way of quantifying the risk for brain death, it is essential to comprehend the physiological changes in brain functions in patients with impending brain death. These contexts greatly encouraged us to assess the dynamic changes in axonal function, one of the major brain functions, in patients at risk for brain death. Such changes have never to our knowledge been assessed because of the extreme and various difficulties in performing the necessary studies.

To date, there is no method capable of noninvasively and directly evaluating axonal function of the brain in humans. Nonetheless, quantitative estimation of diffusion anisotropy of the brain by using DW MR imaging was expected to allow us indirectly to assess changes in axonal function of the brain. Diffusion-weighted MR imaging procedures reflect tissue diffusion, perfusion, and fluid flow. These motions result in a distribution of phases in a single voxel in the presence of magnetic field gradients. Consequently, this distribution produces a spin echo attenuation.9 It is well known that DW MR images of the brain mainly demonstrate the motion occurring along neural bundles as diffusion anisotropy. Therefore, diffusion anisotropy of the brain in some instances might indirectly reflect axoplasmic transport.

To quantify diffusion anisotropy, a number of methods are available: isotropic DW imaging, trace imaging, and full tensor analyses. Of these, the most suitable method in patients suffering from severe brain damage is, we believe, 3DAC MR axonography because of the importance of maintaining good anatomical resolution, which will affect the quality of the final image.9–12 The usefulness of 3DAC MR axonography was previously demonstrated by detecting wallerian degeneration of the corticospinal tract in patients with early-stage stroke and hemiparesis.3,16

The purposes of this study were to evaluate the serial
Axonal function in brain-dead patients

changes in diffusion anisotropy of the brain, likely reflecting axonal function in stroke patients with impending brain death, by using 3DAC MR axonography and thus to explore the possibility of quantitatively estimating the risk of irreversible cessation of brain function, that is, brain death.

### Clinical Material and Methods

#### Healthy Volunteers and Patients

This study was performed according to the human research guidelines of the Internal Review Board of Suiharago General Hospital. Written informed consent was obtained from all healthy volunteers and the relatives of all patients. Seven stroke patients with impending brain death, three stroke patients without impending brain death, and 10 healthy volunteers were recruited into this study. All participants were studied to estimate the normal range of diffusion anisotropy before and after apparent brain death, and to compare patients with and without brain death. Brain death was diagnosed based on the following criteria: 1) deep coma (Glasgow Coma Scale Score 3); 2) apnea; 3) bilaterally fixed pupils larger than 4 mm in diameter; 4) absence of brainstem reactions such as corneal, ciliospinal, oculocephalic, vestibular, pharyngeal, and cough reflexes; 5) abrupt fall in blood pressure followed by persistent hypotension; and 6) isoelectric EEG results and no ABR except for the first wave. Apparent brain death was defined as severe brain damage satisfying all of the aforementioned criteria shortly before actually verifying it with the sixth criterion (EEG and ABR). The onset of apparent brain death was defined as the occurrence of apnea. All seven patients with impending brain death could be diagnosed as having brain death by verifying the sixth criterion by EEG and ABR within 6 hours after diagnosis. The three patients without impending brain death were comatose and experienced respiratory arrest caused by direct damage to the brainstem; none of these patients satisfied the aforementioned criteria. A clinical summary of the 10 stroke patients (seven with and three without brain death) is listed in Table 1.

#### Data Collection

A 1.5-tesla whole-body MR imaging system (Signa Horizon; GE Medical Systems, Milwaukee, WI) was used to perform all studies. A single-shot echoplanar imaging technique was used to obtain anisotropic DW images for 3DAC MR axonography. The parameters for DW images were as follows: TR 10,000 msec; TE 96.8 msec; two acquisitions; matrix 128 × 128; field of view 300 × 190 mm; slice thickness 5 mm; and no gap between slices. Three anisotropic DW images of identical volume, representing each of the orthogonal axes, were obtained using a b value of 750 seconds/mm². The total imaging time for each study was approximately 80 seconds. A 3DAC MR axonogram was obtained in all participants, serially in three of the seven patients.

#### Processing of the 3DAC MR Axonography Image

The 3DAC MR axonography images were obtained using a method reported previously. The images provided sensitive MR imaging contrast for axonal information by displaying qualitative information (directional) through spectral frequency (color) and quantitative information (relative density) through the intensity of each color that appears simultaneously within the same pixel. The colors assigned to each direction were as follows: right to left, red (R); anterior to posterior (up and down), green (G); and orthogonal to axial imaging plane, blue (B). Commercially available computer software (GE Yokogawa Medical Systems, Hino, Tokyo, Japan) was used to produce 3DAC MR axonograms, and image analyses were performed with the aid of a personal computer.

#### Diffusion Anisotropy in Neural Bundles

Diffusion anisotropy of the corticospinal tract, corpus callosum, and optic radiation (including the genu and body of the corpus callosum and the trigone of the lateral ventricle) was evaluated with the aid of axial images obtained using 3DAC MR axonography. The diffusion anisotropy of neural bundles was evaluated by using the mean trichromatic coefficients [max{R, G, or B}/(R + B + G)] × 100 of nine (3 × 3) pixels including the neural bundles on each side (Fig. 1). Values are expressed as the means ± standard deviation.

#### Results

#### Diffusion Anisotropy of Neural Bundles in Healthy Volunteers

On the 3DAC MR axonography images, the corticospinal tract, corpus callosum, and optic radiation exhibited rich blue, red, and green hues, respectively, because of the fibers running in the direction of the z-axis (perpendicular to the axial slice), x-axis (right to left in the axial image), and y-axis (anterior to posterior in the axial slice), respectively (Fig. 2). The trichromatic coefficients of the corticospinal tract, corpus callosum, and optic radiation in healthy volunteers were 94.7 ± 2.8%, 93.5 ± 4%, and 70.3 ± 7.7%, respectively.

#### Changes in 3DAC MR Axonography Findings Related to Apparent Brain Death

Representative 3DAC MR axonography images ob-

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**Table 1**: Clinical summary of 10 stroke patients with or without brain death

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>Stroke Type</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>w/brain death</td>
<td>w/o brain death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70, F</td>
<td>SAH</td>
<td>1, 6, &amp; 48 hrs⁺</td>
</tr>
<tr>
<td>2</td>
<td>48, F</td>
<td>SAH</td>
<td>before &amp; 12 hrs⁺</td>
</tr>
<tr>
<td>3</td>
<td>69, M</td>
<td>SAH</td>
<td>24 &amp; 44 hrs⁺</td>
</tr>
<tr>
<td>4</td>
<td>63, M</td>
<td>ICH</td>
<td>48 hrs⁺</td>
</tr>
<tr>
<td>5</td>
<td>67, M</td>
<td>SAH</td>
<td>4 days⁺</td>
</tr>
<tr>
<td>6</td>
<td>55, M</td>
<td>ICH</td>
<td>4 days⁺</td>
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<tr>
<td>7</td>
<td>46, M</td>
<td>SAH</td>
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<td>8</td>
<td>68, M</td>
<td>ICH</td>
<td>2 hrs⁺</td>
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<tr>
<td>9</td>
<td>48, F</td>
<td>SAH</td>
<td>24 hrs⁺</td>
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<tr>
<td>10</td>
<td>78, M</td>
<td>infarction</td>
<td>48 hrs⁺</td>
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* ICH = intracerebral hemorrhage; SAH = subarachnoid hemorrhage.
⁺ Time after apparent brain death.
‡ Time after respiratory arrest.
tained before and after apparent brain death are shown in Fig. 2. Associations between changes in the imaging findings and the time after apparent brain death were assessed using the trichromatic coefficients. The diffusion anisotropy of the axonal fibers in patients before apparent brain death did not differ from that in healthy volunteers. The anisotropy in short axonal fibers appeared to be reduced immediately after apparent brain death. Although the trichromatic coefficients of the corticospinal tract remained within normal range until 6 hours after apparent brain death, the coefficients dramatically decreased 12 hours after apparent brain death (Fig. 3). On the other hand, the coefficients of the corpus callosum and optic radiation greatly decreased between 1 and 6 hours after apparent brain death (Figs. 4 and 5). Between 24 and 44 hours after apparent brain death, the trichromatic coefficients of almost all of the axonal bundles reached 33.3%, the isotropic line; that is, diffusion anisotropy had disappeared.

In the patients with brain death 2, 24, and 48 hours after respiratory arrest (apparent brain death), all of the trichromatic coefficients of the corticospinal tract, corpus callosum, and optic radiation were also smaller compared with the respective coefficients in the patients without brain death, which remained within normal range (Figs. 3–5).

Discussion

Data from the current study revealed that subtle and dynamic changes in brain structures might occur in stroke patients at risk for brain death. These changes herald definitive dysfunction, that is, probable disturbance of axoplasmic flow, and might be detected as a reduction in diffusion anisotropy on 3DAC MR axonography. We believe that these findings are greatly helpful both in establishing an appropriate method to estimate the risk of brain death quantitatively and in elucidating the mechanism of brain death. This is the first report to date regarding associations between dynamic changes in diffusion anisotropy and time in patients at risk for brain death, although there have been several case reports regarding isotropic DW MR images of brain death.5,7,13
In our study, anisotropy of the corticospinal tract decreased between 6 and 12 hours after the onset of apparent brain death; anisotropy of relatively short bundles, such as the corpus callosum and the optic radiation, rapidly diminished between 1 and 6 hours of the onset of brain death. Subsequently, anisotropy reached the isotropic level between 24 and 44 hours after apparent brain death. A study by Matsuzawa, et al., who also used 3DAC MR axonography, revealed that the anisotropy of the rat spinal cord reduced within the first 2 hours of cardiac arrest and reached a plateau by the 4th hour postmortem, results similar to our findings except for a difference in time scale. In the study of Matsuzawa and colleagues, the short period of time to anisotropic reduction may be ascribable to the short length of the axonal bundles in the rat spinal cord, although there were several differences in study parameters, such as species.

As mentioned earlier, the diffusion anisotropy might indirectly reflect axoplasmic flow, which is driven by axonal transport systems. The disappearance of the transport indicates severe impairment of the axonal function. Data from recent extensive studies have revealed that axonal transport systems are driven by various types of microtubule-associated motors, such as kinesin superfamily proteins, which are enzymes specializing in the generation of force and movement along their cellular tracks (that is, microtubules using hydrolysis of ATP). From our data, one can infer that the ATP exhaustion following complete brain damage is responsible for irreversible damage to the axoplasmic transport of neural bundles, resulting in a reduction in diffusion anisotropy. Furthermore, subsequent destruction of myelin sheaths causes the disappearance of anisotropy. This hypothesis also seems to be consistent with our finding that the time interval from apparent brain death to anisotropic reduction in the corticospinal tract was longer than that in relatively short bundles, likely because of the longer bundles containing greater amounts of ATP. Further studies are highly warranted.

Conclusions

The results of 3DAC MR axonography revealed that diffusion anisotropy of neural bundles diminished between 1 and 12 hours after onset of apparent brain death, probably depending on the length of the bundles, and disappeared between 24 and 44 hours after the onset of brain death, which might reflect subtle and dynamic changes of axonal structure and indirectly heralding axonal dysfunction. These findings seem to be highly relevant both in establishing an appropriate method to estimate the risk of brain death quantitatively and in forming the basis of future definitions of brain death.

References

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