Scalp acupuncture effects of stroke studied with magnetic resonance imaging: different actions in the two stroke model rats

Isao Inoue,1 Mari Fukunaga,2 Keiko Koga,2 Hong-Du Wang,3 Makoto Ishikawa2

ABSTRACT

Background: Scalp acupuncture (SA) therapy on strokes has been empirically established and widely used in clinics in China. The evidence from clinical studies suggests that SA produces significant benefits for some patients with stroke.

Methods: The effect of scalp acupuncture was studied using MRI for two different stroke models: spontaneously hypertensive stroke-prone (SHR-SP) rats and rats with transiently induced focal cerebral ischaemia by middle cerebral artery occlusion for 2 h (MCAO rats).

Results: Stroke onset in SHR-SP rats was characterised by a development of vasogenic oedema without any appearance of cytotoxic oedema. Scalp acupuncture reduced rapidly neurological dysfunction in SHR-SP rats and reduced the volume of the vasogenic oedema during the same period. In contrast, in MCAO rats, focal cerebral ischaemia caused an immediate development of cytotoxic oedema without any appearance of vasogenic oedema. Vasogenic oedema developed after reperfusion. Scalp acupuncture had no significant effects on the cytotoxic oedema, vasogenic oedema or neurological dysfunction of the MCAO rats within the time span examined.

Conclusion: Scalp acupuncture had a rapid and strong effect on neurological dysfunction only in the hypertensive stroke-model by reducing the vasogenic oedema. Our results suggest that, if there are similar underlying mechanisms in human strokes, scalp acupuncture may be more beneficial for patients with strokes of hypertension-caused vasogenic origin than ischaemic origin.

Scalp acupuncture (SA) is a therapy used to treat neurological dysfunction by needling specific stimulation areas in the scalp. This therapy originated in China from “Huangdi’s Internal Classic” over 2000 years ago. However, its rapid development and wide clinical use for therapy in strokes have occurred only since the 1970s. In 1984, the China Association of Acupuncture and Moxibustion standardised these portions onto 14 lines, and these 14 lines have been approved as an “International Standard of Scalp-Acupuncture” at the WHO West Pacific Zone Conference (Japan, 1984). It is now suggested from statistical analyses of clinical results that the effects of scalp acupuncture on strokes are significant. Evidence from clinical studies suggests that SA produces significant benefits for some patients with stroke, with good results reported in 60–80% of patients. Comparative studies have shown that the rate of recovery in SA-treated patients with stroke was approximately twice that in those treated with medication alone.4–6 One remarkable effect of SA is a rapid and phasic recovery from paralyses. The effect appears in 60% of patients with stroke within 10–30 min after an SA treatment, and during the phasic period the muscle force in the paralytic side increases by two grades or more.4–6 Although the mechanisms are unknown at present, these clinical results strongly suggest that the stimulation of SA is transmitted to the brain and activates the inherent self-curing function. In order to elucidate such effects of SA on a scientific basis, it is important to reproduce the SA therapy in experimental animals that provide reliable controls and exclude psychological effects. A genetic strain of rats, spontaneously hypertensive stroke-prone (SHR-SP), is an animal model which develops spontaneous onset of hypertensive strokes. In many aspects, cerebrovascular pathology resembles the human disease.7–8 Rats with surgery-induced focal brain ischaemia by middle cerebral artery occlusion (MCAO) have been used as an animal model of ischaemic strokes.

MRI is one of the most powerful tools for the detection of cerebrovascular abnormalities, especially for seeking the time-dependent changes in abnormalities under non-invasive conditions. Hence, MRI observations of the brain of SHR-SP and that of MCAO rats have been made rather extensively.9–17 Vasogenic oedema that occurs by an increase in the water volume in the extracellular space as a consequence of the impairment of the blood–brain-barrier (BBB) can be detected by T2 imaging as increased values of the T2 relaxation time. Cytotoxic oedema that occurs by swelling of brain cells as the result of energy failure and loss of ion homeostasis can be detected by apparent diffusion coefficient (ADC) imaging as decreased ADC values. Previous reports have shown that the types of cerebrovascular abnormality associated with the stroke onset are different between SHR-SP and MCAO rats. The stroke onset in SHR-SP is characterised by the appearance of vasogenic oedema without any development of cytotoxic oedema.12 In MCAO rats, the brain ischaemia induces cytotoxic oedema but does not induce vasogenic oedema in the early stage of ischaemia.16

We studied the effect of SA using MRI on the two different stroke models: SHR-SP and MCAO rats with transient focal ischaemia for 2 h. This report describes the experimental results showing that SA showed different effects between the two stroke models.

MATERIALS AND METHODS

Animals

This study was performed in accordance with the Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co, 1 April 2004.
Forty male, 8-week-old SHR-SP rats were purchased from Japan SLC Inc (Hamamatsu, Japan). They were kept in an animal room at 22°C, lit for 12 h daily, fed dry foods for SHR-SP (Funabashi Farm, Chiba, Japan) and given distilled water containing 1% (w/v) NaCl. The NaCl water was given throughout the experiments. The blood pressure was measured by the tail-cuff method using an automatic sphygmonanometer (Softron, BP-98A, Tokyo). Rats that suffered a stroke were alternatively separated into two groups: rats in one group were treated with SA and the other used for control without SA treatment.

Twenty-eight male, 9–10-week-old Sprague-Dawley rats purchased from Japan SLC were used for MCAO. Rats were kept in the animal room and fed pellet food (MF, Oriental Yeast, Tokyo) and drinking tap water ad libitum. Rats were anaesthetised with thiopental (42 mg/kg, intraperitoneal), and placed on a heating pad to keep the body temperature at 38°C. Focal cerebral ischaemia was induced by transient occlusion of the left middle cerebral artery (MCA) as described. Briefly, surgical nylon suture thread (3–0 in size) with a rounded tip was advanced from the external carotid artery into the lumen of the internal carotid artery to block the blood flow of MCA. Two hours after MCAO, reperfusion was allowed by withdrawal of the suture thread. Then, each rat immediately underwent MRI observations. Only those rats in which the MCAO successfully induced cytotoxic oedema in the brain were used for experiments.

Grade of paralyses
When SHR-SP rats suffered stroke spontaneously, the animals became “dispirited,” the hair became fluffy and lost its lustre, and paralyses appeared in limbs at different sites between rats. Other dysfunction such as tics, hyperaemia in the eye and incontinence appeared but not in all rats. We classified the paralyses into five grades from observations of expressed paralyses, modifying the examinations of Bederson et al. Table 1 shows the characteristic symptoms of paralyses at each grade.

SA treatment
SA was treated for 10 min a day without anaesthetisation by alternatively changing the side of needle insertion. For SHR-SP SA treatment was started when the stroke onset was observed, and for MCAO rats after reperfusion. After sterilisation of the skin, a stainless steel acupuncture needle (0.3 mm thick and 26 mm long) was inserted from the point of “Baihui” (GV20) to the point that may correspond to “Qubin” (GB7) in the opposite side of paretic limbs between the galea aponeurotica and the periosteum along the cranial bone; electrical stimulation was then applied for 10 min using a pulse generator (Nihon Kohden, SEN-710, Tokyo). One stimulation output was connected to the needle, and the other to a hindlimb via a piece of wet cotton. Pulses were given in sets, each pulse consisting of a bipolar rectangular voltage pulse with 300 μs duration. For electrical stimulation, the pulses at 2.5 Hz were applied for 4 s at every 4 s interval. The pulse intensity was increased gradually and fixed when both the hindlimb and an ear near the needle tip were locally twitched while the pulses were given; the voltage was 3–3.5 V.

MRI observations
For MRI measurements, rats were gas-anaesthetised with isoflurane (1–2%) mixed with 30% O₂ and 70% N₂O, and placed in a stereotaxic head holder. The rectal temperature was feedback-controlled at 38°C by warm/cold water throughout the MRI experiments. MRI observations were performed using an Inova 500 System (7T) with VNMRj 1.1D software (Varian, Palo Alto, California). A volume coil and a surface coil (RAPID Biomedical, Rimpar, Germany) were used for signal transmission and detection, respectively. The bregma position was determined by the coronal images.

The T2 image was obtained by multislice spin echo sequence using the following parameters: pulse repetition time (TR) 5000 ms; echo time (TE) 10, 30, 70 and 90 ms; number of scans (NT) 1. ADC image was obtained by multislice Stejskal-Tanner type pulsed gradient spin echo sequence using the following parameters: TR 1500 ms; TE 40 ms; time between the rising edges of the two diffusion-encoding gradient (A) 22 ms; duration of these gradients (b) 12 ms; b-factors = 55, 400, 700, 1000 and 1300 s/mm²; NT 2. The slice thickness for T2 and ADC measurements for SHR-SP was 1 mm; the images of 13 or 15 slices were obtained. In T2 and ADC measurements for MCAO rats, the slice thickness was 2 mm, and the images of seven slices were obtained. For all T2 and ADC images, the matrix size was 256×128, the images were zero-filled to 256×256, and the field of view was 40×40 mm. The area of vasogenic oedema in a T2 image of SHR-SP was calculated, summing the number of pixels having T2 values greater than 50 ms, and that of the MCAO rat by summing the number of pixels having a deviation more than mean ± 3SD from the mean pixel values of the contralateral hemisphere. The area of cytotoxic oedema in an ADC image was obtained from the number of pixels with ADC values below 80% of each contralateral value. The oedema volume in the brain was calculated by summation of the oedema area of every slice multiplied by the slice thickness.

Dynamic susceptibility contrast MRI was performed to assess a relative cerebral blood volume (CBV) and a cerebral blood flow (CBF). After the precontrast image acquiring, Magnevist (Schering, Berlin), an MRI contrast agent, was administered via the tail vein at a dose of 0.3 mmol/kg. A series of 49 gradient-echo single slice images with TR 7.8 ms, TE 3 ms and flip angle 25° was acquired. Relative CBF and relative CBV were determined with commercially available image analysis software (MEDx, Version 3.45; Medical Numerics, Virginia) to evaluate the leakage of contrast agent caused by impairment of BBB permeability, a postcontrast image was acquired 10 min after Magnevist administration. T1 weighted images for pre- and postcontrast images were acquired by a multislice spin echo sequence with TR 500 ms, TE 12 ms and NT 2; the matrix size was 256×256. The images were zero-filled to 512×512, and the field of view was 25×25 mm. Contrast MRI was obtained by subtracting the precontrast images from the corresponding postcontrast images.

Table 1 Characteristic symptoms of paralyses at each grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>Mild wrist and elbow flexion and adduction of a shoulder appear. Rats can walk normally, but slower than normal rats.</td>
</tr>
<tr>
<td>2</td>
<td>Symptoms at Grade 1 become more pronounced with full flexion of the wrist and the elbow, and adduction of the shoulder with internal rotation. Rats can walk straight but are lame in the paretic limb.</td>
</tr>
<tr>
<td>3</td>
<td>This grade is characterised as being a significant decrease in the resistant strength against pushing toward the paretic side. Rats can walk, but not straight, and deviate toward the paretic side.</td>
</tr>
<tr>
<td>4</td>
<td>Rats cannot walk; they circle toward the paretic side or cannot move.</td>
</tr>
</tbody>
</table>
Some rats were killed using ether after MRI observations for histological examination. The brain was removed and cut into 2 mm thick coronal blocks. The brain slices were immersed in a physiological saline (Otsuka Pharmaceutical, Tokushima, Japan) containing 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC, Sigma-Aldrich, St Louis, Missouri) in the dark at room temperature for 50 min and then fixed in 10% phosphate-buffered formalin at 4°C. TTC-stained brain slices were scanned with a scanner (Color Imaging GT-8700, Seiko Epson, Suwa, Japan).

Numerical values are expressed as mean (SD). The p value was obtained by the Student t test, and p < 0.05 was considered statistically significant.

RESULTS

SA effects on paralyses in SHR-SP rats

The blood pressure of SHR-SP rats gradually increased after switching the drinking-water to one containing 1% NaCl. Thirty-six rats out of 40 suffered a stroke within 7 weeks after NaCl loading. The systolic and the diastolic blood pressures on the day of stroke onset measured for 24 rats were 264.9 (SD 26.4) mm Hg and 174.0 (16.3) mm Hg, respectively. SA dramatically alleviated neurological dysfunction caused by a stroke attack in SHR-SP rats. Figure 1 shows photographs of a rat demonstrating such dramatic effects of SA and representative dysfunction at each grade of paralysis. This rat experienced a severe stroke 27 days after being given NaCl water. Both fore- and hindlimbs were paralysed; the grade of paralyses was 4 at day 0 and day 1. At day 2, the grade decreased to 3, but it could not lift the body. At day 3, it could walk but slowly, and the grade was 2. At day 4, the paralyses were almost cured (grade 0), and the rat could walk normally. This rat suffered a second stroke 10 days after the first stroke onset and died after 12 days. The average values of the grade 1–3 days after stroke onset (day 0) in the SA-treated group of rats and untreated group of rats are summarised in table 2A. The paralysis grade in the SA-treated rats became lower, whereas that in the untreated rats became higher during the study. The effect of SA became statistically significant (p < 0.05) on day 2. SA had no significant effect on the blood pressure as previously reported. Therefore, SA did not reduce the risk of stroke attack. The SA-treated rats were repeatedly attacked by strokes, even after the paralyses were mitigated by SA treatments, and they eventually died. However, as a result of the powerful effect of SA on the neurological dysfunction, the SA-treated group of rats were able to survive much longer (21.7 (14.5) days; n = 11) than the untreated group of rats (4.6 (3.7) days; n = 15) after the first stroke onset.

SA effects on paralyses of MCAO rats

In MCAO rats, neurological dysfunction became apparent 1 day after reperfusion. However, conspicuous paralysis appeared only in a forelimb. The paralysis grade was 1–3 in most cases. SA had no statistically significant effects on the paralysis in MCAO rats during day 1 to day 3 (table 2B).

MRI observations in the brain after stroke onset

Figure 2A shows representative MRI images of the SHR-SP brain demonstrating that the stroke onset is characterised by an increase in the relaxation time measured with T2 images (whitened area) and also the increase in the ADC values measured with ADC images, indicating the appearance of vasogenic oedema without cytotoxic oedema. These images can be interpreted as indicating that the interstitial space in the brain parenchyma and the ventricles were filled with iso-osmotic fluid and serum proteins that leaked out from the bloodstream through impaired BBB, but it was not accompanied by swelling of the brain cells that would be caused by an energy...
Leakage could be detected with the contrast MIR image using Magnevist (fig 2B). The haemorrhage appeared at the focus of the lesion area 2 days after the stroke onset (the black spot in Right of the T2 image in fig 2B). Figure 2C shows the perfusion images indicating that there was no significant change in the cerebral blood flow (CBV) or the cerebral blood volume (CBF), even though the hemisphere was expanded by the vasogenic oedema.

In contrast to the cases of SHR-SP, the transient brain ischaemia by MCAO induces cytotoxic oedema without vasogenic oedema (fig 3A top). The cytotoxic oedema is characterised by the decreased ADC values (darkened area), reflecting the transfer of water from extracellular space into the cells due to cellular energy failure. Both CBF and CBV decreased remarkably during the MCAO (fig 3C). After reperfusion, the volume of the cytotoxic oedema quickly decreased once, then increased again and reached the maximum level at 24 h (fig 3A left, B). On the other hand, vasogenic oedema appeared only after reperfusion and increased to the maximum level at 24 h (fig 3A right, B). The second phase of development of the cytotoxic oedema and the appearance of the vasogenic oedema were obviously not directly induced by the ischaemia. These were induced by blood recirculation. Then, both the cytotoxic oedema and the vasogenic oedema decreased gradually.

Effect of SA on the vasogenic oedema in SHR-SP

It was found that the volume of vasogenic oedema in SHR-SP decreased markedly in a short time after SA treatment. Figure 4

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### Table 2
Grades of paralyses in spontaneously hypertensive stroke-prone and middle cerebral artery occlusion rats

<table>
<thead>
<tr>
<th>A. Grade of paralyses in spontaneously hypertensive stroke-prone rats</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalp-acupuncture-treated (n = 18) (SD)</td>
<td>2.88 (0.85)</td>
<td>2.47 (1.01)</td>
<td>1.88 (1.11)</td>
<td>1.70 (1.33)</td>
</tr>
<tr>
<td>Untreated (n = 15) (SD)</td>
<td>2.43 (1.07)</td>
<td>2.62 (0.51)</td>
<td>2.92 (0.86)</td>
<td>3.15 (0.80)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.90</td>
<td>0.64</td>
<td>0.0094</td>
<td>0.0049</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Grade of paralyses in middle cerebral artery occlusion rats</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalp-acupuncture-treated (n = 12) (SD)</td>
<td>1.00 (0.00)</td>
<td>1.53 (1.00)</td>
<td>1.58 (1.30)</td>
<td>1.27 (1.19)</td>
</tr>
<tr>
<td>Untreated (n = 11) (SD)</td>
<td>1.00 (0.63)</td>
<td>1.27 (0.63)</td>
<td>1.64 (1.20)</td>
<td>0.75 (0.62)</td>
</tr>
<tr>
<td>p Value</td>
<td>1.00</td>
<td>0.50</td>
<td>0.92</td>
<td>0.20</td>
</tr>
</tbody>
</table>

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**Figure 2** MRI images of the brain of a spontaneously hypertensive stroke-prone rat. (A) T2 images (top) and apparent diffusion coefficient (ADC) images (bottom) of the same slice on the day of stroke onset (left) and at 2 days after stroke onset (right). (B) T2 images (top) and contrast MRI images (bottom) of the same slice on the day of stroke onset (left) and at 2 days after stroke onset (right) indicating the leakage from the focus of the oedema. Haemorrhage appeared at day 2 in the lesion area. (C) T2 image (top), relative cerebral blood flow (CBF) (middle) and relative cerebral blood volume (CBV) (bottom) taken from the same slice at 3 days after stroke onset.
shows T2 images of 15 slices on the day of stroke onset (left) and those recorded a day after (right). On the day of stroke onset, the vasogenic oedema increased in the left hemisphere (right side in the T2 images), and the ventricles were filled with fluid. The grade of paralyses was 4, the oedema volume in the brain parenchyma was calculated to be 162 mm$^3$, and the fluid volume in the ventricles was 299 mm$^3$. SA was treated for 10 min after the MRI observations. A day after, the grade of paralyses decreased to 2. The oedema volume decreased to 71 mm$^3$ (44%), and the fluid in the ventricles decreased to 125 mm$^3$.

Figure 5 compares the grade of paralyses and the volume of vasogenic oedema in three SA-treated SHR-SP. Vasogenic oedema appeared in different areas, and the paralysis grade was different between rats. However, the results clearly indicate that there was a close relation between the pattern of changes in grade of paralyses and that of the volume of vasogenic oedema in each rat. In fact, in the SA-treated rats, the oedema volume decreased to 47.9 (26.9)% (n = 7) during 24–48 h after the stroke onset, but in the untreated rats the oedema volume increased to 125 mm$^3$. This rat suffered second attack on day 5, the paralyses grade increased to a level of 4, the oedema volume increased to 179 mm$^3$, and the fluid in the ventricles increased to 282 mm$^3$.

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SA effects on the cytotoxic and the vasogenic oedema in MCAO rats
In contrast to the strong effect of SA on the brain lesion in SHR-SP, SA had no significant effect on either the time-dependent change in the volume of cytotoxic oedema (fig 6 left) or that in vasogenic oedema (fig 6 right) in MCAO rats.

Comparison of infarct between SHR-SP and MCAO rats
TTC staining that measures tissue viability has been used to evaluate infarct size. Figure 7A shows T2 images and TTC staining at nearly the same coronal section of the brain obtained from the SA-treated SHR-SP (SP#7A) used in the experiment of fig 5 (right). The TTC staining indicated that although small spots around the foci of blood serum leakage and/or haemorrhage underwent infarction during 4 days after the stroke onset, the major portion of the brain in the vasogenic oedema seen on the day of stroke onset (day 0) did not. In contrast, in the MCAO rat, the greater portion of the brain in the cytotoxic oedema underwent infarction 1 day after reperfusion (fig 7B).
DISCUSSION

We have shown here that the causes of brain abnormalities in SHR-SP rats are different from those in MCAO rats, and that the effects of SA on neurological dysfunction are different between the causes of strokes, that is hypertension and ischaemia. The brain abnormality in SHR-SP has a hypertension-caused vasogenic origin, and the stroke onset is characterised by the expansion of vasogenic oedema due to leakage of iso-osmotic serum into the interstitial space of the brain parenchyma via BBB with increased permeability that occurs without failure of cell metabolism (figs 2, 7A). SA has strong and rapid effects in reducing oedema size and in mitigating neurological dysfunction (figs 1, 4, 5). In contrast, in MCAO rats, the focal brain ischaemia rapidly induces cytotoxic oedema due to cell swelling by cell energy failure but does not induce vasogenic oedema in the early stage of ischaemia (fig 3). After recirculation of blood, the oxygen supply quickly reduces the cytotoxic oedema. At the same time, recirculation is known to induce intense superoxide, nitric oxide and peroxynitrate synthesis. Overproduction of these radicals leads to reperfusion-induced cell injury. The delayed development of vasogenic oedema occurs as a consequence of the BBB disruption. SA has no significant effect on the vasogenic oedema that has occurred, or the spontaneous decrease in the vasogenic or cytotoxic oedema in the MCAO rats at least within the short time span examined (fig 6), suggesting that SA is not effective for neurological dysfunction caused by neuronal injury. This may account for the different effects of SA between SHR-SP and MCAO rats.

However, it is true that in clinics, SA has been used to treat patients in a chronic stage not only with haemorrhage strokes but also with ischaemic strokes, and yielded better therapeutic results than an SA-untreated group of patients. This suggests that SA has multifarious effects over a short to long time span. In fact, a number of studies on MCAO rats have shown that acupuncture treatments including SA and body acupuncture promote neuronal activity, neuroprotective effects, neurogenesis, etc with a longer timescale. The present study could demonstrate only some of the SA effects, that is the rapid effect on the neurological dysfunction in SHR-SP in relation to the vasogenic oedema that is not accompanied by neuronal injury.

Vasogenic oedema fluid found in the white matter primarily consisting of aligned axonal tracts is higher than that in the grey matter consisting of tangles of neuronal cell processes. Because the brain volume is limited by the skull, the generation of vasogenic oedema in the white matter would compress the neuronal tracts that consist of axons and surrounding glial cells, and narrow the diffusion space between an axon and surrounding glial cells, the so-called periaxonal space or Frankenhaeuser and Hodgkin (F-H) space. If this occurs, $\mathrm{K}^+$ released from axons as the physical consequence of action potential propagation would be accumulated in the F-H space. It is well known that the action potential propagation fails when $\mathrm{K}^+$

Figure 4 T2 images of whole 15 slices obtained from a spontaneously hypertensive stroke-prone rat, demonstrating the appearance of vasogenic oedema at the onset of stroke (left) and a dramatic reduction in the vasogenic oedema in 1 day after scalp acupuncture (SA) treatment for 10 min (right). The grade of paralyses decreased from 4 to 2 on 1 day, and the volume of vasogenic oedema from 162.0 mm$^3$ to 71.3 mm$^3$. The fluid volume in the ventricles changed from 299 to 125 mm$^3$ during the same period.
is accumulated in the F-H space. As the neurons are not injured but simply lost the capability of propagating action potentials by the K\(^+\) depolarisation, the propagation failure can be immediately restored when the F-H space widens by the reduction in vasogenic oedema. This may explain the rapid recovery from neurological dysfunction when the vasogenic oedema was reduced.

It is not understood how SA can reduce so rapidly the vasogenic oedema caused by hypertension. There may be two possible mechanisms that can reduce vasogenic oedema. One is the rapid recovery of microvascular integrity, and the other enhancement of the endogenous potential in draining out the excess water from the brain parenchyma into veins. Previous

**Figure 5** Changes in the grade of paralyses (top) and the volume of vasogenic oedema (bottom) obtained from three spontaneously hypertensive stroke-prone rats treated with scalp acupuncture for 10 min per day, showing a close relationship between grade of paralyses and oedema volume. The rats suffered a second stroke during the experiments. “MRI” means that MRI observations were made on the days indicated, and “TTC” (2,3,5-triphenyltetrazolium chloride) means that a histological examination was performed by TTC staining after the MRI observation.

**Figure 6** Time-dependent changes in the volumes of cytotoxic oedema (left) and vasogenic oedema (right) in middle cerebral artery occlusion rats of the scalp acupuncture (SA) treated group (n = 7) and the untreated group (n = 7), showing that SA had no significant effect on the spontaneous changes in the volume of the oedema in this timescale of observations. The error bars indicate the magnitude of SD.
Figure 7  Generation of infarction in a spontaneously hypertensive stroke-prone (SHR-SP) rat (A) and middle cerebral artery occlusion (MCAO) rat (B) measured with 2,3,5-triphenyltetrazolium chloride (TTC) staining. (A) T2 images (T2) at day 0 and at day 4, and TTC staining (TTC) at day 4 after stroke onset at nearly the same coronal section of the brain obtained from the scalp-acupuncture-treated SHR-SP (SP#7A) used in the experiment of fig 5 (right). (B) Apparent diffusion coefficient (ADC) images (ADC) on day 0 and day 1, and TTC staining on day 1 after reperfusion obtained from a MCAO rat in nearly the same coronal section of the brain.

studies have shown that in SHR-SP, neurological symptoms appear later than abnormalities in the arterial structures, such as vessel wall alterations particularly with medial necrosis and focal degeneration of the medial smooth muscle cells. Together with the results shown in fig 7A, it is unlikely that these long-term degenerations of arteries could be restored so quickly by SA treatment. Therefore, it seems more likely that SA stimulates the endogenous potential in maintaining the brain water homeostasis.

In clinics, the effectiveness of SA is different between patients, and the rapid effects are observed in 60% of patients. If human stroke involves in part a similar mechanism to that which occurs in SHR-SP stroke, the present experiments could explain the clinical results, and SA would be more beneficial for patients with stroke with hypertension-caused vasogenic origin.

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