Long-term changes of diffusion tensor imaging and behavioural status after acupuncture treatment in rats with transient focal cerebral ischaemia

Zhiyuan Wu,1 Jinqing Hu,2 Fang Du,3 Xiaoyan Zhou,4 Qiongyao Xiang,5 Fei Miao1

Abstract

Background The effect of acupuncture treatment in cerebral ischaemia is controversial. A study was undertaken to assess its effects in rats with transient middle cerebral artery occlusion (tMCAO) and discuss its mechanisms.

Methods 21 Sprague–Dawley rats were divided into three groups. Group 1 underwent tMCAO and subsequently received acupuncture treatment, Group 2 underwent tMCAO without treatment and Group 3 only underwent sham operation. The evolution of diffusion tensor imaging (DTI) features in ischaemic lesions from acute to chronic periods was assessed and the correlations with behavioural tests and histopathological changes were examined.

Results tMCAO rats displayed side-specific sensorimotor deficits after occlusion. Behavioural scores of rats in group 1 reduced gradually with acupuncture treatment. No significant difference in lesion size on T2-weighted imaging was found between the two tMCAO groups. Relative apparent diffusion coefficient (rADC) and relative fractional anisotropy (rFA) values in the centre and at the edge of the ischaemic lesions reduced at first and then increased to varying degrees. Only changes in the rFA value at the edge of the ischaemic lesions were significantly different between the two tMCAO groups. A more significant increase in growth-associated protein B-50 and synaptophysin protein expression was found in group 1 than in the other groups. No significant correlations were found between behavioural scores, DTI appearances and immunohistochemical results.

Conclusions The acupuncture points applied were effective, and improving neuronal regeneration may have a role in the mechanism of acupuncture treatment of post-stroke paralysis of the limbs in rats. MRI, particularly the fractional anisotropy value of DTI, is an appropriate parameter to evaluate the recovery status.

INTRODUCTION

Sensorimotor neurological deficits are common sequelae of transient cerebral ischaemia. These impairments have been linked to the presence on MRI of post-ischaemic lesions, and neurological recovery has been accompanied by changes in brain activation patterns on MRI. Diffusion tensor imaging (DTI) can determine the diffusivity of every voxel and fully depict tissue diffusion characteristics. Since 1990 it has been used to detect acute cerebral ischaemia within minutes of stroke onset.1 Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) are the two most common parameters used to evaluate the ischaemic lesions quantitatively. Many authors have addressed the appearance of the acute stage on different sequences of MRI, such as T2-weighted imaging (T2WI) and DTI, with regard to early diagnosis of clinical or experimental cerebral ischaemia.

Although acupuncture therapy is not a panacea for paralysis, it is safe and drug-free. The appropriate use of acupuncture therapy may lead to good outcomes in patients with stroke. However, the clinical effects of acupuncture therapy are uncertain and the mechanisms underlying acupuncture are still poorly understood.2

To examine these issues, the present study investigated long-term changes of T2WI, DTI, behavioural status and histopathology appearances after acupuncture treatment in rats with transient middle cerebral artery occlusion (tMCAO). The major aims of this study were to evaluate the therapeutic effects of acupuncture treatment in tMCAO and to examine the mechanism of acupuncture treatment by evaluating the changes in DTI. It was hypothesised that (1) acupuncture treatment would promote the recovery of post-ischaemic motor function deficits; (2) tMCAO would produce dynamic changes in DTI, behaviour and pathology in the 28 days after tMCAO; and (3) DTI might reflect the changes in behaviour and pathology.

METHODS

Animal preparation

Institutional review board approval was obtained for this study. Twenty-one male Sprague–Dawley rats (3 months old, body weight 250–300 g) were purchased from Laboratory Animal Services Center, Shanghai.
Jiaotong University School of Medicine, Shanghai, China. The animals were housed in rooms maintained at 20±1°C with an alternating 12 h light–dark cycle and were acclimated to their surroundings over 1 week to eliminate the effect of stress prior to initiation of the experiments.

The animals were randomly separated into three groups with seven animals in each group. One day after the first MRI examination, groups 1 and 2 underwent intraluminal suture 30 min tMCAO with reperfusion and group 3 underwent a sham operation. In groups 1 and 2 the right middle cerebral artery (MCA) was occluded with a transvascular approach as described by Dzialowski et al.\(^3\) The right common carotid artery and external carotid artery were exposed through a midline neck incision and ligated with a 4.0 monofilament nylon suture (Nitcho Kogyo, Tokyo, Japan). A 4.0 monofilament nylon suture with a silicone-coated tip (Xantopren L Blue, Heraeus Kulzer Dental, Hanau, Germany) was then inserted into the common carotid artery and gently advanced into the internal carotid artery to a point approximately 17 mm distal to the carotid bifurcation. Mild resistance to this advancement indicated that the suture had entered the anterior cerebral artery and the posterior communicating artery. The nylon suture was pulled out 30 min later for reperfusion. In group 3 the suture was only inserted 10 mm above the carotid bifurcation without occlusion of the intracerebral vessels. During surgery, anaesthesia was induced with 5.0% isoflurane (Forane; Abbott, Shanghai, China) under spontaneous respiration and maintained at 2.0% during surgery with a small animal anaesthetiser (Model TK-4, Bio Machinery, Funabashi, Japan). Physiological parameters (respiration, heart rate and arterial oxyhaemoglobin saturation) were non-invasively recorded throughout surgery. The temperature was maintained at 37.0±0.5°C with a feedback-controlled heating pad. All surgeries were performed by a neurosurgeon (HJQ).

**Acupuncture treatment**

After the operation, interdermal needles (36#, Suzhou Gusu Acupuncture and Moxibustion Appliance, Suzhou, Jiangsu, China), diameter 0.20 mm, length 7.0 mm, were subcutaneously inserted into Baihui (DU20), Dazhui (DU14), Shousanli (LI10) and Zusanli (ST36) simultaneously, with the handles covered in a sterile adhesive plaster, for 30 min of stimulation each day for 28 days in group 1. Acupuncture was performed by a certified acupuncturist (XQY). The rats were anaesthetised with isoflurane prior to acupuncture. Groups 2 and 3 were given no treatment.

**MRI measurements**

After anaesthesia with 40 mg/kg pentobarbital sodium intraperitoneally, an MRI scan was performed with a 1.5-T magnetic resonance system (Excite HD, General Electric Medical System, Milwaukee, Wisconsin, USA). A 3 inch surface coil was used for brain imaging. Animals were imaged before occlusion and 1, 4, 7, 14 and 28 days after reperfusion.

Prior to scanning, head movement was limited by vacuum fixation cushions. Coronary T1-weighted imaging (T1WI), fast spin echo T2WI, traditional FLAIR and DTI were used. Consecutive slices were acquired in an identical location for all sequences with a 2 mm slice thickness. Typical acquisition parameters were: T1WI (TR 540.0 ms/TE 15.4 ms), fast spin echo T2WI (TR 3500.0/TE 89.5 ms) and traditional FLAIR (TR 2600.0/TE130.0/TI 2100.0 ms). For DTI, an echo planar imaging sequence was used (TR 5000.0/TE 84.1 ms, NEX=4.0, matrix 96×96, field of view 10×10 cm). Diffusion-weighted images were obtained with \( b=600 \text{ s/mm}^2 \) and diffusion sensitive gradients were applied along 13 gradient directions. In addition, a reference image without diffusion weighting (\( b=0 \text{ s/mm}^2 \)) was acquired.

**MRI data analysis**

Images were analysed with Func tool 2.3.1 software (ADW4.2, General Electric Medical System) and interpreted retrospectively and jointly in consensus by two radiologists (WZY and MF). Regions of interest (ROIs) were manually defined as an ellipse with an area of 2 mm\(^2\), first on T2WI images and then on the corresponding DTI images. ROIs were placed in the brain area within the MCA territory: the edge and the centre of ischaemic lesions. To ensure correct placement and to minimise partial volume effects, ROIs were drawn carefully and conservatively with reference to anatomical images and a stereotaxic rat brain atlas. The ADC value and the FA value were measured on the ADC images and anisotropy images, respectively. Relative ADC (rADC) and relative FA (rFA) were analysed. The rADC and rFA were calculated according to the formulae: rADC=(ipsilateral ADC)/(contralateral ADC) and rFA=(ipsilateral FA)/(contralateral FA). The ipsilateral area was the ischaemic brain area within the MCA territory of the right hemisphere and the contralateral area was the corresponding area in the left hemisphere.

**Behavioural test**

Longa’s scores,\(^4\) as the behavioural tests, were evaluated 30 min before each MRI examination by two radiologists (WZY and MF) who were not blinded to group. The neurological findings were scored on a 5-point scale where 0 indicated no neurological deficit, 1 (failure to extend left forepaw fully) indicated a mild focal neurological deficit, 2 (circling to the left) indicated a moderate focal neurological deficit and 3 (falling to the left) indicated a severe focal deficit; rats with a score of 4 did not walk spontaneously and had a depressed level of consciousness.

**Histological evaluation**

Twenty-eight days after tMCAO following the behavioural test and MRI examination, the animals were anaesthetised with 40 mg/kg pentobarbital sodium intraperitoneally, an MRI scan was performed with a 1.5-T magnetic resonance system (Excite HD, General Electric Medical System, Milwaukee, Wisconsin, USA). A 3 inch surface coil was used for brain imaging. Animals were imaged before occlusion and 1, 4, 7, 14 and 28 days after reperfusion.
intraperitoneally and perfused with 500 ml phosphate-buffered saline solution (0.1 M) through the ascending aorta followed by 250 ml phosphate-buffered 4% paraformaldehyde. The brains were removed and underwent overnight fixation at 4°C in the same paraformaldehyde solution. The following day the brains were sectioned coronally into coronal slices 2.0 mm thick corresponding to the MR slices. After dehydration, clearing and infiltration with paraffin solvent, the tissue was embedded in paraffin. One 4 μm thick section from each of the second, third and fourth most anterior slices was stained with haematoxylin and eosin for microscopic (×100) counting and classification of neurons (ie, intact, necrotic or apoptotic) in five non-overlapping fields in each ROI, performed by one individual (ZXY) blinded to the imaging data. In brief, neurons were classified as necrotic when they exhibited pyknosis, karyorrhexis, cytoplasmic eosinophilia ('red neuron') or loss of affinity for haematoxylin ('ghost neuron'). The total number of neurons was recorded and the numbers of apoptotic and necrotic neurons within an ROI were summed to derive the number of non-intact neurons; this was done for each ROI.

Immunohistochemistry was performed with two antibodies of the growth-associated protein B-50 (GAP-43) and synaptophysin. Briefly, after deparaffinisation and rehydration, 4 mm sections were subjected to heat-induced epitope retrieval in 0.01 mol/l citrate buffer (pH 6.0). The slides were incubated overnight with primary antibodies including polyclonal rabbit anti-GAP-43 (1:200, Chemicon, Hofheim, Germany) and monoclonal mouse anti-synaptophysin (1:200, Sigma-Aldrich Chemi, Buchs SG, Switzerland), respectively. Slides processed with normal rabbit serum (Dako, Glostrup, Denmark) in place of the primary antibody were used as negative controls.

Immunodetection was performed with the EnVision +System. 3.3’-diaminobenzidine was used for colour development and haematoxylin was used for counterstaining. The slides were observed under a Zeiss Axioplan 2 imaging microscope with AxiosVision 3.0 imaging software, AxioCam HRc camera and Zeiss objectives (Carl Zeiss Vision, Oberkochen, Germany). The area of the lesion and the positive expression of GAP-43 and synaptophysin were measured on the computer screen. This procedure was conducted by an investigator (DF) who was blind to the grouping of the rats.

Statistical analysis
Data are presented as mean±SD unless otherwise stated. Statistical comparisons of these measurement data were performed by multivariate analysis of variance (MANOVA) using the program package SAS (V6.12). Correlation analyses used the Spearman rank order test. p<0.05 was considered significant.

RESULTS
Behavioural testing
Basal physiological parameters did not differ significantly between the groups. Compared with pre-occlusion, rats that underwent tMCAO displayed significant post-ischaeimic side-specific sensorimotor deficits after occlusion. The behavioural scores in all rats were 0 before occlusion and increased significantly after occlusion. In tMCAO rats the behavioural score was not significantly different in groups 1 and 2 at 1 day and 4 days after tMCAO (p>0.05). At 7, 14 and 28 days after reperfusion, a significant difference in the behavioural score was found between these two groups (p<0.05). In group 1 the behavioural score reduced gradually with acupuncture treatment but no significant change was seen in group 2. In sham-operated rats (group 3) there were no significant differences in behavioural parameters between time points and the score was 0 at all time points (p<0.05). The behavioural scores of the three groups at each time point are shown in figure 1.

MRI results
T2WI revealed post-ischaeimic swelling and lesions in the ipsilesional hemisphere. The size of the ischaemic lesions on T2WI reduced gradually during the follow-up period in both groups 1 and 2 but no significant difference was seen between the two groups (p>0.05). In sham-operated rats there was no significant signal difference in T2WI between hemispheres or time points (p>0.05). The size of the ischaemic lesions on T2WI is shown in figure 2.

The spatiotemporal analyses of diffusion tensor parameters (rADC and rFA) are summarised in figure 3. Regional rADC and rFA values were significantly reduced relative to corresponding pre-occlusion values (p<0.05) in the centre and at the edge of the ischaemic lesions at all time points after tMCAO. In tMCAO rats, rADC values in the centre of the ischaemic lesions within ROIs reduced at first and then increased gradually, but there was no significant difference between the two groups at all time points after reperfusion (p>0.05). The same changes were seen at the edge of ischaemic lesions.

Figure 1 Changes in behavioural scores. The Longa’s scores in all rats were 0 pre-occlusion and increased after occlusion. There was no significant difference between groups 1 and 2 at 1 day and 4 days after transient middle cerebral artery occlusion (tMCAO) but a significant difference was found at 7, 14 and 28 days after reperfusion (marked with an asterisk). In group 3 the scores were 0 at all time points.
rFA values in the centre of the ischaemic lesions also showed a reduction at first and a slight increase later. No significant difference was seen between the two tMCAO groups (p>0.05). At the edge of the ischaemic lesions the changes in rFA values also showed a similar pattern, with a reduction at first and then a gradual increase later but, at 7 and 28 days after reperfusion, a significant difference was found between the two tMCAO groups (p<0.05). Group 1 recovered much better than group 2. In sham-operated rats (group 3), no significant change was seen between the different time points (p>0.05).

Histopathology
No significant postoperative histological abnormalities were demonstrated in the contralesional hemisphere of tMCAO rats or in either hemisphere of sham-operated rats, nor was grossly visible tissue swelling, pallor and/or cavitation present. In tMCAO rats a significant number of irreversibly damaged neurons was found in all ipsilesional ROIs. Isolated necrotic or apoptotic neurons were surrounded by normally appearing neuropil, glia and endothelium, suggesting selective neuronal death (ie, incomplete infarction). Immunohistochemical results are shown in figure 4 and table 1. A more significant increase in GAP-43 and synaptophysin protein expressing cells was found in group 1 than in groups 2 and 3 (p<0.05). The number of cells expressing GAP-43 and synaptophysin protein was greater in group 2 than in group 3 but the differences were not significant (p>0.05).

Correlations
No significant correlations were found between behavioural test results (Longa’s scores) and ipsilesional MRI (size of ischaemic lesions, rADC or rFA values) and/or immunohistochemical results (level of GAP-43 or SYN expression) at individual time points and when the data were grouped (r2 range −0.21 to 0.82, p>0.05 for all; data not shown).

DISCUSSION
Acupuncture treatment has been used as a safe and drug-free intervention in the treatment of post-stroke paralysis.

Figure 2 Changes in lesion size on T2-weighted imaging. High signal intensity lesions with tissue swelling were found in the right hemisphere in group 1 and group 2. No abnormality was found in group 3. The size of the ischaemic lesions reduced gradually in groups 1 and 2 and no significant difference was seen between the two groups. No significant difference in signal was seen between the hemispheres or time points in group 3.
of the limbs in China for several years, but its effects are often questioned and disputed by modern medical science. Many authors consider that the appropriate and informed use of acupuncture therapy might lead to better outcomes in stroke patients. Some authors⁵ think that acupuncture might be helpful in alleviating pain, anxiety, depression, sleep disturbance or other conditions that are common sequelae of stroke and that are often

**Figure 3** Changes in relative apparent diffusion coefficient (rADC) and relative fractional anisotropy (rFA) values in the centre and at the edge of ischaemic lesions. Both in the centre and at the edge of the ischaemic lesions, rADC values reduced at first and then increased gradually in groups 1 and 2. No significant difference was seen between the two groups. In the centre of the lesions rFA values reduced at first and then increased slightly later. No significant difference was seen between the two groups. At the edge of the lesions, rFA values also reduced at first and then gradually increased but, at 7 and 28 days after reperfusion, a significant difference was found between the two groups (marked with an asterisk). Animals in group 1 recovered much better than those in group 2. In group 3 no significant change was seen between different time points.

**Figure 4** Expression of growth-associated protein B-50 (GAP-43) and synaptophysin protein (SYN). Cells with positive expression of GAP-43 and SYN stained as light brown (arrow, original magnification ×400). A more significant increase in GAP-43 and synaptophysin protein expressing cells was found in group 1 than in groups 2 and 3.
The four acupuncture points selected in this experiment were those typically used in the treatment of post-stroke paralysis of the limbs. DU20 is needed in order to ‘activate spirit and resuscitate the brain’ in traditional Chinese medicine. Some authors have found that electroacupuncture at DU20 is effective in extenuating cerebral ischaemic injury and upregulating endogenous insulin-like growth factor 1 expression in the acute phase after MCAO, with increased dopamine levels and reduced brain atrophy in the chronic phase. DU14 is thought to play a role in regulation, and stimulation of DU14 could induce sympathetic and parasympathetic nerve activity. LI10 is used clinically for hemiplegia and atrophic disorders, although this is supported by only limited evidence. ST36 is a well-known acupuncture point in animals and humans and has been commonly used for the enhancement of functional recovery in stroke patients. For example, the finding of Siu et al suggested that acupuncture treatment at ST36 could increase thioredoxin and oxidative stress in cerebral infarction rats by increasing the activities of superoxide dismutase and glutathione peroxidase and the expression of CuZnSOD mRNA and protein in the hippocampus of impaired rats. The underlying mechanisms of acupuncture in general are still not clearly delineated.

The phenomenon of neuronal death was observed after occlusion of the MCA, which is consistent with observations made in numerous other species including humans. Selective neuronal cell death was consistently observed in ipsilesional regions where T2WI, ADC and FA had previously normalised. Although the frequency of cerebral cell death increased over time, concomitant neuroplastic events occurred so the behavioural functions could steadily recover. A longitudinal multiparametric MRI study by Van der Zijden et al suggested that resolution of early ischaemic damage and reorganisation of white matter in perilesional tissue was chronically accompanied by preservation or restoration of neuronal connectivity, which might significantly contribute to post-stroke functional recovery.

Table 1 Count of cells with positive expression of GAP-43 and synaptophysin in regions of interest (N/mm²)

<table>
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<th></th>
<th>GAP-43</th>
<th>Synaptophysin</th>
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<tr>
<td>Group 1</td>
<td>1739.14±136.79</td>
<td>128.16±12.09</td>
</tr>
<tr>
<td>Group 2</td>
<td>1227.59±94.64</td>
<td>85.11±10.52</td>
</tr>
<tr>
<td>Group 3</td>
<td>941.54±535.38</td>
<td>46.62±36.92</td>
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GAP-43 is a neuron-specific protein preferentially distributed in the growth cone and elongating axon during development and regeneration. Neurons developing both in vivo and in vitro express high levels of GAP-43 coincident with the initiation and elongation of neurite outgrowth. Due to its characteristics and its pattern of expression, GAP-43 is frequently used as a marker for axonal sprouting and growth. Increased GAP-43 was described in neurons that extend axons during the terminal phase of development. Synaptophysin is a member of a family of phosphoproteins that are localised to the presynaptic membrane and regulate neurotransmitter release. It has been shown to play a role in both axonogenesis and synaptogenesis. More synaptophysin expressing cells were observed in group 1 than in groups 2 and 3. A significant increase in GAP-43 and synaptophysin expressing cells in group 1 in the present study might be explained by greater neuroregeneration occurring after acupuncture treatment. Improving neuronal regeneration might be a mechanism of acupuncture treatment in post-stroke paralysis of the limbs in these rats, in accordance with the results of Yang et al.

Early reports showed the ADC value reduced in both the ischaemic grey matter and white matter in the acute stage. Guadagno et al considered that the degree of restricted water diffusion reliably reflected the severity of agreement in the literature. In the present experiment the size of the ischaemic lesions in T2WI reduced gradually during the follow-up period in both groups 1 and 2, but no significant difference was seen between the two groups and no significant correlation was found between the size of the ischaemic lesions in T2WI and behavioural scores. It may be that lesion volume is not an appropriate method to evaluate the recovery status.

DTI is widely recognised as a powerful tool for the rapid detection of clinical and experimental cerebral ischaemia and is more sensitive than DWI in white matter ischaemia. It has also often been used in vivo to assess the longitudinal and regional microstructural changes occurring after MCA infarcts and is regarded as a surrogate marker in treatment trials. However, there are few reports on research into the effective mechanism of acupuncture treatment.

Measuring functional outcome is a relevant and necessary means to assess the consequences of cerebral ischaemia. To evaluate the value of acupuncture treatment in ischaemic rats, we selected Longa’s score as a behaviour measurement. In group 1 the behavioural score reduced gradually with acupuncture treatment, but no significant change was seen in group 2. The rats with acupuncture treatment recovered better than those without treatment, indicating that the acupuncture treatment was effective for post-stroke paralysis of the limbs in these rats.

Many previous studies have attempted to show relations between lesion volume and behavioural tests, and although some have found a significant correlation between the two, there remains a major lack of...
the edge of the ischaemic lesions reduced at present experiment, rFA values both in the centre and at that the degree of impairment in diffusion anisotropy of ischaemic lesions but a significant difference between the two tMCAO groups in the centre of the neuronal regeneration induced by acupuncture treatment, ischaemic lesion in group 1 might be due to improved grey matter were not significantly, but changes in anisotropy in ischaemic white matter reduced significantly, but in the acute stage. Yang et al29 identified three phases of diffusion abnormalities: (1) reduced ADC and elevated apparent diffusion anisotropy; (2) reduced ADC and reduced apparent diffusion anisotropy; and (3) elevated ADC and reduced apparent diffusion anisotropy. Many authors31 consider that the degree of impairment in diffusion anisotropy of stroke has the potential to predict motor outcome. In the present experiment, rFA values both in the centre and at the edge of the ischaemic lesions reduced at first and then increased gradually, but there was no significant difference between the groups with or without acupuncture treatment. We therefore consider that the ADC value does not predict tissue outcome, nor could we find any difference between rats with and without acupuncture treatment.

The anisotropy of ischaemic white matter reduced significantly, but changes in anisotropy in ischaemic grey matter were not significant in the acute stage.27 Yang et al29 identified three phases of diffusion abnormalities: (1) reduced ADC and elevated apparent diffusion anisotropy; (2) reduced ADC and reduced apparent diffusion anisotropy; and (3) elevated ADC and reduced apparent diffusion anisotropy. Many authors31 consider that the degree of impairment in diffusion anisotropy of stroke has the potential to predict motor outcome. In the present experiment, rFA values both in the centre and at the edge of the ischaemic lesions reduced at first and then increased later. No significant difference was seen between the two tMCAO groups in the centre of the ischaemic lesions but a significant difference was found at the edge. Better recovery of rFA at the edge of the ischaemic lesion in group I might be due to improved neuronal regeneration induced by acupuncture treatment, and this phenomenon was revealed by DTI, especially the FA value.

Limitations of the study
This experiment had several weaknesses: (1) MRI data were acquired using a 1.5T clinical scanner and the quality of the MRI data was relatively poor; (2) the size of the ischaemic lesions between rats varied even though the weights of the rats were similar and the occlusion times were identical, and this might have influenced the outcomes of post-stroke paralysis; (3) no appropriate method could be found to evaluate whether the needles stimulated the exact positions of the four acupuncture points to the rats; (4) interdermal needles might simulate the technique of traditional Chinese acupuncture well but it was not as standardised as electroacupuncture.

Conclusions
These results suggest that the acupuncture points applied in this study are effective for functional recovery and the mechanism of acupuncture treatment might be due to improved neuronal regeneration in these rats with post-stroke paralysis of the limbs. MRI, particularly the FA value of DTI, is an appropriate parameter to evaluate the recovery status. However, several factors were difficult to control and many of them need continued study.

Summary points

- Acupuncture is often used to treat post-stroke paralysis
- We tested its effect on a rat model of transient ischaemia
- Acupuncture improved motor function, brain microscopy and MRI changes.

Contributors ZW: design of the study, acquisition and analysis of MRI and behavioural test data, drafting and revising the article. JH: design of the study, building animal models and drafting the article. FD: acquisition and analysis of immunohistochemistry data, drafting the article. XZ: acquisition and analysis of histological data, drafting the article. OX: design of the study, performing acupuncture treatment and drafting the article. FM: design of the study, acquisition and analysis of MRI and behavioural test data, drafting and revising the article. All authors approved the publication of the final version.

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