The effects of acupuncture on rats with brain ischemia-reperfusion

Ahmet Kavakli, MD, PhD, Evren Köse, MD, Nusret Akpolat, MD, Mustafa Sarsılmaz, MD.

ABSTRACT

Objectives: To investigate the damage of brain ischemia-reperfusion (I/R) caused by occlusion of the middle cerebral artery (MCA), and the effects of acupuncture on this damage.

Methods: This investigation took place in the Experimental Research Unit of Firat University, Elazig, Turkey in January-February 2007. For this aim, 14 rats were divided into 2 groups: I/R (control) and I/R+acupuncture (experiment). In the I/R group, the MCA was occluded for 60 minutes, after this reperfusion was applied. In the I/R+acupuncture group, dry needle acupuncture was applied after reperfusion for 10 days. At the end of the experiment, all rats were sacrificed. The brain tissues were examined after staining with hematoxylin and eosin.

Results: In the samples belonging to the I/R group, widespread necrotic areas, red neurons, vacuolization, congestion, and edema were observed. In the I/R+acupuncture group, the findings of ischemia were significantly decreased when compared with the I/R group.

Conclusion: The damage caused by I/R was decreased by manual acupuncture therapy, however, further clinical studies are needed to determine the mechanism of acupuncture treatment, the optimal timing, and duration of acupuncture treatment in such disorders.

Cerebrovascular diseases represent one of the main causes of death and long-term disability. Thromboembolic occlusion of the artery is the most important cause of ischemia in patients. In brain ischemia, cerebral blood flow is reduced in brain regions that are supplied with oxygen by the occluded vessels. In addition to the lack of blood flow and oxygen delivery, the restoration of blood flow has also been reported to contribute to cell damage due to the generation of free radicals. Several drugs were studied for their neuroprotective effects in the ischemia-reperfusion (I/R) models of animals. Some studies show that over production of free radicals plays an important role in the pathogenesis of the cerebral damage induced by I/R. In addition, some of the drugs were evaluated for the treatment of stroke to reduce the cerebral damage and the recovery of motor and cognitive functions after an I/R attack. Acupuncture, a technique stimulation on acupoints by acupuncture needles, is a physical treatment in Chinese Traditional Medicine.
There are 2 acupuncture methods. One of them is the manual acupuncture (dry needle), where mechanical stimulation is applied to the acupoints. In another technique called electroacupuncture (EA), electrical stimulation is delivered to acupoints. Clinically, acupuncture has beneficial effects on stroke patients. For example, acupuncture can minimize speech retardation, improve locomotion, and increase muscle strength, and stimulate cerebral blood flow. In addition, experimental studies reported that acupuncture attenuated edema formation, lipid peroxidation, and cerebral infarction. The aim of the current study was to investigate the effects of manual acupuncture after I/R performed in rats.

**Methods.** Fourteen adult male Wistar rats, from the Experimental Animal Center of Firat University, Medical Faculty, Elazig, Turkey in January-February 2007, weighing 280-320 g, comprised the study material. All the protocols in the present study were performed according to the guidelines of the local ethics committee. The animals were maintained under controlled temperature (21±1°C) and controlled light conditions (light 07:00-19:00 hours). Food (standard pellet diet) and tap water were supplied ad libitum. The 14 rats were divided into 2 groups I/R and I/R+ acupuncture. Occlusion of the right MCA was performed by a nylon filament as described previously. The middle cerebral artery (MCA) was occluded for 60 minutes followed by perfusion. Briefly, the right common carotid artery was exposed through a midline incision and carefully dissected from the surrounding tissue using the microsurgery technique. The external carotid artery (ECA) was dissected further distally and coagulated along with the occipital and superior thyroid artery branches, which were then divided. The internal carotid artery (ICA) was isolated and carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was ligated close to its origin with a 7-0 silk suture. Next, a 7-0 silk suture was tied loosely around the mobilized ECA stump, and a piece of 4-0 monofilament nylon suture, with its tip rounded by gentle heating, was inserted into the lumen of the right ECA stump and gently advanced via the right ICA to embed into the right anterior cerebral artery so that the right MCA was occluded at its origin. Reperfusion was accomplished by pulling the filament. Acupuncture was applied at the anatomical points of DU-20, DU-26, St-36 and GB-20. The acupuncture treatment was started after reperfusion for 30 minutes and continued for 10 days (30 minutes a day). On the tenth day, at the end of the reperfusion, all rats were sacrificed. The brains were quickly removed from the skull and placed in neutral formalin (10%) and then cut into 2 mm thick coronal slices for routine histopathological examination by light microscopy. Sections (5 µm-thick) from the paraffin–blocks were stained with hematoxylin and eosin. Structural alterations in the brain tissues were determined as semi-quantitative + sign (0: absent, +: slight, ++: moderate, +++: severe).

**Results.** In this light microscopic study, widespread necrotic areas, red neurons, vacuolization, congestion, and edema were observed in the I/R group. In the I/R+ acupuncture group, findings of ischemia were not widespread and necrotic areas were absent. While edema, vacuolization, and eosinophilic degeneration in the I/R group were severe (+++), in the I/R+ acupuncture group the edema, eosinophilic degeneration, vacuolization, and congestion were slight (+) and the necrotic areas were determined as absent (0). The light microscopic results were shown in Table 1 and Figures 1 & 2.

**Table 1 - Light microscopic findings in the brain tissue of group I (I/R) and group II (I/R+ acupuncture) rats (n=7 in each group).**

<table>
<thead>
<tr>
<th>Light microscopic findings</th>
<th>I/R (control)</th>
<th>I/R + acupuncture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrotic areas (infarct)</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Eosinophilic (red neurons)</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Edema</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Vacuolization</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Congestion</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

I/R - ischemia-reperfusion

**Figure 1** - Ischemia-reperfusion group: red neurons (arrows) and infarct (I) area are seen (Hematoxylin & Eosin x 100).

**Figure 2** - Ischemia-reperfusion + acupuncture (experimental) group: neurons with slight edema, congestion, and red neuron (arrow) are seen (Hematoxylin & Eosin x 100).
Discussion. Acupuncture, one of the alternative and complementary treatment methods, has been frequently used in the treatment of various diseases. It has provided a positive outcome in the treatment of many neurological disorders. In addition, it has been experimentally shown to be an efficient treatment method. In a clinical study by Chen et al,17 on stroke patients, routine therapy was complemented by acupuncture at ST 36 and GB 39 points. In these patients, the brain blood flow and collateral circulation were significantly better after acupuncture than in the routine treatment group. Similarly, Si et al18 showed that EA along with routine treatment significantly increases the rate of functional recovery in patients of acute cerebral infarct. Liu et al,19 in a study on rats with multi infarcts of the brain, applied dry needle acupuncture at CV17, CV12, CV6, ST36, and SP10 points for 21 days and determined that the procedure increased the activities of superoxide dismutase and glutathione peroxidase, which are antioxidant enzymes. Furthermore, they found significant improvement in the Morris water-tank experiment used in the evaluation of spatial memory. In our study, dry needle acupuncture applied to rats after brain I/R provided marked histopathological improvement when compared with the control group. It has been claimed that free oxygen radicals cause tissue damage following I/R. Improvement after acupuncture may be associated with increased antioxidant activity by acupuncture. Liu et al20 developed a rat model of transient ischemic damage by middle cerebral artery occlusion (MCAO) and applied EA at GV 14 and GV 20 points. They reported improvement in the neurological deficit and reduction in the size of the pathomorphological lesions. In our study, after I/R, acupuncture was performed at DU 20, DU 26, St-36, and GB-20 points for 10 days, the histopathological evaluations showed significant improvement in the I/R+ acupuncture group, which was compatible with the earlier studies conducted by similar methods. Wang et al21 applied EA at D20 and D26 points and at G4, G5, G6, and G7 points of the rats that were subjected to MCAO. After a 12-day treatment, significantly improved functional recovery was noted in the treatment group. They reported significant reduction in the area of infarct on the thirtieth day of treatment. In the immunohistochemical evaluation, vascular endothelial growth factor (VEGF) in the peri-infarct area astrocytes of the acupuncture group had increased, thus, they suggested that this increase might have played a role in the improvement noted. Likewise, Ma and Luo22 applied EA at the LI 4 point in the rats inflicted with focal cerebral I/R and reported neurological improvement, increased VEGF expression, and reduced anti-VEGF expression. In another study, it was found that interleukin-1 beta (IL-1 B) levels that increased after I/R and played an important role in pathogenesis were reduced following acupuncture treatment.23 In our study, histopathological improvement might have been associated with increased VEGF expression or decreased IL-1 B level. Yang et al24 applied EA treatment of 10 mA for 20 minutes at GV 16 and GV 8 points of the rats subjected to 30 minutes of MCAO. They determined in the histopathological evaluation that cell regeneration and new cell migration had increased. In another study, it was immunohistochromically shown that EA performed 90 minutes after MCAO reduced the neuron count in apoptotic cell death.25

In conclusion, clinical and experimental studies have shown positive effects of acupuncture in the treatment of various neurological disorders or damage. Acupuncture procedure, particularly when it is applied following brain I/R, provides both functional and histopathological improvement. Electroacupuncture has been the common procedure in earlier studies. However, it has been shown in both our studies, and other studies using dry needle acupuncture that this procedure is as effective as the EA procedure in achieving positive outcome. Further clinical studies are needed to determine the mechanism of acupuncture treatment, and the optimal timing and duration of acupuncture treatment in such disorders.

References


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