Effects of Electroacupuncture on the Contents of NO, ET and T-AOC in the Brain Tissues of the Cerebral Hemorrhage Model Rats

Dai Gaozhong 戴高中 1, Chen Yuelai 陈跃来 2, Gu Falong 顾法隆 2 & Chen Ruxing 陈汝兴 1

1Longhua Hospital Affiliated to Shanghai University of TCM, Shanghai 200032, China
2Yueyang Hospital Affiliated to Shanghai University of TCM

In the cerebral hemorrhage model rats established by injection of collagenase and heparin into caudate nucleus, the effects of electroacupuncture (EA) on the contents of nitric oxide (NO) and endothelin (ET), and total anti-oxidation capability (T-AOC) in the brain tissues were investigated. It is found that the content of NO in the Shuigou EA group lowered, ET decreased and the T-AOC raised significantly in both the Fengfu EA group and the Shuigou EA group (all $P<0.05$) as compared with the model group, indicating that acupuncture can reduce the contents of ET and NO, and increase the T-AOC in the brain tissues of the rats with cerebral hemorrhage.

It was found in our previous study that electroacupuncture (EA) in both the "Suigou" and "Fengfu" groups can obviously improve the morphological lesions of the cerebral tissues and the defect of nervous function, and decrease the edema in the surrounding tissues with hematoma caused by hemorrhage in the cerebral hemorrhage model rats induced by a combined injection of collagenase and heparin. In the present experiment, the effects of EA on the contents of NO, ET, and T-AOC in the cerebral tissues of the cerebral hemorrhage model rats were investigated in order to probe into the mechanism of EA in improving cerebral hemorrhage.

Materials and Methods

Experimental animals: Male Sprague-Dawley rats, weighing 250±15g, were purchased from the Experimental Animal Center, Shanghai University of Traditional Chinese Medicine.

Reagents and drugs: Collagenase VII (1500U) was made by Sigma Company; NO, T-AOC and Coomassie brilliant blue agent kits by Nanjing Jiancheng Bioengineering Company; ET radioimmunoassay regent kit by Beijing Dongya Immunologic Technique Institute; and heparin was purchased from the market.

Establishment of the animal model: Referring to the method of Ren Zeguang et al., the rats were anesthetized by intraperitoneal injection of 2% phenobarbital sodium(40mg/kg), and fixed on a stereotaxic instrument in prone position, making the anterior and posterior fontanelles at a same level. The parietal hair was cut off, and the local skin was disinfected. And then, a 0.8cm incision was made at the median of the scalp, and the anterior fontanelle was exposed, with a hole drilled at 0.2 cm behind the fontanelle and 2.9mm right lateral to the midline. A syringe needle 0.7mm in diameter of the 5μl microinjector fixed on the stereotaxic instrument was inserted along the hole to a depth of about 6mm (The caudate nucleus is located here), then 1.2μl normal saline containing collagenase VII 0.5U/μl and heparin 7U/μl was injected slowly. The needle was withdrawn after a retaining for 8 min, and then the
scalp was sutured. In the false operation group, only an equal volume of the normal saline was injected.

Grouping and treatment: The rats were divided into 5 groups, the normal group (N=8), the false operation group (N=8), the model group (N=7), the Shuigou EA group (N=8) for which "Shuigou" (GV 26) and "Shangxing" (GV 23) were punctured, and the Fengfu EA group (N=7) for which "Fengfu" (GV 16) and "Yamen" (GV 15) were needled. The conscious rats were fixed, and the location of acupoints was referred to the methods described in the book Experimental Acupuncture and Maxibustion combined with imitate location in the human body. "Fengfu" (GV 16) and "Yamen" (GV 15) were perpendicularly punctured, and "Shangxing" (GV 23) and "Shuigou" (GV 26) were horizontally needled with the disinfected 1-cun filiform needles. Parameters of EA: using the disperse-tense waves at the intensity that may induce a slight trembling of the rats’ limbs. The treatment was given once a day, with the needles retained for 30 min. The normal group, the false operation group and the model group were only fixed without any treatment.

The corresponding treatments were given 2h after the model establishment for 3 consecutive days. Before the rats were sacrificed, they were fasted for 12 hrs with a free access to water. And at the 1st hr after the last treatment, the rats were killed by decapitation, and then the brains were taken. The brain tissues were incised by coronal section with the micro-injecting hole as the center, and the surrounding tissues of the hematoma were taken for respective determination of NO, ET and T-AOC.

Determination of NO, T-AOC and ET: 10% homogenate of the brain tissues was prepared with cold saline in ice bath, and then centrifugalized (at 4 °C, 3000rpm) for 15min. The supernatant fluid was stored in a refrigerator at ~70 °C for determination of NO and T-AOC. The determination of NO and T-AOC was followed the operating manual. The ET content was determined by radioimmunoassay, following the operating manual. And the data were processed by the single factor analysis of variance.

**Results**

Effects of EA on NO content in the brain tissues:
After the model was established, NO content of the brain tissues in the model group increased significantly as compared with that in the false operation group ($P<0.05$). However, after treatment, the NO content in the Shuigou EA group decreased significantly ($P<0.05$); but in the Fengfu EA group, there was no such a significant change ($P>0.05$) as compared with that in the model group (see Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>NO content (μmol/g prot)</th>
<th>ET content (pg/mg tissue)</th>
<th>T-AOC (U/mg prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>4.229±0.811</td>
<td>114.57±64.55</td>
<td>2.298±0.719</td>
</tr>
<tr>
<td>False operation</td>
<td>8</td>
<td>4.833±0.466*</td>
<td>145.81±108.01*</td>
<td>2.253±0.314*</td>
</tr>
<tr>
<td>Model</td>
<td>7</td>
<td>10.256±1.569*</td>
<td>377.74±137.67*</td>
<td>0.982±0.062*</td>
</tr>
<tr>
<td>Shuigou EA</td>
<td>8</td>
<td>6.630±0.975**</td>
<td>135.32±42.76**</td>
<td>1.617±0.184**</td>
</tr>
<tr>
<td>Fengfu EA</td>
<td>7</td>
<td>9.116±1.295**</td>
<td>183.65±55.16**</td>
<td>1.589±0.268**</td>
</tr>
</tbody>
</table>

Note: Compared with the normal group, *$P<0.05$; **$P>0.05$; compared with the model group, $^*P<0.05$; $^*P>0.05$; and compared with the Shuigou EA group, $^*P<0.05$. The data of T-AOC were transferred by lg10().

Effects of EA on ET content in the brain tissues:

ET content in the brain tissues increased significantly in the model group ($P<0.05$) as compared with the false operation group ($P<0.05$); but in both the Shuigou EA group and the Fengfu EA group it decreased significantly ($P<0.05$) as compared with that in the model group (see Table 1).

Effects of EA on T-AOC content in the brain tissue:
T-AOC content in the brain tissues of the model group decreased significantly as compared with the false operation group; but in both the Shuigou EA group and the Fengfu EA group, it increased significantly as compared with the model group \( (P<0.05) \), with no significant difference between the two EA groups \( (P>0.05, \text{ see Table 1}) \).

**Discussion**

After cerebral hemorrhage, the secondary cerebral ischemia in the surrounding tissues of hematoma is a pathological change, to which the nerve functional defect is attributed. This is caused by the harmful network chain reaction due to a series of pathophysiological changes.

The destruction of the brain tissues, with edema, ischemia and anoxia of the surrounding tissues induced by cerebral hemorrhage, may lead to production of a large number of free radicals in the brain tissues, which is a result of the cerebral ischemia and anoxia, and can further aggravate the condition. Attention should be paid to NO, a kind of free radicals can induce the cerebral lesions, manifested by 1) inactivating enzymes to inhibit mitochondrial respiration, leading to rapid exhaustion of energy in the target cells, hence the death of neurons; 2) influencing repair of the target cells and the protein synthesis; 3) promoting the production of free radicals. The neurotoxicity induced by NO depends on its reduced state. The reduced type NO can rapidly react with superoxic anion to produce superoxidized nitrous acid, which is further resolved in acid environment into free radicals, hydroxyl group and nitrogen dioxide, with very strong toxicity. The former is the main oxygen free radical in NO inducing the cerebral lesions; and the toxicity of the latter, as a strong stimulator of lipid peroxidation, exceeds far than the hydroxyl group, and aggravates the lesions of the cerebral tissues. It has been confirmed that the key to cell death induced by cerebral ischemia is the increase of intercellular \( Ca^{2+} \) concentration and the activation of NO pathway. In comparison of the plasma NO levels in 133 cases of cerebral hemorrhage with that in 100 cases of healthy persons, Jiang Yajun et al.\(^4\) found that the patients with cerebral hemorrhage had abnormal NO metabolism, with severe imbalance of oxidation and anti-oxidation. This pathological change plays an important role in secondary cerebral lesions after cerebral hemorrhage. The present study indicated that 3 days after hemorrhage, the NO content in the cerebral hemorrhage model group was significantly higher than that in the normal group and the false operation group \( (P<0.05) \); and that the NO content in the Shuigou EA group was lower than that in the model group \( (P<0.05) \), but there was no significant difference between the Fengfu EA group and the model group \( (P>0.05) \). It is suggested that EA at "Shuigou" (GV 26) and "Shangxing" (GV 23) can decrease the NO content in the brain tissues, thus improving lesions of the hemorrhage tissue. Additionally, T-AOC in the cerebral hemorrhage model group decreased as compared with that in the normal group and the false operation group \( (P<0.05) \). In both the Shuigou EA group and the Fengfu EA group, the decreased T-AOC induced by cerebral hemorrhage were significantly increased \( (P<0.05) \), indicating that EA at "Shuigou" (GV 26) and "Shangxing" (GV 23) can decrease the production of NO and the other free radicals, and increase the anti-oxidation capability in rats, thus protecting the secondary cerebral lesions after cerebral hemorrhage; and that the protective action of EA at "Fengfu"(GV 16) and "Yamen"(GV 15) in the brain tissues is possibly related to the increase of anti-oxidation capability in rats.

Vascular endotheliocytes have wide physiological functions. Lesion of endotheliocytes can induce abnormal expression and increase release of ET. In a normal condition, ET can be degraded by endothelial cells, keeping at a very low level. In addition, the closed connection among the endothelial cells can arrest the leakage of ET from the blood vessels, acting on the vascular matrix membrane area. After cerebral hemorrhage, thrombosis and the other toxic materials released from hematoma can cause lesions of the vascular endotheliocytes, increasing vascular permeability. Production of ET is positively
correlated with lesions of the vascular endothelium. Increase of the ET expression and release can promote contraction of the vascular smooth muscles and the blood vessels, aggravate ischemia and anoxia of the brain tissues, increase in-flow of the extracellular Ca++, stimulate release of the intracellular Ca++, and activate excitant amino acids, making an overload of Ca++ and leading to direct lesions of the neurons and degeneration and necrosis of the brain tissues. The present study indicates that 3 days after hemorrhage, the ET content of the brain tissues in the cerebral hemorrhage model group is significantly higher than that in the normal group and the false operation group (P<0.05); but in the Shuigou EA group and the Fengfu EA group, it is significantly lower than that in the cerebral hemorrhage model group (P<0.05), suggesting that EA can improve the increase of ET expression and release, thus exerting a protective action on the brain tissues.

In brief, EA at "Shuigou" (GV 26) and "Shangxing" (GV 23), or at "Fengfu" (GV 16) and "Yamen" (GV 15) can improve the increase of the ET expression and release in the surrounding tissues of the hematoma induced by cerebral hemorrhage, and enhance the anti-oxidation capability; and EA at "Shuigou" (GV 26) and "Shangxing" (GV 23) can increase the anti-oxidation capability and decrease the production of NO free radicals in the surrounding tissues of the hematoma. Our previous experiment indicated that EA at "Shuigou" (GV 26) and "Shangxing" (GV 23), or at "Fengfu" (GV 16) and "Yamen" (GV 15) can significantly improve the cerebral morphological lesions and the deficit of the nervous and behaviour functions in the cerebral hemorrhage model rats induced by a combined injection of collagenase and heparin, and decrease the edema in the surrounding tissues of hematoma. However, EA at "Shuigou" (GV 26) and "Shangxing" (GV 23) is superior to that at the "Fengfu" (GV 16) and "Yamen" (GV 15) in improving the cerebral morphological lesions and deficit of the nervous and behaviour functions, indicating that a different combination of acupoints is of different specificity in action.

Reference
1. 任泽光，吴建中. 大鼠脑出血模型. 中华神经外科杂志 1993;9(4):205
2. 陆文注，王佩. 实验针灸学. 上海: 上海科学技术出版社 1999:288
4. 姜亚军，何家声，周冠富. 脑出血患者氧化抗氧化状态研究. 铁道医学 1997;25(6):335

(Translated by Wang Youjing 王友京)