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Metabolic Changes in Rats with Photochemically Induced Cerebral Infarction and the Effects of Batroxobin: A Study by Magnetic Resonance Imaging, ^{1}H- and ^{31}P- Magnetic Resonance Spectroscopy

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Metabolic changes in rats with photochemically induced cerebral infarction and the effects of batroxobin were investigated 1, 3, 5 and 7 days after infarction by means of magnetic resonance imaging (MRI), ^{1}H- and ^{31}P- magnetic resonance spectroscopy (MRS). A region of T_2 hyperintensity was observed in left temporal neocortex in infarction group and batroxobin group 1, 3, 5 and 7 days after infarction. The volume of the region gradually decreased from 1 day to 7 days after infarction. The ratio of NAA/Cho+Cr in the region of T_2 hyperintensity in the infarction group was significantly lower than that in the corresponding region in the sham-operated group 3, 5 and 7 days after infarction respectively (P<0.05). Lac appeared in the region of T_2 hyperintensity in the infarction group 1, 3, 5 and 7 days after infarction, but it was not observed in the corresponding region in sham-operated group at all time points. Compared with the sham-operated group, the ratios of βATP/PME+PDE and PCr/PME+PDE of the whole brain in the infarction group were significantly lower 1, 3 and 5 days after infarction respectively (P<0.05), and the ratio of βATP/PCr also was significantly lower 1 day after infarction (P<0.05). Batroxobin significantly decreased the volume of the region of T_2 hyperintensity 1 and 3 days after infarction (P<0.05), significantly increased the ratio of NAA/Cho+Cr in the region 5 and 7 days after infarction (P<0.05), significantly decreased the ratios of Lac/Cho+Cr and Lac/NAA in the region 5 and 7 days after infarction (P<0.05), and significantly increased the ratios of βATP/PME+PDE and βATP/PCr in the whole brain 1 day after infarction (P<0.05). The results indicated that the infracted region had severe edema, increased Lac and apparent neuronal dysfunction and death, and energy metabolism of the whole brain decreased after focal infarction, and that batroxobin effectively ameliorated the above-mentioned abnormal changes.

When the photosensitizing dye rose Bengal is intravenously injected into rats and the intact skull surface is focally illuminated, cerebral blood vessels in a confined area sustain photochemical injury. Singlet oxygen molecules generated by the interaction of the dye and light cause peroxidation of

endothelial cell membranes and occlusive platelet aggregation, and subsequent thrombus formation leads to cerebral infarction[1]. This photochemical model of cerebral infarction is virtually noninvasive, and allows for reproducible infarct size and location.

Magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are noninvasive techniques that allow longitudinal studies of biochemical changes in vivo after cerebral infarction. MRI can show well the changes of brain water after infarction and quantitatively reflect histopathological changes[2]. MRS can provide information about the changes of several other metabolites after infarction.[3,4] Both of them have been proved to be of great value in the investigation of cerebral infarction.

Batroxobin is abstracted from the venom of botrops moojeni, and has the activity of the thrombin-like enzyme because it can convert fibrinogen to fibrin by splitting off fibrinopeptide. Results of clinical trials indicate it is an effective drug for treating cerebral infarction.[5]

This study was designed to investigate metabolic changes of cerebral infarction and the effects of batroxobin in a photochemically induced cerebral infarction rat model by methods of MRI, ^{1}H- and ^{31}P-MRS.

MATERIALS AND METHODS

18 male Sprague-Dawley rats weighing 180-220g each were assigned randomly and equally into the sham-operated group, infarction group and batroxobin group. Each rat was anesthetized with chloral hydrate (100 mg/ml, 400 mg/kg,i.p.), then placed in stereotaxic apparatus. Rectal temperature was kept constant at 37.0±0.2°C with a homeothermic blanket. With the use of aseptic techniques, a midline incision was made in the scalp between left eye and left ear, and the pericranium was displaced to provide a clear surface of left temporal bone. Rose bengal (Sigma Chemical Ltd; 5 mg/ml, 20 mg/kg) was injected slowly (90 seconds) via a femoral vein, and the skull was immediately illuminated for 20 minutes by means of a fiber-optic bundle. Cold white light originated from a fiber-optic light source that contained a 120W halogen bulb regulated to provide a constant 67% of its maximal nominal power. The illuminated area was a circular area of 3 mm in diameter and the center of the area was stereotaxically positioned on the skull surface 2 mm posterior to the bregma, 6.5 mm left of the midline and 2.6 mm downward, that is, aimed at left temporal neocortex. The light was then interrupted, the scalp wound closed with sutures, and the animal allowed to recover. Rats in the sham-operated group were prepared with the same procedure, but given no rose bengal and illumination. Rats in the batroxobin group were treated with batroxobin (Tobishi Pharmaceutical Co Ltd; 8 BU/kg, i.p.) three times (10 minutes before illumination, 24 and 48 hours after illumination); and rats in the sham-operated group and infarction group were treated with the same volume of saline.

1, 3, 5 and 7 days after operation, rats were anesthetized and placed in a Bruker 4.7T spectrometer. Firstly, rats underwent T_2 weighted MRI by means of a transaxial T_2 weighted spin-echo sequence (TR, 2500 ms; TE, 20 ms; slice thickness, 1mm; interstice gap, 1 mm; 16 slices; FOV, 30×30 mm^2, matrix, 128×128). Then, ^{1}H-MRS was performed with a single volume technique. A 4×4×4 mm^3 region of interest was selected in the center of the region of T_2 hyperintensity of each rat in the infarction group and batroxobin group and in the

corresponding region of each rat in the sham-operated group. The sequence (TR, 1000 ms; TE, 135 ms; data points, 1024; iterations, 2048; spectral width, 1500 Hz) was applied. Finally, ^{31}P-MRS of the whole brain of each rat was performed with a sequence (TR, 1000 ms; data points, 2048; iterations, 1024; spectral width, 5000 Hz).

Based on T_2 weighted images, the volume of the region of T_2 hyperintensity was calculated as follows: calculating the area of the region of T_2 hyperintensity in each slice of each rat with the use of imaging processing software; then, adding up the areas and multiplying the sum and slice thickness. After Fourier transformation and phase correction, each spectrum was baseline corrected. The area of the peak was calculated by integration. We assigned four peaks in ^{1}H-magnetic resonance spectra according to their positions: choline-containing compounds (Cho), at 3.25 ppm; creatine and phosphocreatine (Cr), at 3.05 ppm; N-acetylaspartate (NAA), at 2.00 ppm; lactate (Lac), at 1.33 ppm (see Fig. 1), and the ratios of NAA/Cho+Cr, Lac/Cho+Cr and NAA/Lac were calculated. We assigned six peaks in ^{31}P-magnetic resonance spectra according to their positions: phos-phomonoester (PME), at 6.7 ppm; phosphodiester (PDE), at 5.0 ppm; phosphocreatine (PCr), at 0.0 ppm; γATP, at-2.4 ppm; αATP, at −7.6 ppm; βATP, at −16.2 ppm (see Figure 2), and the ratios of βATP/PME+PDE, PCr/PME+PDE and βATP/PCr were calculated.

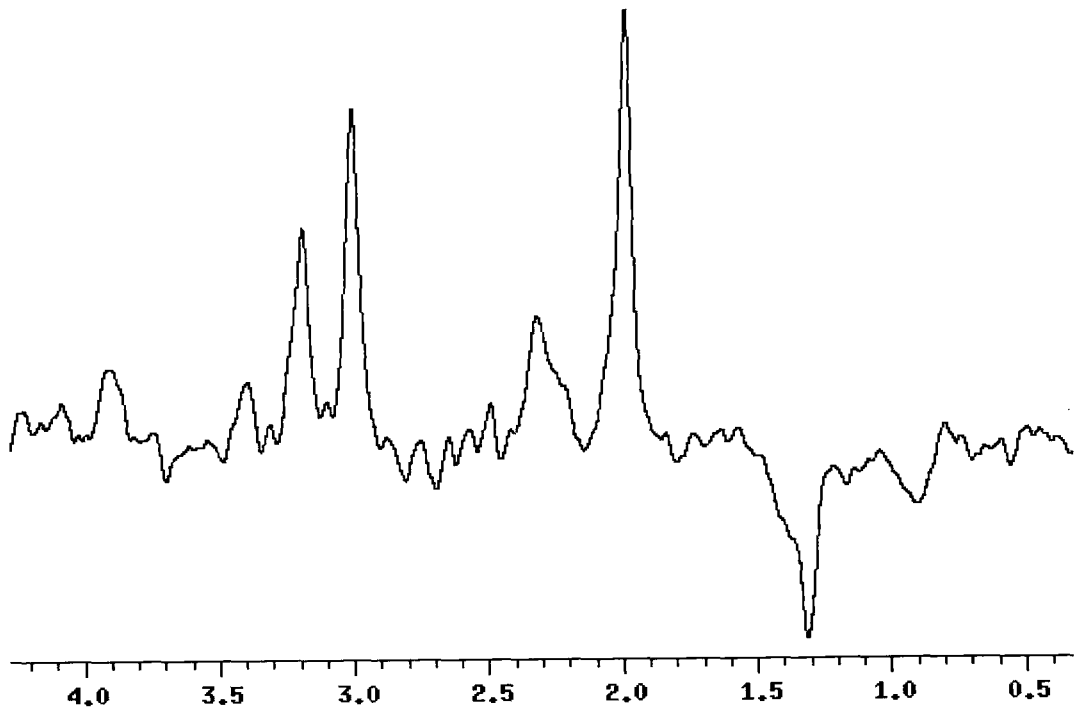

Fig 1. ^{1}H-spectrum from the region with T_2 hyperintensity of a rat in cerebral infarction group: Cho at 3.25 ppm, Cr at 3.05 ppm, NAA at 2.00 ppm and Lac at 1.33 ppm

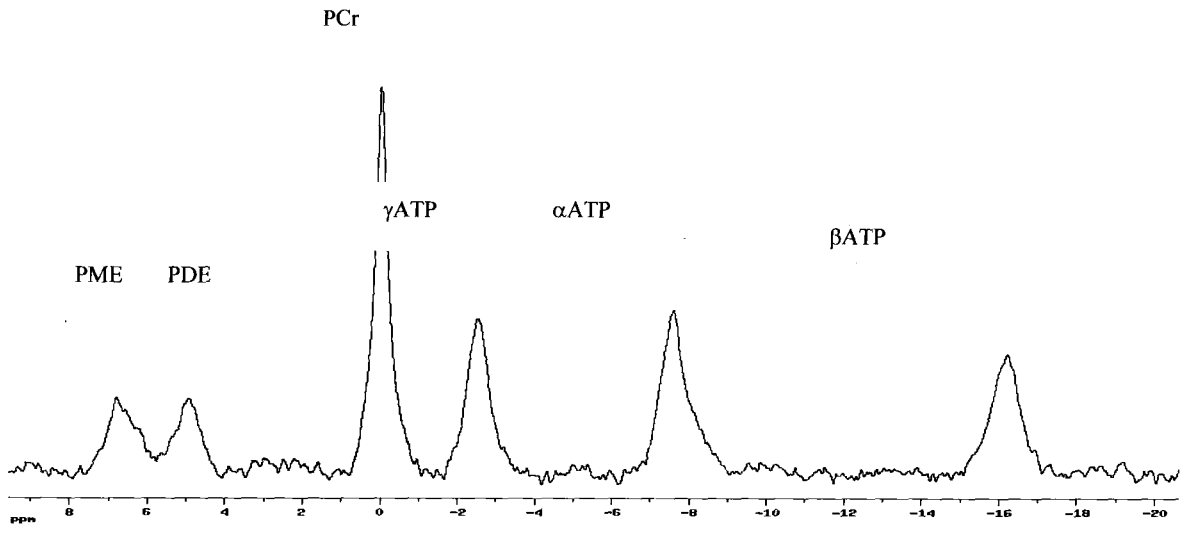

Fig 2. 31P-spectrum from the whole brain of a rat in cerebral infarction group: PME at 6.7 ppm, PDE at 5.0 ppm, PCr at 0.0 ppm, γATP at-2.4 ppm, αATP at –7.6 ppm and βATP at –16.2 ppm

Statistics: Statistical differences were assessed by the use of unpaired *t* test, one-way ANOVA and rank test. Values are mean±SD.

RESULTS

The Volume of the Region of T_2 Hyperintensity

In the sham-operated animals, no pathological changes were detected at all time points examined with T_2 weighted MRI. In contrast, a lesion, which T_2 weighted MRI revealed a hyperintense region was clearly visible in left temporal neocortex in each rat of the infarction group and batroxobin group at all time points. The volume of the region of T_2 hyperintensity gradually decreased from 1 day to 7 days. Compared with the infarction group at the corresponding time point, the volume of the region of T_2 hyperintensity of the rat in the batroxobin group significantly decreased 1 and 3 days after infarction respectively ($P<0.05$), as shown in Table 1.

Table 1. The volume of the region of T_2 hyperintensity (mm^3)

Group	n	1d	3d	5d	7d
Infarction	6	158.3±27.9	114.3±11.7	79.5±7.6	60.8±9.4
Batroxobin	6	107.5±24.2*	92.5±12.8*	78.8±24.2	60.5±15.1

Data are mean ±SD. By *t* test

* $P<0.05$ in comparison with the corresponding infarction group

The Ratios of NAA/Cho+Cr, Lac/Cho+Cr and NAA/Lac

Peaks corresponding to NAA, Cho, and Cr were readily assigned in all rat brains, but Lac peak was too low to identify in the sham-operated group. Compared with the corresponding region of rats in the sham-operated group at the corresponding time point, in infarction group, the ratio of NAA/Cho+Cr

of the region of T_2 hyperintensity was significantly lower 3, 5 and 7 days after infarction respectively (P<0.05). Lac appeared in the region of T_2 hyperintensity in the infarction group 1, 3, 5 and 7 days after infarction, and it was not observed in the corresponding region in the sham-operated group at all time points. Compared with the infarction group at the corresponding time point, in batroxobin group, the ratio of NAA/Cho+Cr significantly increased 5 and 7 days after infarction respectively (P<0.05), and the ratios of Lac/Cho+Cr and Lac/NAA significantly decreased 5 and 7 day after infarction respectively (P<0.05), as shown in Table 2 and 3.

Table 2. The ratio of NAA/Cho+Cr

Group	n	1d	3d	5d	7d
Sham-operated	6	0.92±0.03	0.92±0.04	0.92±0.04	0.92±0.01
Infarction	6	0.76±0.19	0.64±0.13#	0.49±0.09#	0.51±0.05#
Batroxobin	6	0.73±0.09	0.58±0.11	0.62±0.05*	0.65±0.05*

Data are mean ±SD. By one-way ANOVA

* P<0.05 in comparison with the corresponding infarction group

# P<0.05 in comparison with the corresponding sham-operated group

Table 3. The ratios of Lac/Cho+Cr and Lac/NAA

Group	Lac/Cho+Cr					Lac/NAA			
	n	1d	3d	5d	7d	1d	3d	5d	7d
Infarction	6	0.25±0.07	0.16±0.09	0.46±0.49	0.47±0.40	0.34±0.09	0.27±0.15	1.14±1.44	0.75±0.95
Batroxobin	6	0.35±0.17	0.11±0.10	0.05±0.11*	0.04±0.07	0.49±0.25	0.21±0.21	0.10±0.19*	0.05±0.10*

Data are mean ±SD. By rank test

* P<0.05 in comparison with the corresponding infarction group

The Ratios of βATP/PME+PDE, PCr/PME+PDE and βATP/PCr

Compared with the sham-operated group at the corresponding time point, in infarction group, the ratios of βATP/PME+PDE and PCr/PME+PDE of the whole brain significantly decreased 1, 3 and 5 days after infarction respectively (P<0.05), and the ratio of βATP/PCr significantly decreased 1 day after infarction (P<0.05). Compared with the infarction group, the ratios of βATP/PME+PDE and βATP/PCr of the whole brain in the batroxobin group significantly increased 1 day after infarction (P<0.05), but the ratio of PCr/PME+PDE showed no significant difference between the two groups at all time points, as shown in Table 4 and 5.

Table 4. The ratios of βATP/PME+PDE and PCr/PME+PDE

Group		βATP/PME+PDE				PCr/PME+PDE			
	n	1d	3d	5d	7d	1d	3d	5d	7d
Sham-operated	6	1.14±0.07	1.17±0.06	1.19±0.04	1.16±0.07	1.75±0.28	1.81±0.19	1.88±0.19	1.83+0.29
Infarction	6	0.64±0.06#	0.88±0.17#	0.96±0.09#	1.20±0.37	1.30±0.19#	1.44±0.17#	1.46±0.15#	1.56±0.64
Batroxobin	6	1.03±0.18*	1.38±0.64	1.01±0.32	1.05±0.03	1.54±0.16	2.22±1.04	1.33±0.18	1.50±0.21

Data are mean ±SD. By one-way ANOVA and rank test

* $P<0.05$ in comparison with the corresponding infarction group

# $P<0.05$ in comparison with the corresponding sham-operated group

Table 5. The ratio of βATP/PCr

Group	n	1d	3d	5d	7d
Sham-operated	6	0.66±0.07	0.65±0.07	0.64±0.07	0.64±0.07
Infarction	6	0.50±0.10#	0.62±0.18	0.66±0.10	0.68±0.19
Batroxobin	6	0.66±0.05*	0.63±0.16	0.75±0.15	0.71±0.10

Data are mean ±SD. By one-way ANOVA

* $P<0.05$ in comparison with the corresponding infarction group

# $P<0.05$ in comparison with the corresponding sham-operated group

DISCUSSION

Edema is an important event in the development of cerebral infarction. Because T_2 weighed imaging is based on the signals derived from hydrogen nuclei in brain water, it can show well the extent of edema of infarcts. By means of T_2 weighed imaging, this study demonstrates the edema of photochemically induced cerebral infarction is gradually abated from 1 to 7 days which is consistent with the previous report.[2] The serious edema of cerebral infarcts at the early stage indicates ameliorating the edema of infarcts is an important principle in the treatment of cerebral infarction at the early stage. In this study, by effectively lessening the edema of infarcts, batroxobin shows its good efficacy in the treatment of cerebral infarction.

Metabolic changes occur after cerebral infarction. Peaks in magnetic resonance spectra can show the changes of several metabolites after cerebral infarction.

NAA is the most important visible metabolite in ^1H MRS. Although NAA is present at the highest level of any amino acid in the brain except glutamate, its function is still not well known. NAA is found almost exclusively in neurons, where it has been shown to be produced in the mitochondria. It has also been found in the oligodendrocyte type II astrocyte progenitor cells, but these cells only represent very small part of the glial population. Consequently, NAA is considered to be the marker of neurons.[6] NAA concentration of the brain tissue is relative to its synthesis in neurons. Neuronal dysfunction and

death lead to the reduction of NAA synthesis, which results in the decrease of NAA concentration, so the change of NAA concentration may reflect the changes of the number and function of neurons. Lac is the production of anaerobic metabolism, so its concentration may reflect the severity of ischemia. Because Lac is also produced by macrophages that migrate to the site of ischemia,[7] its concentration also reflects the invasion of macrophages. Cr consists of creatine and phosphocreatine; and they are found in both neurons and glial cells and act as a phosphate transport system and energy buffer within cells. Cho consists predominantly of glycerophosphocholine and phosphocholine, and both compounds are involved in membrane synthesis and degradation. Cho is also a precursor and degradation product of acetylcholine, an important neurotransmitter.

This study has clearly shown that cerebral infarction results in a reduction of NAA/Cho+Cr with an increase of Lac/ Cho+Cr and Lac/NAA, and that batroxobin ameliorates the abnormal changes of these ratios. During the period of cerebral infarction, the decrease of Cho and Cr is less than that of NAA,[3,8] so this study not only indicates the reduction of NAA and the increase of Lac occur in infarcts, which is consistent with results of many clinical trials and experimental studies on cerebral infarction,[6-12] but also demonstrates batroxobin can lead to the increase of NAA and the reduction of Lac of infarcts. This exposes that neuronal death and dysfunction, the intensifying of anaerobic metabolism and macrophages' invasion take place after cerebral infarction, and that batroxobin can effectively ameliorate these abnormal changes.

A gradual decrease of NAA has been observed within 5 days of infarction onset in this study. It suggests that neuronal dysfunction and death in infarcts are progressive several days after infarction. This implicates that there is a longer period than expected for the treatment of saving the neurons in infarcts. In this study, the further increase of Lac, which results from the invasion of macrophages, has also been observed 5 days after infarction. This implicates inhibiting the invasion of macrophages is helpful to relieve the acidosis of Lac after cerebral infarction.

There are three peaks of ATP in ^{31}P-MRS: αATP, βATP and γATP. The peak of βATP represents ATP best, so βATP peak is used to represent ATP in this study. PCr peak represents phosphocreatine. Both ATP and PCr contain high energy phosphate which can be transferred between them. They are the important ways for the storage of energy in body and play a key role in energy metabolism. PME consists of phosphorylcholine and phosphorylethanolamine, PDE consists of phosphatidylcholine and phosphatidylethanolamine, and they are the main composition of cerebral membrane phospholipids and myelin.

This study has clearly shown that cerebral infarction results in the reduction of βATP/PME+PDE、PCr/PME+PDE and βATP/PCr in the whole brain, and that batroxobin relieves the decrease of βATP/PME+PDE and βATP/PCr in the whole brain. Concequently, this study not only indicates that the reduction of ATP and PCr in the whole brain occur and the reduction of ATP is more than that of PCr after focal infarction, but also demonstrates batroxobin can lead to the increase of ATP in the whole brain after focal infarction. This exposes that focal infarction results in low level of energy metabolism in the whole brain, and that batroxobin can effectively improve energy metabolism of the whole brain with focal infarction.

A rapid decrease of ATP and PCr of the whole brain 1 day after infarction and a gradual increase of them afterwards have been observed in this study. It indicates that the level of energy metabolism of the whole brain rapidly decreases at the early stage of focal infarction and gradually recovers afterwards. This implicates that attention should be paid to improving energy metabolism of the whole brain after focal infarction, especially at the early stage in the treatment of cerebral infarction.

This study shows batroxobin may ameliorate the abnormal metabolic changes after cerebral infarction. Batroxobin is one of glucoproteins, and its molecular weight is about 36,000 daltons. It has three kinds of pharmacological effects: decreasing plasma fibringen and inhibiting blood coagulation and thrombosis; promoting the release of t-PA, intensifying the effect of t-PA, and increasing the activity of fibrinolysis and promoting thrombolysis; decreasing blood viscosity and vascular resistance, and improving microcirculation. These pharmacological effects are the basic factors for treating cerebral infarction.

Our previous studies[13-17] found that batroxobin could decrease the severity of edema and the content of arginine-vasopressin and nitric oxide of ischemic cerebral tissue, inhibit peroxidation of lipid and the expression of c-fos ,c-jun, heat shock protein 70, basic fibroblast growth factor and endothelin, and that batroxobin was able to directly protect hypoxic hippocampal neurons cultured or in brain slice by its neuroprotective effects. In the present study, we find that batroxobin can ameliorate edema of infarcts, decrease the severity of acidosis of Lac and the neuronal dysfunction and death of infarcts, and improve energy metabolism of the whole brain with focal infarction. All of our studies indicate that batroxobin is an effective drug for cerebral infarction; but its effects on cerebral infarction are very complicated, which needs to be further studied.

References

1 Dietrich WD, Watson BD, Busto R, et al. Photochemically induced cortical infarction I. Early microvascular alterations. Acta Neuropathol (Berl), 1987; 72: 315-325.

2 Lee VM, Burdett NG, Carpenter TA, et al. Evolution of photochemically induced focal cerebral ischemia in the rat: magnetic resonance imaging and histology. Stroke, 1996; 27: 2110-2119.

3 Higuchi T, Graham SH, Fernandez EJ, et al. Effects of severe global ischemia on N-acetylaspartate and other metabolites in the rat brain. Magn Reson Med, 1997; 37: 851-857.

4 De la Torre JC, Butler K, Kozlowski P, et al. Correlates between nuclear magnetic resonance spectroscopy, diffusion weighted imaging, and CA1 morphometry following chronic brain ischemia. J Neurosci Res, 1995; 41: 238-245.

5 匡培根, 王国平, 郎森阳, 等. 东菱精纯克栓酶治疗缺血性脑血管病. 中国新药杂志, 1994; 3 (6): 36.

6 Saunders DE, Howe FA, van den Boogaart A, et al. Continuing ischemic damage after acute middle cerebral artery infarction in humans demonstrated by short-echo proton spectroscopy. Stroke, 1995; 26: 1007-1013.

7 Lanfermann H, Kugel H, Heindel W, et al. Metabolic changes in acute and subacute cerebral infarctions: Findings at proton MR spectroscopic imaging. Radiology, 1995; 196: 203-210.

8 Bruhout S,Loubinoux MI, Borredon J, et al. A one-dimensional (proton and phosphorus) and two-dimensional (proton) in vivo NMR spectroscopic study of reversible global cerebral ischemia. J Neurochem, 1996; 66: 2491-2499.

9 Sappey-Marinier D, Chileuitt L, Weiner MW, et al. Hypoglycemia prevents increase in lactic acidosis during reperfusion after temporary cerebral ischemia in rats. NMR-Biomed, 1995; 8 (4): 171-178.

10 Higuchi T, Fernandez EJ, Maudsley AA, et al. Mapping of lactate and N-acetyl-l-aspartate predicts infarction during acute focal ischemia: in vivo 1H magnetic resonance spectroscopy in rats. Neurosurgery, 1996; 38 (1): 121-130.

11 Gyngell ML, Busch E, Schmitz B, et al. Evolution of acute focal cerebral ischaeimia in rats observed by localized 1H MRS, diffusion-weighted MRI, and electrophysiological monitoring. NMR-Biomed, 1995; 8 (5): 206-214.

12 Gillard JH, Barker PB, van Zijl PCM, et al. Proton MR spectroscopy in acute middle cerebral artery stroke. AJNR Am J Neuroradiol, 1996; 17: 873-886.

13 Wu WP, Kuang PG, Jiang SJ, et al. Effect of batroxobin on expression of c-Jun in left temporal ischemia rats with spatial learning and memory disorder. J Trad Chin Med, 2000; 20 (2): 83-86.

14 刘军, 匡培根, 张凤英, 等. 巴曲酶的神经保护作用: 缺血再灌注不同时程热休克蛋白 70 及病理组织学变化的相关性研究. 中风与神经疾病杂志, 1996; 13 (3): 137-139.

15 匡培根, 刘军, 吴卫平, 等. 巴曲酶对局灶性脑缺血再灌注大鼠成纤维细胞生长因子样免疫反应的影响. 中风与神经疾病杂志, 1996; 13 (2): 78-80.

16 刘军, 匡培根, 王福庄, 等. 缺氧对海马神经元培养的影响及巴曲酶的保护作用: HSP70 的表达及细胞形态学的研究. 中风与神经疾病杂志, 1997; 14 (1): 8-10.

17 匡培根, 黄燕华, 吴卫平, 等. 巴曲酶对海马脑片缺氧损伤的保护作用. 中风与神经疾病杂志, 1997; 14 (6): 325-326.