Effects of *Ginkgo biloba* extract on acute cerebral ischemia in rats analyzed by magnetic resonance spectroscopy

PENG Hai[1], LI Yue-Fen, SUN Sheng-Gang

Department of Neurology, Union Hospital of Tongji Medical Colleg, Huazhong University of Science and Technology, Wuhan 430022, China

KEY WORDS *Ginkgo biloba*; magnetic resonance spectroscopy; cerebral ischemia

ABSTRACT

AIM: To study the effect of *Ginkgo biloba* extract (*Gb*E) on acute cerebral ischemia in rats. **METHODS**: The rats were randomly divided into four groups: sham-operated group (group I as control), ischemic group (group II), the prophylactic (*Gb*E premedication) group (group III) and *Gb*E-treatment group (group IV). Magnetic resonance spectroscopy (MRS) was carried out to dynamically monitor the changes in biochemical metabolic variations 48 h after cerebral ischemia and effects of *Gb*E (100 mg/kg, ip, qd). **RESULTS**: (1) Lactate (Lac) peak could be detectable at the infarction area 90 min after acute cerebral ischemia and increased with time. Lac peak in the prophylactic group was elevated slightly ($P<0.01$, $n=6$), whereas in the treatment group the elevation of Lac was more remarkable than that in the prophylactic group ($P<0.05$, $n=6$). (2) In the ischemic group, the level of *N*-acetyl aspartate (NAA) was decreased within 4 h after ischemia ($P<0.05$, $n=6$), and the decline persisted ($P<0.01$, $n=6$). In the treatment group and prophylactic group, NAA was decreased slightly after 24 h ($P<0.05$, $n=6$). (3)Twenty-four hours after ischemia, in both ischemic group and treatment group, choline (Cho) was elevated slightly ($P<0.05$, $n=6$) and creatine (Cr) was decreased slightly ($P<0.05$, $n=6$), but in the prophylactic group these changes occurred only after 48 h. **CONCLUSION**: *Gb*E could prevent and treat acute cerebral ischemia. The effectiveness was more satisfactory when *Gb*E was used preventively.

INTRODUCTION

A series of chain reactions occur in brain after acute ischemia. Of all the changes, the biochemical metabolic change happens earliest. So, the study on the early biochemical metabolic change after acute cerebral ischemia may play a very important role in the diagnosis, treatment and prognosis of cerebrovascular diseases.

Ginkgo biloba extract (*Gb*E) is a kind of Chinese herb, consisting of 24 % flavonoid glycosides, 6 % terpene lactones (including ginkgolides A, B, C, J and bilobalide) and 70 % other substances (such as proanthocyanidins, organic acids, and sugars, *etc*). *Gb*E has extensive protective effects on central nerve system and circulation system. *Gb*E can eliminate free oxygen radicals[1], reduce lipid peroxidation and facilitate the synthesis and/or release of epoprostenol[2]. It was reported that *GbE* could diminish the coagulation of platelets to inhibit the thrombosis by antagonizing platelet activating factor. *Gb*E is capable of inhibiting the adhesion of monocytes and neutrophils to cultured cerebral microvascular endothelial cells[3]. *Gb*E has therapetic effects on peripheral arterial occlusive dis-

[1] Correspondence to Assoc Prof PENG Hai.
Phn 86-27-8572-6055. E-mail penghai493@sohu.com
Received 2002-08-22 Accepted 2002-10-22

ease[4]. Though *Gb*E has been widely applied to treat vascular ischemia, its effects on the biochemical metabolism following cerebral ischemia and the interference on the prognosis were unclear. The present study was to observe the dynamic changes of biochemical metabolism after acute cerebral ischemia and validate the effects of *Gb*E on acute cerebral ischemia during early stage by magnetic resonance spectroscopy (MRS).

MATERIALS AND METHODS

Animals Twenty-four healthy Sprague-Dawley rats weighting 200-250 g (provided by Experimental Animal Centre of Tongji Medical College of Huazhong University of Science and Technology, Certificate No 19-053) were randomly divided into 4 groups: sham-operated group (group I as control), ischemic group (group II), prophylactic (*Gb*E premedication) group (group III) and *Gb*E-treatment group (group IV). There were 6 rats in each group.

Reagents *Gb*E was prepared and kindly provided by Calculation Test-Analysis Center of Guangxi Normal University (Guilin, China). The contents of flavonoid glycosides and terpene lactones were used as quality control standard (24 %±1 % and 6.1 %±0.5 % , respectively).

Animal experiment The rats were fasted overnight before the experiment but allowed free access to water according to the method of Nakasawa *et al*[5]. The rats were anesthetized with 10 % chloral hydrate (3.5 mL/kg, ip). Temperature was kept at 37 ° C with a heat lamp and a piece of heating pad during operation. Their right proximal common carotid artery (CCA) and the root of the external carotid artery (ECA) were separated and ligated. The occluder was intromitted from CCA near the branch, passed through internal carotid artery (ICA) to middle cerebral artery (MCA). The depth of the occluder was (18.5±0.5) mm. The rats in the sham-operated group were subject to the same procedure, but the depth of the occluder was less than 9 mm and did not take any drugs, those in the ischemic group received isometric normal saline just at the moment of ischemia for 3 consecutive days, those in the prophylactic group had been premedicated with *Gb*E 3 d before ischemia (*Gb*E 100 mg/kg, ip, qd), while those in the treatment group took *Gb*E just at the moment of ischemia.

Magnetic resonance spectroscopy MRS spectra obtained on a Bruker Biospec200 spectrometer (Biospec 47/30, Bruker/spetrospin, Switzerland). The probe was inserted into a Bruker Biospec200 spectrometer with a vertical bore and a magnetic field strength of 4.7T. Homogeneity of 0.11 parts per million (ppm) was obtained in all experiments, leading to a constant line width. The excitation pulse was optimized through maximizing the water signal in the ^{1}H spectral[6].

Statistical analysis The spectra were analyzed by measuring the peak heights of choline (Cho) 3.15, creatine (Cr) 3.05, *N*-acetyl aspartate (NAA) 2.02 and lactate (Lac) 1.32. ^{1}H spectra and magnetic resonance imaging (MRI) were obtained respectively at the 4 h, 24 h, and 48 h after ischemia. To compare individual experiments, the areas of Lac, Cho, Cr and NAA under the peaks and the ratios of the Lac/(Cho+Cr), Cho/(Cho+Cr), Cr/(Cho+Cr) and NAA/(Cho+Cr) were measured by integral method respectively. Semi-quantitative analysis was performed. All data were expressed as mean±SD. Comparison among groups was made by analysis of variance.

RESULTS

Changes in the cerebral infarction volume There was no infarction locus formation in each group within 4 h after ischemia. Infarction loca emerged after 24 h, and enlarged as time went on. The infarction volume was smaller both in the prophylactic group and the treatment group, especially in the former, than in the ischemic group (Tab 1). The infarction volume was expressed as physical unit (pu).

Changes of lactate Lac could be detected in the infarct lesion at 90 min after acute cerebral ischemia and was elevated with time. Lac was elevated slightly in the prophylactic group, but moderately in the treat-

Tab 1. Influence of *Ginkgo biloba* extract (*Gb*E) on the infarction volume in rat hippocampus. *n*=24. Mean±SD. ^{b}*P*<0.05, ^{c}*P*<0.01 *vs* ischemia.

Time after ischemia /h	Sham-operation	Ischemia	Premedication	Treatment
4	ND	ND	ND	ND
24	ND	4806±241	1399±508^c	3690±153^b
48	ND	64381±71	2422±41^c	5823±65^c

ND: not detected

ment group. No Lac signal was found in the sham-operation group (Tab 2).

Tab 2. Influence of *Ginkgo biloba* extract (*Gb*E) on lactate in rat hippocampus. *n*=24. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* ischemia.

Time after ischemia /h	Sham-operation	Ischemia	Premedication	Treatment
4	ND	1.57±0.21	0.41±0.09^{c}	1.23±0.07^{b}
24	ND	4.9±0.5	0.95±0.03^{c}	2.6±0.6^{b}
48	ND	8.29±0.11	2.19±0.05^{c}	5.29±0.11^{b}

ND: not detected

Changes of NAA NAA in the ischemic group was decreased within 4 h. As time went on, NAA was decreased obvioulsy. However, in the prophylactic group and the treatment group, NAA did not change within first 4 h, and decreased slightly after 24 h (Tab 3).

Changes of Cho and Cr There was no significant change of Cho and Cr within 4 h after ischemia in all groups. Twenty-four hours after ischemia, Cho was slightly elevated and Cr slightly decreased after 24 h in both the ischemic group and the treatment group, but in the prophylactic group the above changes occurred after 48 h (Tab 3).

Changes of Lac peak in MRS Normally, there is no Lac in the brain, so that there is no Lac peak in MRS (Fig 1). Lac peak could be detected in the infarct lesion at 90 min after acute cerebral ischemia (Fig 2) and was elevated as time went on (Fig 3, 4).

DISCUSSION

Several studies have reported the presence of Lac signal after acute cerebral ischemia at various time points when monitored with MRS (from several minutes to 28 h[7]). Our present data revealed that Lac could be detected at 90 min after ischemia and was elevated as time went on. Lac generated in the ischemic brain remained for a long time. The reasons might be that: (1) Lac is produced in acute stage of ischemia and anoxia; (2) Lac was accumulated in the ischemic tissue in acute stage, where there was not enough blood flow to wash out local Lac[7]. The levels of Lac in the prophylactic group or the treatment group was not as high as that in the ischemic group, which might be related to the effects of *Gb*E by which the blood circulation was improved and the brain blood flow was increased so the glucose and oxygen intake was enhanced.

It was also found in this study that the level of Lac was related to the infarction volume, which is consistent with the result of Graham[8], suggesting that the

Tab 3. Influence of *Gb*E on NAA, Cho, and Cr in rat hippocampus. *n*=24. Mean±SD. $^{b}P<0.05$, $^{c}P<0.01$ *vs* sham-operation.

Groups	Time after ischemia/h	NAA	Cho	Cr
Sham-operation	4	0.91±0.14	0.35±0.05	0.64±0.12
	24	0.87±0.16	0.34±0.07	0.64±0.12
	48	1.1±0.4	0.32±0.15	0.77±0.10
Ischemia	4	0.65±0.16^{b}	0.38±0.06	0.62±0.06
	24	0.120±0.010^{c}	0.625±0.021^{b}	0.375±0.021^{b}
	48	0.010±0.010^{c}	0.780±0.014^{c}	0.150±0.014^{c}
Premedication	4	0.86±0.17	0.38±0.03	0.60±0.04
	24	0.630±0.060^{b}	0.44±0.09	0.56±0.09
	48	0.530±0.030^{b}	0.56±0.04^{b}	0.46±0.09^{b}
Treatment	4	0.79±0.07	0.35±0.04	0.65±0.04
	24	0.53±0.05^{c}	0.61±0.05^{c}	0.44±0.06^{b}
	48	0.220±0.010^{c}	0.69±0.06^{b}	0.270±0.014^{c}

ND: not detected; Cho: the peak heights of choline; Cr: creatine; NAA: *N*-acetyl aspartate.

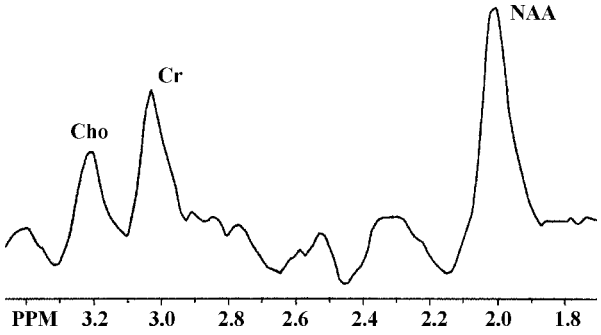

Fig 1. Normal spectra in sham-operation group (NAA 2.02, Cr 3.05, Cho 3.15, no Lac peak). Cho: the peak heights of choline; Cr: creatine; NAA: *N*-acetyl aspartate.

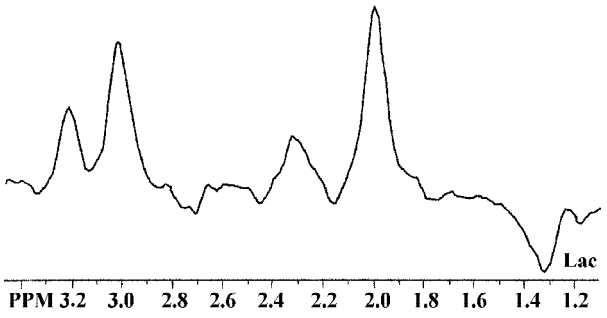

Fig 2. Lac peak occurred within 90 min after ischemia (Lac 1.32).

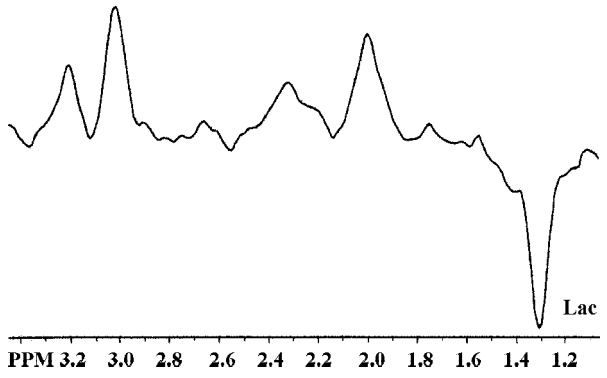

Fig 3. Lac peak increased within 24 h after ischemia.

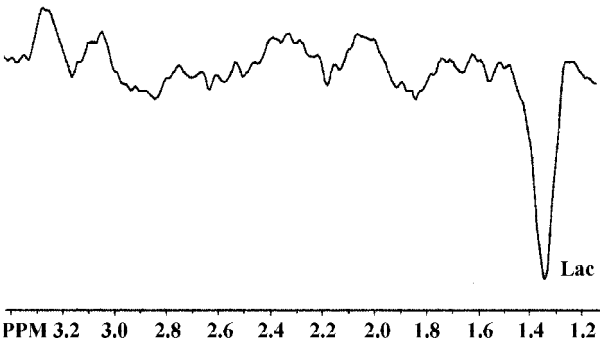

Fig 4. Lac peak was more obvious within 48 h after ischemia.

severity of ischemia could be estimated by quantifying the change of Lac.

NAA mainly exists in the brain neurons during normal condition as a marker of neurons[9]. In brain injury and brain tumor, NAA is reduced markedly. In this study, the results showed that NAA was reduced within 4 h after ischemia, indicating the starting of neuron injury. In the prophylactic group and the treatment group, Lac emerged at 90 min after ischemia, but the level of NAA was not reduced, demonstrating that there was a time course from ischemia to necrosis. If active treatment was implemented early, damage caused by ischemia can be alleviated. So early diagnosis and treatment in the "time window" are very important for the prognosis.

Cho is one of the main compositions of cell membrane, and also the precursor and metabolite of acetylcholine. Although there was greatly controversy about the increase or decrease of the Cho levels following ischemia at present, most researchers believed that Cho was increased[10,11]. Our results demonstrated that Cho did not change within 4 h after ischemia, but it was elevated after 24 h, which might be contributed to the facts that ischemia and anoxia of brain tissue and the reducation of ATP could result in the decrease of the activities of cholinekinase and phosphocholine cytidine transacetylase, leading to the disturbance of Cho metabolism.

Cr exists both in neurons and gliocyte. It serves as a reserve for high-energy phosphates most prominently in neurons and buffers cellular energy reservoirs[12]. We found that there was no apparent change of Cr within 4 h after ischemia, but Cr levels both in the ischemic group and the treatment group after 24 h of cerebral ischemia were reduced, which might reflect the changes in energy metabolism. It was considered that Cr was probably a marker of cell injury too.

Lac appears within 90 min after ischemia in this study, indicating that the local ischemia existed in the brain at that moment even MRI did not show any infarction till 24 h. This evidences that MRS finds the phathological changes earlier than MRI, which was consistent with that reported by Wardlaw *et al* [13]. MRS can early supply the information for acute cerebral ischemia. This study found that the Lac level in the treatment group was signficant lower than that in the ischemic group, but the Lac level in the prophylactic group was the lowest among the groups. NAA reduction in the prophylactic group and the treatment group

was decreased as compared with that in the ischemic group, demonstrating that *Gb*E could protect the cerebral ischemic neurons, which provides the theoretical basis for clinical prevention and treatment. It was also found that the infarct volume in the prophylactic group was reduced more significantly as compared with that in the treatment group, implying that prophylatic *Gb*E can enhance the tolerance of the neurons to ischemia and anoxic damage.

It was concluded that *Gb*E interfered with the development and deterioration of rat acute cerebral ischemia. The effectiveness of prophylatic *Gb*E was more satisfactory.

REFERENCES

1 Nin YH, Yang XY, Bao WS. Protective effects of *Ginkgo biloba* extract on cultrued rat cardiomyocytes damaged by H_2O_2. Acta Pharmcol Sin 1999; 20: 635-8.

2 Chen LX, Chen WZ, Huang HL, Chen LX, Xie ZZ. Protective effects of *Ginkgo biloba* extract against lysophosphatidylcholine-induced vascular endothelial cell damage. Acta Pharmacol Sin 1998; 19: 359-63.

3 Xu JP, Rui YC, Li TJ. Antagonistic effects of *Ginkgo biloba* extract on adhesion of monocytes and neutrophils to cultured cerebral microvascular endothelial cells. Acta Pharmacol Sin 1999; 20: 423-5.

4 Li AL, Shi YD, Landsmann B, Schanowski-Bouvier P, Dikta G, Bauer U, *et al*. Hemorheology and walking of peripheral arterial occlusive diseases patients during treatment with *Ginkgo biloba* extract. Acta Pharmacol Sin 1998; 19: 417-21.

5 Nagasawa H, Kogure K. Correlation between cerebral blood flow and histologic changes in a new rat model of middle cerebral artery occlusion. Stroke 1989; 20: 1037-43.

6 Rijen PC, Verheul HB, Echteld CJA, Balazs R, Lewis P, Nasim MM, *et al*. Effect of dextromethorphan on rat brain during ischemia and reperfusion assessed by magnetic resonance spectroscopy. Stroke 1991;22:343-9.

7 Graham GD, Blamine AM, Rothman DL, Brass LM, Fayad PB, Petroff OAC, *et al*. Early temporal variation of cerebral metabolites after human stroke: a proton magnetic resonance spectroscopy study. Stroke 1993; 24: 1891-6.

8 Graham GD, Blamire AM, Howseman AM, Rothman DL, Fayad PB, Brass LM, *et al*. Proton magnetic resonance spectroscopy of cerebral lactate and other metabolite in stroke patients. Stroke 1992; 23: 333-40.

9 Kugel H, Heindel W, Ernestus RI, Bunke J, Mesnil RD, Friedmann G. Human brain tumors: spectral patterns detected with localized ^{1}H-MR spectroscopy. Radiology 1992; 183: 701-9.

10 Lanferman H, Kugel H, Heindel W, Herholz K, Heissn WD, Lackner K. Metabolic changes in acute and subacute cerebral infarctions: findings at proton MR spectroscopic imaging. Radiology 1995; 196: 203-10.

11 Scremin OU, Jenden DJ. Effects of middle cerebral artery occlusion on cerebral cortex choline and acetylcholine in rats. Stroke 1989; 20: 1524-30.

12 Barker PB, Gillard JH, Vanzijl PC, Soher BJS, Hanley DF, Agildere AM, *et al*. Acute stroke: evaluation with serial proton MR spectroscopic imaging. Radiology 1994;192: 723-32.

13 Wardlaw JM, Marshall I, Wild J, Dennis MS, Cannon J, Lewis SC. Studies of acute ischemic stroke with proton magnetic resonance spectroscopy: relation between time from onset, neurological deficit, metabolite abnormalities in the infarct, blood flow, and clinical outcome. Stroke 1998; 29: 1618-24.