Effect of *Ginkgo biloba* leaf extract on electroencephalography of rat with cerebral ischemia and reperfusion

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KEY WORDS *Ginkgo biloba*; fast Fourier transform; electroencephalography; brain ischemia; reperfusion injury

ABSTRACT

AIM: To test the effect of *Ginkgo biloba* leaf extract on electroencephalography (EEG) during cerebral ischemia and reperfusion. **METHODS:** Based on the quantitative analysis of EEG using the fast Fourier transform (FFT), the effect of *Ginkgo biloba* extract (*Gb*E) on rat EEG was surveyed in the model of middle cerebral artery (MCA) occlusion and global cerebral ischemia. **RESULTS:** In the global cerebral ischemia, *Gb*E 8 and 16 mg/kg could accelerate the recovery of EEG after reperfusion, and *Gb*E 4 mg/kg had the same effect but much weaker. In the MCA occlusion model, *Gb*E 16 and 32 mg/kg greatly suppressed the drop of power spectrum of EEG. **CONCLUSION:** *Gb*E could mitigate the cerebral damage caused by ischemia.

INTRODUCTION

The leaves of *Ginkgo biloba* are used to treat many diseases by the traditional Chinese medicine. Much research have been carried out since 1960s and it was found that *Ginkgo biloba* extract (*Gb*E) could increase the rat cerebral blood flow[1], improve the mice memory impaired by ischemia[2,3], and protect the cerebral function[4,5]. But few research was focused on the effect of *Gb*E on cerebral function following ischemic insult using electroencephalography (EEG), which reflects cerebral excitation. By using digital signal processing technique, this paper aimed to test the effect of *Gb*E on EEG during cerebral ischemia and reperfusion.

MATERIALS AND METHODS

Materials *Gb*E, brown powder, produced by Shanghai Zhongwei Biochemistry Co, Ltd, patch number 960706, was diluted by distilled water after drops of alcohol and sorbitol (3:4) mixture was added. The pH of solution was adjusted to 7.4 by adding NaOH 1 mol/L. Nimodipine, white powder, gifted by Dr ZHANG Rui-Wen, Shanghai New Drug Research & Developing Center, was diluted in 0.5 % carboxycellulose just before use. The solution was kept on sting to prevent precipitating.

Effect of *Gb*E on rat after middle cerebral artery (MCA) occlusion[6] SD rats, weighing (180± 20)g, were anesthetized by ip urethane 1 g/kg. The skulk was exposed then cleaned using H_2O_2. Drilling was performed at 4 points: 2-mm left and right to the sagittal suture and 2-mm in front of and behind coronal suture. Four electrodes were inserted into the holes and fixed. The left 2 electrodes formed one recording pare, the right 2 formed the other. An 0.2-mm nylon thread was inserted into right internal carotid artery to about 1.2 cm. The temperature of rectum was maintained at (37.0±0.5) ° C.

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Drugs were given as follows: *Gb*E 8, 16, and 32 mg·kg^{-1} iv, NS iv as negative control, nimodipine 20 mg/kg as positive control. Ten minutes later (20 min for nimodipine), EEG was recorded. Then the MCA was blocked by inserting the nylon thread to 2.2 cm. Meanwhile the left common carotid artery was blocked. EEG was recorded at 1, 5, 10, 15, 30, 45, and 60 min after occlusion.

Effect of *Gb*E on rat global cerebral ischemia Spraw-Dawley rats, weighing (260±54) g, were inserted 2 electrodes at the points in front of the coronal suture for EEG recording. The 2 femoral arteries were used separately for blood pressure measuring and for bleeding. The rectum temperature was monitored and maintained at (37.0±0.5) °C. The animals were left to rest for 30 min.

After blood pressure and EEG were recorded, the rats were given drugs as follows: NS, *Gb*E 4, 8, and 16 mg·kg^{-1} iv and nimodipine 10 mg·kg^{-1} ig. Fifteen minutes later, blood was bleeding from femoral artery until the systolic blood pressure dropped to about 40 mmHg. Then the two aortors were clamped. The systolic blood pressure was maintained to 38-48 mmHg by bleeding or refilling the blood during the occlusion. The BP and EEG were recorded at 1, 5, 10, and 15 min after the occlusion. The reperfusion was established by refilling all blood and re-opening the aorta. The BP and EEG were recorded at 1, 5, 10, 15, and 30 min after reperfusion.

Data process and statistics The subgroup of EEG waves are defined as follows: waves that have frequency between 1-3 Hz are defined as δ, waves that have the frequency between 3-7 Hz are θ, waves that have the frequency between 7-13 Hz are α, and waves have the frequency between 13-40 are β[7].

The EEG signals were analyzed with computer after A/D convert (sample rate 200 points per second). By means of 1024-point fast Fourer transform (FFT), the power spectrum was calculated[8]. Then it was summed according to the EEG wave definition above. The data were expressed as the percentage of the value before drug. For it was not in normal distribution so the significance was evaluated using Mann-Whitney test. The software used for statistic calculation was StatView ver 5.0.1, SAS Institute Inc (Cary, NC27513, USA).

RESULTS

Effect of *Gb*E on MCA blocking model The EEG from left side did not change greatly, but at the right electrode pair, the power spectrum dropped greatly when MCA was blocked (Fig 1). All waves in *Gb*E 32 mg/kg-treated group were significantly higher than those of NS group at 1-30 min occlusion except δ wave at 15 min and α wave at 30 min. When rats were given *Gb*E 16 mg/kg, the power spectrum of EEG was also higher than that of the NS group, but the improvement was weaker. *Gb*E 8 mg/kg did not have any effect. Nimodipine 20 mg/kg had the effect on δ, θ, and α wave sometimes but not on β wave (Tab 1). When right MCA was occluded, the power spectrums of the waves from left electrode pair dropped slightly also. The change was smaller than that of right pair and there was no significant difference between NS and drug values.

Action of *Gb*E on global cerebral ischemia During ischemia and reperfusion, the power spectrums of NS group droped quickly. The higher the wave frequency, the more the amplitude dropped. For the latent electrode pair, the δ, α, and β waves in *Gb*E 16 mg/kg-treated group and α wave in *Gb*E 8 mg/kg-treated group were significantly higher than those in control group at 10 min after drug administration. During ischemia, the power spectrum of all groups was reduced rapidly. Though they were slightly higher than those of NS group, no statistic difference was found. After reperfusion, the EEG power spectrum of all *Gb*E groups recovered rapidly. In *Gb*E 16 mg/kg-treated group, the power spectrum was significantly higher than that of NS group in all waves from 5 min after reperfusion and on. In *Gb*E 8 mg/kg-treated group, the change of δ wave was aparent at 30 min after reperfusion, but the change of θ, α, and β waves were observed at 5 min after reperfusion. In *Gb*E 4 mg/kg treated-group only α and β waves were higher at 5 min after reperfusion. These results showed that higher frequency waves were more sensitive to the drug treatment. This phenomenon was more prominent in nimodipine-treated group (Tab 2).

The mean blood pressure was restored after reperfusion and reached the peak at 10 min. *Gb*E 8 and 16 mg/kg had a significant effect occasionally but *Gb*E 4 mg/kg and nimodipine did not (Tab 3).

DISCUSSION

FFT is a tool that combines computer science and modern signal processing technique. It converts sig-

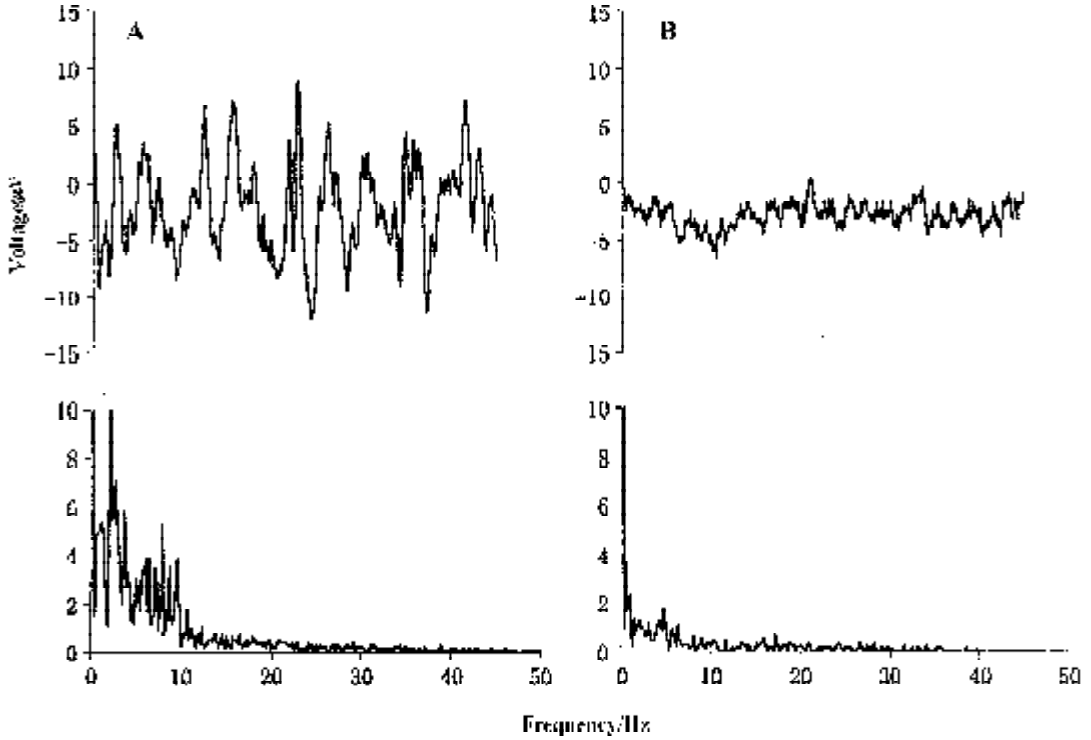

Fig 1. EEG wave (up) and its power spectrum (down) before MCA occlusion (A) and at 10 min after MCA occlusion (B). The data were from a rat of NS group and contained 5-s period of EEG. Compared with A, EEG and power spectrum of B were much smaller.

nals to a serious of different frequency sine waves. Counting these sine waves can quantitatively define the detected signals. Normally, EEG waves were divided into some groups according to its frequency. The altitude and duration, but not the shape, of individual wave were measured. That is to say the high frequency subwave was discarded. Besides, the individual EEG wave is repeatless and could not represent the outlook of EEG. And, even the same piece of EEG was studied, there would be a large deviation between data sets, different observers, and different time of study. Converting EEG, by FFT, to the sine waves and then counting them can be an easy and automatic step in EEG analysis. Because FFT uses a relatively long period of original EEG for calculation, the results would be more reliable and accurate. In this paper, the original EEG was used in FFT without any pre-processing. That is to say the original EEG was cut by a square window. The sudden truncation brings distortion to the results at the edge of high frequency[8]. Though the overload is very high which could be 10 % of the normal, it would be located in a narrow high frequency band on the condition that the number of sample data is large. Here, 1024-point FFT was used and sine waves below 40 Hz were summed, so the overload was excluded and could be neglected.

Results of this paper showed that the power spectrum of EEG dropped slowly when global cerebral ischemia was formed. In the model of MCA blocking, though the power spectrum decreased rapidly, it remained in a higher level, for *Gb*E could increase cerebral blood flow[1]. And there may be blood flowing from unblocked side to the ischemia zone in an MCA model. But in the model of global cerebral ischemia, the blood supply was extremely low and may not be increased any more. It may explain the fact why *Gb*E 16 mg/kg improved the EEG changes at the early stage of MCA blocking but not at the other one.

*Gb*E suppressed the decrease in SOD and MDA accumulations that caused by ischemia[9,10]. These phenomena reflected that *Gb*E had an anti-oxidation effect. *Gb*E helps alleviate the subcellular damages of cerebral ischemia[11] and allows mitochondria to maintain their respiratory activity under ischemic conditions as long as some oxygen is present, thus delaying the onset of ischemia-induced damage[12]. The data of this paper showed that the *Gb*E increased EEG power spectrum, it was more effective on high frequency waves than on

Tab 1. Effect of *Ginkgo biloba* extract (*Gb*E) on EEG power spectrum of rats with MCA occlusion. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* normal saline (NS) group.

	Time	NS	Nimodipine 20 mg/kg	*Gb*E/mg·kg^{-1} 32	16	8
n		10	10	12	12	10
	After drug	93±20	119±83[a]	104±26[a]	100±40[a]	84±20[a]
	Occlusion 1	37±24	72±48[a]	76±51[b]	81±39[c]	57±56[a]
	Occlusion 5	36±40	73±52[b]	74±57[b]	61±30[b]	53±41[a]
δ	Occlusion 10	30±31	63±48[b]	87±68[c]	58±22[b]	45±52[a]
	Occlusion 15	36±33	66±54[a]	67±73[a]	64±19[b]	41±46[a]
wave	Occlusion 30	33±28	78±70[b]	65±58[b]	56±26[b]	36±42[a]
	Occlusion 45	35±24	85±91[a]	67±56[a]	52±27[a]	35±39[a]
	Occlusion 60	39±26	82±63[a]	66±62[a]	60±44[a]	38±44[a]
	After drug	107±31	113±58[a]	112±32[a]	90±52[a]	102±13[a]
	Occlusion 1	25±14	50±33[b]	64±39[c]	62±34[c]	42±38[a]
	Occlusion 5	28±28	58±39[b]	58±33[b]	48±27[a]	46±39[a]
θ	Occlusion 10	25±23	50±39[b]	61±37[c]	46±20[b]	38±42[a]
	Occlusion 15	24±17	45±23[b]	51±36[b]	57±24[c]	30±29[a]
wave	Occlusion 30	30±20	45±25[a]	52±27[b]	49±25[b]	30±26[a]
	Occlusion 45	31±17	60±55[a]	58±42[a]	50±40[a]	32±33[a]
	Occlusion 60	36±37	56±25[b]	54±36[a]	54±41[a]	46±58[a]
	After drug	110±38	127±92[a]	111±54[a]	82±28[a]	97±21[a]
	Occlusion 1	30±14	52±33[a]	68±35[c]	59±36[b]	47±38[a]
	Occlusion 5	32±34	54±44[b]	63±42[b]	48±31[a]	45±31[a]
α	Occlusion 10	32±28	57±60[a]	65±31[b]	53±30[a]	35±35[a]
	Occlusion 15	25±15	41±15[b]	60±43[b]	56±31[c]	31±24[a]
wave	Occlusion 30	28±15	49±28[a]	59±39[a]	52±32[b]	36±30[a]
	Occlusion 45	34±20	58±56[a]	63±34[b]	62±67[a]	40±43[a]
	Occlusion 60	46±45	59±32[a]	66±31[a]	50±37[a]	61±82[a]
	After drug	106±25	135±110[a]	94±28[a]	84±33[c]	99±25[a]
	Occlusion 1	31±19	47±21[a]	73±44[c]	65±47[b]	58±55[a]
	Occlusion 5	36±34	57±49[a]	65±38[b]	54±27[a]	51±40[a]
β	Occlusion 10	44±47	62±61[a]	69±28[b]	60±29[a]	38±27[a]
	Occlusion 15	33±20	40±12[a]	71±47[b]	62±35[b]	39±22[a]
wave	Occlusion 30	37±22	49±26[a]	71±43[b]	58±27[a]	42±33[a]
	Occlusion 45	51±42	54±35[a]	76±39[a]	67±72[a]	47±36[a]
	Occlusion 60	56±43	50±19[a]	84±65[a]	54±36[a]	84±108[a]

low frequency ones. The more active the cerebral cells are, the higher the EEG frequency they elicite. We may conclude that *Gb*E protects the cerebral cell function against loss during ischemia. Once the normal blood flow was restored, the cerebral cell function could recover rapidly. The fact that the blood pressure of animals treated with *Gb*E was significantly increased when reperfusion was started following the global cerebral ischemia also suggested that the function of central nervous system was strengthened and regained the control over peripheral tissues.

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Tab 2. Effect of *Gb*E on EEG power spectrum of rats with global cerebral ischemia. Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* NS group.

		NS	Nimodipine 10 mg/kg	*Gb*E/mg·kg^{-1} 16	8	4
	10 min after drug	113±40	108±50[a]	165±51[b]	135±78[a]	136±59[a]
	Ischemia 1 min	45±22	51±29[a]	67±47[a]	82±79[a]	62±35[a]
	Ischemia 5 min	41±21	42±26[a]	80±84[a]	48±32[a]	46±30[a]
δ	Ischemia 10 min	41±29	49±46[a]	41±31[a]	48±31[a]	47±20[a]
	Ischemia 15 min	34±20	67±67[a]	60±35[a]	44±33[a]	42±22[a]
wave	Reperfusion 1 min	49±44	56±51[a]	58±47[a]	62±46[a]	44±28[a]
	Reperfusion 5 min	39±25	67±53[a]	69±40[b]	67±59[a]	57±23[b]
	Reperfusion 10 min	48±25	71±52[a]	73±44[a]	79±59[a]	43±13[a]
	Reperfusion 15 min	46±27	60±47[a]	82±45[b]	76±49[a]	56±37[a]
	Reperfusion 30 min	52±19	68±30[a]	100±61[b]	103±48[b]	65±26[a]
	10 min after drug	102±34	100±52[a]	133±37[a]	128±55[a]	111±30[a]
	Ischemia 1 min	35±14	38±17[a]	55±30[a]	62±39[a]	44±30[a]
	Ischemia 5 min	24±16	29±18[a]	48±47[a]	46±41[a]	29±24[a]
θ	Ischemia 10 min	25±20	29±18[a]	22±18[a]	41±36[a]	26±15[a]
	Ischemia 15 min	20±11	35±25[a]	30±21[a]	39±38[a]	22±14[a]
wave	Reperfusion 1 min	33±35	40±24[a]	44±40[a]	44±39[a]	26±12[a]
	Reperfusion 5 min	25±12	49±27[b]	65±32[c]	59±56[b]	38±31[a]
	Reperfusion 10 min	34±22	56±28[a]	70±35[b]	63±41[b]	30±10[a]
	Reperfusion 15 min	37±24	58±39[a]	83±51[c]	76±55[b]	46±22[a]
	Reperfusion 30 min	42±19	76±45[a]	92±33[c]	90±27[c]	68±46[a]
	10 min after drug	86±27	96±25[a]	119±26[b]	118±35[b]	113±39[a]
	Ischemia 1 min	34±15	39±24[a]	54±27[a]	57±22[b]	40±18[a]
	Ischemia 5 min	20±12	24±15[a]	36±26[a]	42±36[a]	26±20[a]
α	Ischemia 10 min	20±14	27±21[a]	20±17[a]	35±30[a]	25±21[a]
	Ischemia 15 min	14±8	29±25[a]	24±13[a]	35±28[b]	22±11[a]
wave	Reperfusion 1 min	34±54	32±26[a]	44±48[a]	40±32[a]	23±12[a]
	Reperfusion 5 min	21±9	46±35[a]	60±23[c]	55±42[c]	40±27[b]
	Reperfusion 10 min	9±14	86±78[a]	86±22[c]	88±17[c]	62±30[a]
	10 min after drug	83±25	97±33[a]	102±16[b]	101±17[a]	88±21[a]
	Ischemia 1 min	32±24	29±14[a]	38±15[a]	49±25[b]	34±24[a]
	Ischemia 5 min	16±9	19±11[a]	24±19[a]	32±31[a]	22±18[a]
β	Ischemia 10 min	19±15	22±15[a]	15±7[a]	27±24[a]	19±19[a]
	Ischemia 15 min	13±7	23±16[b]	20±6[b]	32±25[b]	20±11[a]
wave	Reperfusion 1 min	21±22	29±19[a]	37±50[a]	33±24[a]	24±12[a]
	Reperfusion 5 min	16±6	36±26[b]	38±17[c]	41±33[c]	30±17[b]
	Reperfusion 10 min	23±13	43±24[b]	41±18[b]	41±24[b]	23±12[a]
	reperfusion 15 min	25±9	54±40[a]	52±22[c]	56±20[c]	33±18[a]
	reperfusion 30 min	39±17	64±23[b]	70±13[c]	76±14[c]	49±22[a]

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Tab 3. Effect of *Gb*E on mean blood pressure of rat (mmHg). Mean±SD. [a]*P*>0.05, [b]*P*<0.05, [c]*P*<0.01 *vs* NS group.

Time	NS	Nimodipine	*Gb*E/mg·kg^{-1}		
		10 mg/kg	16	8	4
Normal	63±12	56±13 [a]	56±11 [a]	61±15 [a]	67±17 [a]
10 min after drug	57±18	50±15 [a]	69±12 [a]	66±16 [a]	66±16 [a]
Ischemia 1 min	35±12	35±5 [a]	35±5 [a]	36±6 [a]	37±12 [a]
Ischemia 5 min	31±5	33±5 [a]	33±4 [a]	33.6±2.7 [a]	30±4 [a]
Ischemia 10 min	30±4	29±5 [a]	32±4 [a]	32.6±2.8 [a]	31±4 [a]
Ischemia 15 min	30±5	30±4 [a]	32±4 [a]	33±2.7 [a]	33±6 [a]
Reperfusion 1 min	51±12	49±10 [a]	64±13 [b]	66±19 [b]	55±19 [a]
Reperfusion 5 min	59±18	52±11 [a]	72±10 [a]	73±13 [a]	65±14 [a]
Reperfusion 10 min	60±21	54±12 [a]	74±12 [a]	79±12 [b]	66±12 [a]
Reperfusion 15 min	53±20	54±12 [a]	69±16 [a]	74±13 [b]	61±10 [a]
Reperfusion 30 min	47±15	47±8 [a]	63±12 [b]	70±13 [c]	53±14 [a]

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