

Effect of *Ginkgo biloba* extracts on preventing and curing wistar's cerebral angiospasm after subarachnoid haemorrhage.

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Abstract

Objective: To study the preventive and therapeutic effect of *Ginkgo biloba* extract on wistar's cerebral angiospasm of different phases.

Method: Double cisterna magna blood injection method was adopted to set up Wistar SAH model. Then, 56 healthy male SD wistars were divided into 7 groups according to each time point (sham operation group, SAH 3d group, SAH 5 d group, SAH 7 d group, *Ginkgo biloba* extract 3 d group, *Ginkgo biloba* extract 5 d group, and *Ginkgo biloba* extract 7d group). *Ginkgo biloba* extract groups were treated with *Ginkgo biloba* extract injections once a day after the operation. Each group of wistars was perfused and fixed at each time point. After putting them to death, basilar artery sections were taken and observed by conventional HE staining. Via the image analysis system, the arterial diameter and the vascular wall thickness were measured.

Result: After the treatment of *Ginkgo biloba* extract, the perimeter of the inner diameter of the basilar artery in 7 d treatment group was significantly larger than that in subarachnoid haemorrhage group ($p < 0.05$). It was indicated by pathologic histology that the morphology of the spasm vessel had been improved.

Conclusion: *Ginkgo biloba* extract has a certain role in improving tardive cerebral angiospasm after wistar's subarachnoid haemorrhage.

Keywords: Subarachnoid haemorrhage, Cerebral angiospasm, Basilar artery, *Ginkgo biloba* extract.

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Introduction

Cerebral angiospasm is one of the common complications of subarachnoid haemorrhage, which is the relevant cause of patients' local brain tissue ischemia, tardive ischemic brain damage and cerebral infarction [1,2]. Statistic data have shown that the occurrence rate of cerebral angiospasm of the patients with subarachnoid haemorrhage within one week is as high as 30%~70% [3]. Moreover, cerebral angiospasm is likely to result in rise of patients' intracranial pressure and do damage to neurological function. Clinical studies have shown that early-stage intervention treatment given to patients with cerebral angiospasm can lower the occurrence rate of functional disabilities in the far and near future [4,5]. However, the occurrence of cerebral angiospasm after subarachnoid haemorrhage has not yet been clearly grasped and accurately predicted.

In recent years, a large number of studies have achieved many breakthroughs based on CVS, but the clinical efficacy has never been satisfactory due to the reason that CVS is an extremely complicated pathological process caused by many factors and links. In this study, Double cisterna magna blood injection method was adopted to set up SAH Wistar Model,

observe the changes of the wistar's perimeter of the inner diameter of basilar artery, the vascular wall thickness and the morphology before and after the treatment of GBE, and analyse the related risk factors of cerebral angiospasm after the subarachnoid haemorrhage, providing a reference to improve the prognosis of patients with cerebral angiospasm.

Materials and Methods

Animals and medicament

56 male SD wistars (clean grade) with the weight of (250 ± 20) g were provided by Zhejiang Laboratory Animal Center. Trial drug: GBE injection (injection with the concentration of 1.5 g/100 ml was prepared by utilizing 50% 1, 2-propylene glycol), which was supplied by Youcare Pharmaceutical Group Co., Ltd.

Model building and grouping [6]

After the SAH of wistars, CVS animal model was built by utilizing "double cisterna magna blood injection method"; male wistars with the weight of (250 ± 20) g were selected;

intraperitoneal injection of 3% pentobarbital sodium was adopted for narcosis with the dosage of 2.0 ml/kg; the place that was 3 mm–4 mm below the tuberosity of occipital bone was targeted; the need of 1ml syringe was used to puncture pierce atlantooccipital membrane and slightly push into cisterna magna; 1 ml disposable injector was used to draw 0.3 ml of femoral artery blood when cerebrospinal fluid flew out; within one minute, femoral artery blood was injected into cisterna magna; afterwards, prone position head was lowered by 30° for 30 minutes. After 40 hours, the aforementioned process was repeated. In the sham-operation group, the method was the same as the above case; 0.3 ml of warm normal saline was injected into cisterna magna twice.

Groups were divided after the completion of first blood injection. Animals were randomly divided into SAH 3 d group with 10 wistars, SAH 5 d group with 9 wistars, SAH 7 d group with 8 wistars and GBE 5 d group with 8 wistars, GBE 5 d group with 8 wistars as well as GBE 7 d group with 7 wistars. Medication administration groups were respectively given with GBE injections through abdominal cavity in the dosage of 15 mg/100 g; the sham operation groups were given with normal saline of the same dosage.

Specimen acquiring and processing [7]

After each group of animals was narcotized by intraperitoneal injection of 3% pentobarbital sodium (2.0 ml/kg), their skulls were opened and the basilar artery as well as their adherent brainstems and the entire brains were dissected and taken; the brainstems and the basilar artery were separated; 1/2 segment of basilar artery that contained brainstems was taken to make the specimen; the basilar artery was placed and fixed in 10% formaldehyde for 24 hours; the fracture surface of vascular wall and the vascular wall were kept upright while drawing materials; paraffin embedding, section and HE staining were carried out for pathological examination.

HE stained sections were selected and photographed under Olympus optical microscope. By referring to the documentary method, the build-in computer image analysis system of the Olympus optical microscope was utilized to calculate the perimeter of the inner diameter and the wall thickness of basilar artery. When measuring the vascular wall thickness, the values of wall thickness around the blood vessels were measured and then the average value was taken.

Statistics

The data were expressed by average value \pm standard deviation ($\bar{x} \pm s$). Variance analysis was conducted by utilizing the build-in computer image analysis system (Dp2BSW) of Olympus optical microscope.

Experimental Result

Influence of GBE treatment on BA morphology

In sham operation group: the basilar artery was located within basilar artery groove of the brainstem. The diameter of

arterial wall was small; the arterial wall was comprised of endarterium, tunica media and adventitia. There were mono-layer endothelial cells on cavosurface and the endothelial cells had no degeneration, necrosis and ecclasis; the internal elastic lamina had no obvious incassation; the ruga had not been increased significantly with certain diopter; tunica media and adventitia had no evident lesions.

In SAH group: this group was divided into three time slots of 3 d, 5 d and 7 d with the histological structure identical to that of the sham operation group. Along with the extension of time, the number of endothelial cells was gradually decreasing. Several cells had vacuolated changes; the internal elastic lamina had incassation; the ruga had been increased. The thickness of vascular wall slighted increased. Compared to the sham operation group, the average perimeter of blood vessel diameter decreased. Along with the prolonging of time, the aforementioned lesions had become obvious gradually. Whereby, the lesions were the most obvious ones at 7 d.

In the medication administration group: this group was divided into three time slots of 3 d, 5 d and 7 d. The histological structure was identical to that of the blank group. Along with the extension of time, the number of endothelial cells increased to some extent; the cells' morphological structure was basically similar to that of the blank group with no obvious vacuolated changes. Some internal elastic laminas of wistars' basilar artery were slightly thick and some were basically normal; the ruga decreased compared to that of the SAH group. Along with the prolonging of time, the vascular wall thickness had also gradually thinned, which was slightly thinner compared to that of the SAH group and was similar to that of the sham operation group and the blank group; the perimeter of blood vessel diameter increased slightly. Whereby, it was relatively obvious at 7 d.

Influence of GBE treatment 3 d, 5 d and 7 d on perimeter of basilar artery inner diameter

Table 1. Influence of GBE treatment 3 d on perimeter of basilar artery inner diameter (P1) ($\bar{x} \pm s$).

Group	Animal quantity	Dosage/mg • kg ⁻¹	P1/ μ m
sham operation	6	NA	260.51 \pm 35.26
3dSAH	10	NA	220.43 \pm 18.67
3d GBE	8	150	237.95 \pm 35.74

Compared to the sham operation group, \blacktriangle p<0.05, GBE.

The results showed that the perimeter of basilar artery inner diameter in SAH group had significantly decreased compared to that in sham operation group with statistical difference (p<0.05); there was no statistical difference when comparing GBE group with SAH group (p>0.05) (Table 1).

Influence of GBE treatment 5 d on perimeter of basilar artery inner diameter

Table 2. Influence of GBE treatment 5 d on perimeter of basilar artery inner diameter (P) ($\bar{x} \pm s$).

Group	Animal quantity	Dosage/mg • kg ⁻¹	P1/ μ m
Sham operation	6	NA	262.38 \pm 32.53
5 d SAH	9	NA	215.75 \pm 25.64
5 d GBE	8	150	241.56 \pm 31.21

Compared to the sham operation group, p<0.05.

The results showed that the perimeter of basilar artery inner diameter in SAH group had significantly decreased compared to that in sham operation group with statistical difference (p<0.05); there was no statistical difference when comparing GBE group with SAH group (p>0.05) (Table 2).

Influence of GBE treatment 7 d on perimeter of basilar artery inner diameter

Table 3. Influence of GBE treatment 7 d on perimeter of basilar artery inner diameter (P) ($\bar{x} \pm s$).

Group	Animal quantity	Dosage/mg • kg ⁻¹	P1/ μ m
sham operation	6	NA	258.34 \pm 30.21
7 d SAH	8	NA	223.29 \pm 28.37
7 d GBE	7	150	245.76 \pm 23.60*

Compared to the sham operation group, p<0.05. Compared to SAH group, *p<0.05.

The results showed that the perimeter of basilar artery inner diameter in SAH group had significantly decreased compared to that in sham operation group with significant statistical difference (p<0.01); there was statistical difference when comparing GBE group with SAH group (p<0.05) (Table 3).

Influence of GBE treatment on wall thickness of basilar artery

Table 4. Influence of GBE treatment on wall thickness of basilar artery (P2) ($\bar{x} \pm s$).

Group	Animal quantity	Dosage/mg • kg ⁻¹	P2/ μ m
Sham operation	6	NA	20.25 \pm 1.78
3 d SAH	10	NA	21.31 \pm 2.07
5 d SAH	9	NA	22.64 \pm 1.35
7 d SAH	8	NA	21.95 \pm 1.46
3 d GBE	8	150	19.01 \pm 1.25
5 d GBE	8	150	20.34 \pm 1.26

7 d GBE	7	150	20.95 \pm 2.79
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The results showed that the wall thickness of basilar artery in SAH group had increased compared to that in sham operation group without statistical difference (p>0.05); the wall thickness in ginkgo group was smaller compared to that in SAH group without statistical difference (p>0.05) (Table 4).

Discussion

Cerebral angiospasm is the severest complication of subarachnoid haemorrhage; the cerebral angiospasm after subarachnoid haemorrhage is divided into early-onset and late-onset cerebral angiospasm [8,9]. The latter is more commonly seen clinically, which usually takes place and reaches at the peak time 3 d, 5 d and 7~10 d after the SAH. Currently, it is believed that multiple factors collectively participate in the generating process of DCVS. According to pathogenesis and pathological progress, prevention and cure of late-onset cerebral angiospasm after SAH should include the three key points of blocking of pathogenic factors for angiospasm, reversing of cerebral angiospasm that has taken place, and control of cerebral ischemia caused by arteriarteria etc. Currently, Ca²⁺ antagonist Nimodipine has been mainly utilized as the medicine for resisting cerebral angiospasm at home and abroad [10]. Other medicines such as Fasudil, statins, Mg²⁺ formulations, endothelin receptor antagonists/inhibitors, drugs that facilitate synthesis of Nitric Oxide (NO), oxygen free radical scavengers and peroxidation inhibitors etc. are still on clinical trial and not yet widely popularized [11-13]. The drugs used for inhibiting the generation of vascular immune inflammation and improving cerebral ischemia after angiospasm are extremely significant for lowering morbidity of cerebral angiospasm, improving quality of life and reducing mortality rate [14-16].

In this experiment, double cisterna magna blood injection method was adopted to build the model of tardive cerebral angiospasm. In this experiment, intraperitoneal injection of GBE was taken as the administration route, selecting 3 d, 5 d and 7 d after the first blood injection as the observation of efficacy. The experiment found that basilar artery diameter in various SAH groups turned smaller and the vascular wall thicker. After 7 d of early application of *Ginkgo biloba* extract injection treatment, wistars' spasm blood vessel wall thickness and diameter were obviously improved with significant statistical difference. The morphological structure was close to that of the normal group. It could be seen that *Ginkgo biloba* extract treatment 7 d was significantly effective, which conformed to the documentary report. After *Ginkgo biloba* treatment for 3 d and 5 d, the wall thickness had no significant difference compared to that of SAH group, but there was a tendency of relieving cerebral angiospasm entirely; basilar artery lumen was relatively expanded to some extent and the diameter was increased.

In the experiment, it could be seen that SAH wistars' perimeter of basilar artery inner diameter had increased to some extent after the treatment of *Ginkgo biloba* extract, but statistical

difference could only be seen in 7 d group. The reasons were as follows: (1) the weight difference was big after wistar modelling; the weight had a big influence on wall thickness and perimeter of inner diameter of basilar artery, which would lead to high sample dispersion; (2) during the modelling process, the extent of SAH would be different due to animals' individual difference; (3) after completion of modelling, some wistars had respiratory arrest; during the emergency treatment, wistars were in supine position where injected arterial blood might leak out and eventually lead to relatively few wistar samples in each group; (4) death rate increased after the second blood injection.

The result of this experiment shows that GBE has a comparatively obvious ameliorative function on tardive cerebral angiospasm of SAH wistars and that it has a certain application prospect in the intervention treatment at the early stage of subarachnoid haemorrhage. However, due to the relatively few experimental samples and changes of ultrastructure of the blood vessel, none of the relevant indexes like immune biochemistry have been detected. It is worthy of further study on questions whether the efficacy is related to the dosage and whether it has synergistic effect while using together with Nimodipine.

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