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Email address:- lwang@csu.edu.au

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Effects of extract of Ginkgo biloba on intracranial pressure, cerebral perfusion pressure, and cerebral blood flow in a rat model of subarachnoid haemorrhage

Bao-liang Sun¹, Hui Yuan¹, Ming-feng Yang², Zuo-li Xia¹, Su-ming Zhang³, Le-xin Wang, MD, PhD⁴

1. Department of Neurology & Institute of Microcirculation, Affiliated Hospital, Taishan Medical College, Shandong, China

2. Institute of Cerebral Microcirculation, Taishan Medical College, Shandong, China

3. Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

4. School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW Australia

Corresponding author.

1. Dr Lexin Wang, School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, NSW Australia 2650. Tel +61 2 69332905; Fax: +61 2 69332587; email: lwang@csu.edu.au

Or

2. Prof Bao-liang Sun, Department of Neurology & Institute of Microcirculation, Affiliated Hospital of Taishan Medical College, No.706, Taishan Avenue, Taian, Shandong 271000, China. Tel: +86 538 6236001. Fax: +86 538 8420042. *Email:* blsun@tsmc.edu.cn

Abstract

The purpose of this study was to investigate in effect of extract of *Ginkgo biloba* (EGb) on cerebral blood perfusion in a subarachnoid haemorrhage (SAH) rat model. SAH lead to an increase in intracranial pressure and decrease in cranial perfusion pressure and regional cerebral blood flow in all groups. However, the intracranial pressure increases in EGb groups were less than that of the vehicle group ($p<0.01$), whereas the reduction in cranial perfusion pressure and regional cerebral blood flow in the EGb group was less than that of the vehicle and SAH groups ($p<0.01$). We concluded that EGb attenuates the increase in intracranial pressure and reduction in cerebral blood perfusion after SAH.

Key words: subarachnoid haemorrhage; intracranial pressure; cerebral perfusion pressure; cerebral blood flow; *Ginkgo biloba*.

Introduction

Subarachnoid haemorrhage (SAH) is mainly due to rupture of cerebral aneurysms [Manno, 2004; van Gijn et al, 2001]. The high incidence of morbidity and mortality from this disease is one of the major challenges in medicine today [Manno, 2004; van Gijn et al, 2001; Sun et al, 2003]. Extract of *Ginkgo biloba* (EGb) possesses a variety of pharmacological effects that are potentially beneficial to several conditions, such as dementia, depression, cerebral or peripheral artery disease [Zhou et al., 2004; Zhang et al., 2000; Itil et al., 1996; Kanowski, et al., 2003; Muir et al., 2002]. Our previous studies have demonstrated that EGb improves cerebral microcirculation, antagonizes the abnormality of somatosensory evoked potential, and supplements the deficiency of nitric oxide derived from vascular endothelial cells [Sun et al., 2000; 2003]. In order to further illustrate the protective effect of EGb on SAH, the effects of EGb on intracranial pressure, cerebral perfusion pressure, and cerebral blood perfusion were evaluated in a rat SAH model.

Materials and methods

Animal preparations

This study was approved by the Institutional Review Board of Taishan Medical College. Wistar rats of both sexes (Experimental Animal Center of Shandong University) weighing 330g to 380g were housed in groups of five per cage at a

constant temperature ($24\pm 1^{\circ}\text{C}$) and humidity ($60\pm 5\%$), on a 12-h light-dark cycle.

The rats were given free access to food and water before and after experiment. The room temperature was kept at $26\pm 2^{\circ}\text{C}$ by an air-conditioner.

The rats were anaesthetized by intraperitoneal injection of chloral hydrate (10% solution, 350 mg/kg) and normal body temperature was kept with a heating lamp. The left femoral artery was cannulated and 0.4 ml blood was drawn into a heparinized microinjector (310, Stoelting Co. Ltd, USA). The blood was frozen at -80°C for 15 min. Then, the frozen blood was thawed at 37°C and the autologous arterial haemolysate was obtained. The rat's head was fixed in a stereotaxic frame (51600, Stoelting Co. Ltd, USA) to maintain a head down position of 30 degree. An incision was made in the midline and the skin, musculature were carefully separated using an operating microscope to expose the atlantooccipital membrane. The microinjector was mounted on the manipulating arm of the stereotaxic instrument with a needle connected to a three-way stopcock to allow the measurement of intracranial pressure. The needle was lowered into cisterna magna under direct vision. To induce SAH, 0.3 ml arterial hemolysate was injected into the cistern very slowly over 20 min at a constant rate. Some rats showed respiratory arrest during the cisternal injection, which was immediately reversed by withdrawing 0.05ml haemolysate from the cistern. The non-SAH rats were manipulated in the same way as the SAH rats, except that the same volume of saline, but not arterial haemolysate, was injected into the cistern.

After separation of the skin and muscles, the right femoral artery was exposed

and cannulated with a cannula connected to a three-way stopcock. The cannula was advanced distally into the abdominal aorta. The pressure module 1 (DA100C) of a Biopac system (MP150, USA) was connected to the cannula. The mean arterial blood pressure was monitored and calculated automatically by the software AcqKnowledge ver3.7.2 of the instrument.

At different time points, blood samples were collected from the left femoral artery via the three-way stopcock. The blood samples were used for the measurements of arterial pH, partial pressure of oxygen (PaO₂), and partial pressure of carbon dioxide (PaCO₂).

Drug administration

Thirty rats were randomly divided into non-SAH, SAH, vehicle, EGb1 and EGb2 groups. EGb was obtained from Lvyuan Pharmaceutical Co. Ltd (Shanghai, China), standardized on the amount of flavone glycosides $\geq 24\%$ and ginkgolide $\geq 6\%$ by a HPLC determination. EGb was solubilized with physiological saline. The stock solution of EGb was prepared just before use. Rats in EGb1 and EGb2 groups received intraperitoneal injection of EGb at a dose of 100mg/kg and 200mg/kg, respectively, 30 min before cisternal injection of autologous arterial haemolysate. The intraperitoneal injection of EGb was repeated twice daily at a dose of 50mg/kg and 100mg/kg, respectively, in EGb1 and EGb2 groups. In the vehicle group, normal saline was injected intraperitoneally in the same fashion as the EGb groups.

Measurements of intracranial pressure and cerebral perfusion pressure

The outflow of the clear and transparent cerebrospinal fluid could be seen after successful puncture of the atlantooccipital membrane. Then, a needle was connected to a three-way stopcock to allow the measurement of intracranial pressure using the pressure module of Biopac system. The baseline values of intracranial pressure before cisternal injection of autologous arterial haemolysate or saline were recorded by the software AcqKnowledge ver3.7.2. The recording was continued for 12 hrs. Cerebral perfusion pressure was calculated using the mean artery blood pressure from the femoral artery and intracranial pressure [Chan et al., 1992].

Measurement of regional cerebral blood flow

After general anaesthesia, the animals were fixed in a stereotactic frame and the parietal bones were exposed. A small hole, 3.0 mm in diameter, was drilled on the skull about 3.0 mm behind the fonticulus anterior and 3.0 mm to the left of the midline. The thin inner bone layer was dissected carefully and the dura was kept intact. A probe of laser Doppler flowmetry (LDF, Periflux PF150, Perimed Co., Sweden) was placed on the dura in the small hole. Regional cerebral blood flow was recorded continuously for 12 hours. Once a stable and satisfactory LDF signal was obtained, the probe was fixed by the manipulating arm of the stereotactic instrument throughout the observation period [Maeda et al., 1999].

Statistical analysis of the data

The data were expressed as means \pm SD. Comparisons of data between groups were made with one-way analysis of variance (ANOVA). Comparison of data within groups was made by student *t* test. $P < 0.05$ was considered to be statistically significant.

Results

General findings

There was no significant difference in the blood pH, PaO₂ and PaCO₂ between the non-SAH and SAH groups ($p > 0.05$, Table 1). Thirty minutes after cisternal injection of autologous arterial haemolysate or saline, the mean arterial blood pressure was increased in the two groups ($p < 0.01$, table 1). However, the increased mean arterial pressure returned to the baseline values 30 min later (Table 1).

Changes of intracranial pressure

The baseline values of intracranial pressure in all groups ranged from 3.2 to 4.68 mmHg without significant differences among groups ($p > 0.05$). In non-SAH group, intracranial pressure was substantially increased 5 min after the cisternal injection of saline (Fig 1, $p < 0.01$) and recovered to the baseline value 10 min later. In SAH group, there was also a significant increase in intracranial pressure 5 min after the cisternal injection of haemolysate (Fig 1, $p < 0.01$). The increase in the SAH group at different time points after the injection was greater than that of the non-SAH group (Fig 1, $p < 0.01$).

In the EGb groups, there was also an increase in the intracranial pressure after the injection of haemolysate (Fig 1, $p<0.01$); however, the increases within the first 240 min were significantly lower than those of the vehicle group (Fig 1, $p<0.01$).

The post-injection intracranial pressure in the Egb2 group was lower than that of the Egb1 group (Fig 1, $p<0.05$).

Changes of cranial perfusion pressure

At baseline, there was no significant difference in the cranial perfusion pressure between groups (Fig 2, $p>0.05$). In the non-SAH group, cranial perfusion pressure was decreased 5 to 10 min after the cisternal injection of saline (Fig 2, $p<0.01$); it recovered to normal level in 10 min. In SAH group, cranial perfusion pressure reduced remarkably and persistently, with its nadir occurring at 5 min after the end of cisternal injection of haemolysate (a 30.7% reduction) (Fig 2, $p<0.01$). The values of cranial perfusion pressure at all time points after the end of cisternal injection in SAH group were lower than those in non-SAH group (Fig 2, $p<0.05$).

There was no significant difference in cranial perfusion pressure between EGb1 and vehicle groups after cisternal injection (Fig 2, $p>0.05$). However, in EGb2 group, the values of cranial perfusion pressure at 10min, 20min, and 30min after the cisternal injection were higher than those of the vehicle group (Fig 2, $p<0.05$).

Changes of regional cerebral blood flow

In non-SAH group, regional cerebral blood flow was decreased by 3-5% during

the process of cisternal injection of saline. Regional cerebral blood flow in this group returned to the normal range immediately after the end of injection.

The pattern of changes in regional cerebral blood flow was similar between SAH and vehicle groups. In these two groups, regional cerebral blood flow was decreased at the beginning of cisternal injection of haemolysate. The values of regional cerebral blood flow 10 min after the cisternal injection were 37.6% and 36.9% of baselines, respectively, in these two groups ($p < 0.01$). The lower levels of regional cerebral blood flow in these two groups maintained within the whole observing period.

In EGb1 and EGb2 groups, the values of regional cerebral blood flow were significantly higher than those of the vehicle group (Fig 3, $p < 0.01$). At the nadirs of regional cerebral blood flow reduction, the values of the blood flow were 43.3% and 45.7%, respectively, of baseline values ($p < 0.01$). SAH-induced decreases of regional cerebral blood flow were less than those of the vehicle group or SAH group (Fig 3, $p < 0.01$). Regional cerebral blood flow in the EGb groups started to recover from 5 min after the completion of cisternal injection of haemolysate (Fig 3, $p < 0.01$).

Discussion

Despite the fact that there is a drastic decline in re-bleeding during the progress of SAH due to the improved surgical management of cerebral aneurisms, the treatment of SAH remains one of the major challenges in both neurology and neurosurgery because of the occurrence of cerebral ischaemia [Manno, 2004; Gijn et al., 2001; Sun

et al., 2003]. Spasm of large cerebral vessels, namely cerebral vasospasm, has been regarded as the major cause of SAH-related cerebral ischaemia and the poor outcomes of this disease [Sun et al., 2000]. Disturbances of cerebral microcirculation also contribute to the development of cerebral ischaemia following SAH [Park et al., 2001]. In addition, significant increases of intracranial pressure to the brain, as well as decreases in cranial perfusion pressure, are also involved in the development of SAH-related cerebral ischaemia and cerebral injury, contributing to the death and neurological deficits of SAH [Bederson et al., 1995].

Ginkgo biloba is one of the oldest living tree species. The seed of *Ginkgo biloba* is an herbal drug with a long history of clinical uses in traditional Chinese medicine. In recent years the standardized extract of the leaves of *Ginkgo biloba* has been widely used as a phytomedicine around the world. The main active constituents of *Ginkgo biloba* include flavonol glycosides, terpene trilactons and proanthocyanidines [Zhou et al., 2004; Zhang et al., 2000; Itil et al, 1996]. Due in part to its potent antioxidant properties and ability to enhance peripheral and cerebral circulation, its primary application lies in the treatment of cerebrovascular dysfunctions and peripheral vascular disorders. It has recently been used in patients with a series of cerebral disorders associated with the insufficiency of cerebral blood flow [Gertz et al., 2004]. Clinical studies have shown that *Ginkgo biloba* exhibits therapeutic activity in a variety of disorders including Alzheimer's disease [Colciaghi et al., 2004; DeLaGarza, 2003], failing memory [McKenna et al., 2001], age-related

dementias [Williams et al., 2004], poor cerebral [Kriegelstein et al., 1986] and ocular blood flow [Chung et al., 1999].

Our previously studies have showed some beneficial effects of *Ginkgo biloba* on SAH-related brain injury, in which the devastated balance between vascular relaxing factor nitric oxide and vascular contracting factor could partly be restored [Sun et al., 2000]. The present experiments have demonstrated that *Ginkgo biloba* extracts attenuate the increase of intracranial pressure following SAH. In addition, SAH induced reduction of cranial perfusion pressure and regional cerebral blood flow were also alleviated in Ginkgo-biloba treated animals. Although the mechanisms of these beneficial pharmacological actions remain uncertain, the results from the present study provide further evidences to the protective effects of *Ginkgo biloba* on cerebral ischaemia and cerebral injury after SAH.

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Table 1. Arterial blood gas analysis after cisternal injection.

Groups	pH	PaO ₂ (mmHg)	PaCO ₂ (mmHg)	MABP (mmHg)
non-SAH				
Baseline	7.39±0.02	126.9±8.2	34.2±3.5	102.2±9.5
0.5h	7.40±0.02	124.2±9.3	32.4±5.7	120.2±8.5*
1 h	7.39±0.01	123.6±4.8	33.8±4.8	101.7±6.7
SAH				
Baseline	7.38±0.02	125.9±3.8	32.5±4.9	100.3±10.1
0.5h	7.39±0.02	123.6±5.0	33.3±3.1	133.3±7.6*
1 h	7.38±0.03	122.3±5.1	35.3±4.2	100.1±9.8

SAH: subarachnoid haemorrhage group; non-SAH: non-subarachnoid haemorrhage group; * $p < 0.01$ vs Baseline

Figure legends

Fig 1. Changes of intracranial pressure (ICP) after cisternal injection.

Fig 2. Changes of cranial perfusion pressure (CPP) after cisternal injection.

Fig 3. Changes of regional cerebral blood flow (rCBF) in the five groups.

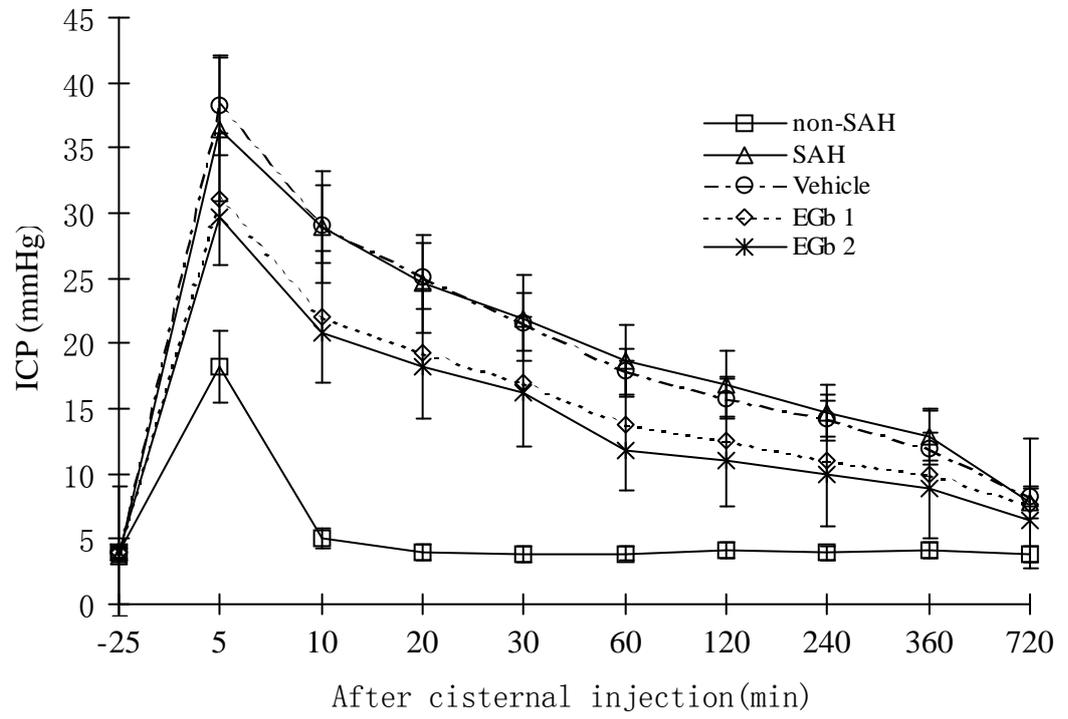


Fig. 1.

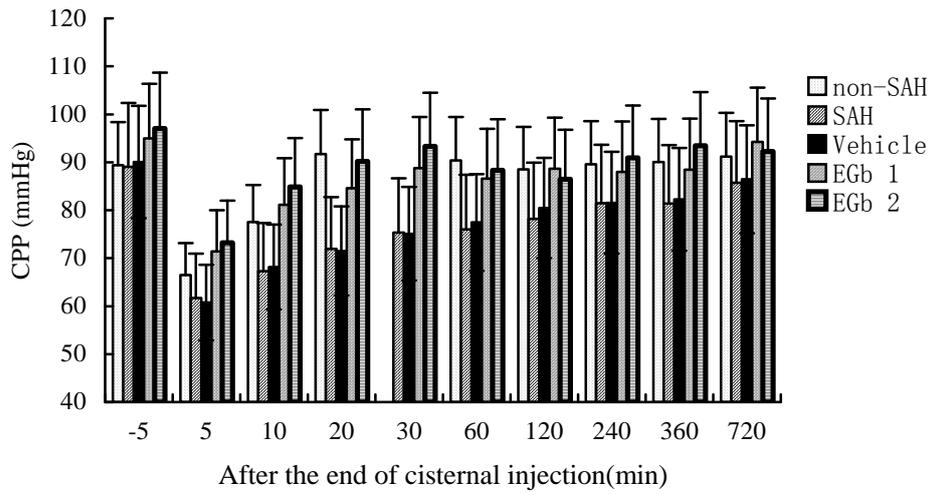


Fig. 2.

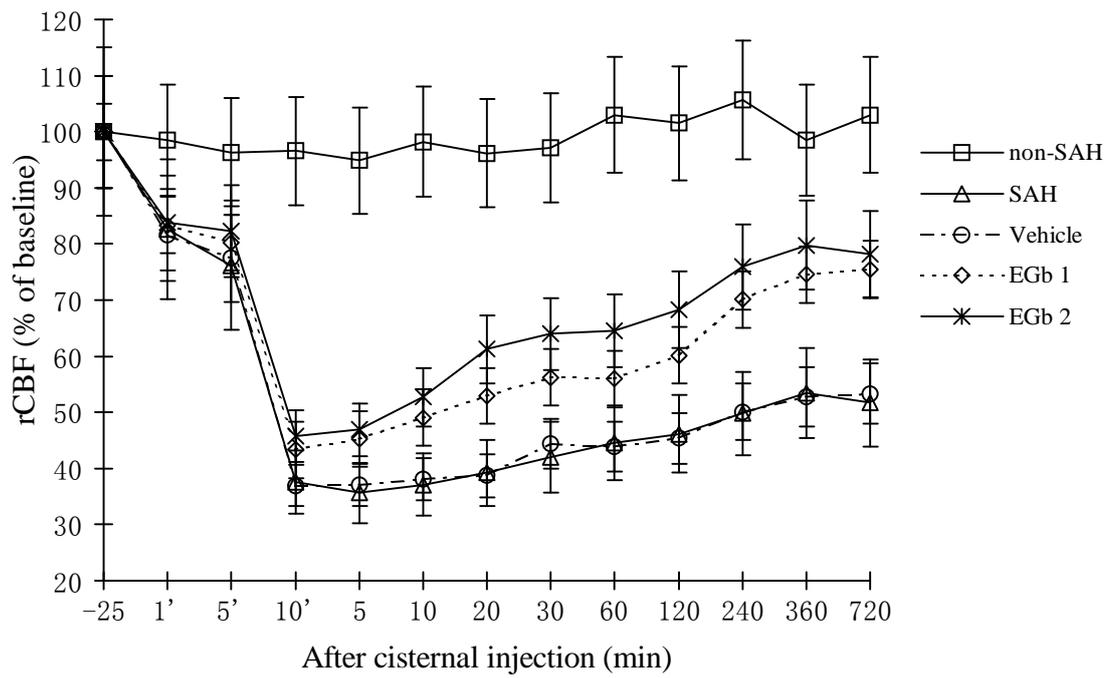


Fig. 3.