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INVESTIGATION OF THE EFFECT OF GINGKO BILOBA ON CHRONIC MORPHOLOGICAL VASOSPASM THROUGH MORPHOMETRIC ANALYSIS IN A RAT MODEL

Ahmet Sengoz* Neurosurgery Specialist, Safa Hospital, Istanbul, Turkey *Corresponding Author

Zafer Berkman Professor, Neurosurgery, Acıbadem Hospital, Istanbul, Turkey

ABSTRACT Vessel diameter is one of the most important parameters affecting the bloodstream. Numerous vasoactive substances are suggested to be involved in the pathogenesis of vasospasm after subarachnoid hemorrhage. In the study, the effect of Ginkgo biloba extracts on chronic morphological vasospasm was investigated in a rat model. Control and experimental groups were subdivided into three: M1 vasospasm model with a silicon cover filled with autologous blood, M2 vasospasm model with just the silicon cover, and the non-operated group. The wall thickness and lumen area were quantified as the number of µm2 units counted in the lumen. For both variables, the differences among the control M1 group, the experimental M1 and control non-operated groups were found statistically significant ($p<0.0001$). These results suggest that Gingko biloba may have a protective effect on subarachnoid hemorrhage-induced vasospasm as an antioxidant.

KEYWORDS : Vasospasm, Gingko biloba, morphometric analysis

INTRODUCTION

Cerebral vasospasm can be asymptomatic, leading to various clinical conditions ranging from mild neurological deficits to mortality. Despite the 200 years time period passed since its first identification by the English physicist Gull, the mechanism of cerebral vasospasm is still not entirely elucidated (1). Up until today, numerous studies on the etiology of vasospasm continue to be undertaken even though various factors have been suggested to be the cause, ranging from hypertension to spasmogenic agents such arachidonic acid, prostaglandin E2, and platelet activating factors. As a result, no widely accepted therapeutic approach for preventing vasospasm has been established yet, and the current treatment methods are palliative rather than curative. The main active constituents of *Gingko biloba* leaf extract, lavonoid glicosides, and terpene lactones, have been shown to exert a significant influence on the cardiovascular system and central nervous system as free radical scavengers and antagonists of platelet activating factors (2,3). The neuroprotective effect of *Gingko biloba* has also been confirmed (4,5). In this study, the anti-vasospasm efficacy of *Gingko biloba* extract was measured using morphometric methods.

MATERIALS AND METHODS

All experiments were conducted in accordance with the rules of Board of Ethics and the protocols set by the Animals Protection Society. The vasospasm model utilized in this study was developed by Okada in rat femoral arteries. Okada had demonstrated that whole blood or washed erythrocyte suspension applied around femoral arteries led to maximum vasospasm in 7 days (6).

60 male Sprague-Dawley rats weighing 144 to 215 g were randomly divided into two groups (control and experimental groups). Each group was further divided into two subgroups, in one of which the vasospasm model was induced. For M1, one cm in length a segment at the inguinal area of the proximal femoral artery was exposed using sterile microsurgical techniques without damaging the vessel wall. Then a 1 cm2 silicon cover was placed around the adventitia. Fresh autologous whole blood was injected between the adventitia and the cover. For M2, same procedures were followed but nothing was put between the adventitia and the cover. No treatment was undertaken in the non-operated groups.

In all groups, one cm length a segment was removed from each left and right femoral artery for histopathological examination after one week. The morphometric analysis was made based on vessel lumen area and wall thickness. The lumen area and wall thickness were determined as the number of µm2 unit in the lumen. Mann-Whitney U-test was used in the statistical analysis of all data. The p value of <0.05 was accepted as statistically significant.

RESULTS

Vessel samples of lumen cross-sections dyed with hematoxylin and eosin were investigated under the light microscope. Explicit decrease in lumen diameter and increase in wall thickness were observed at vessel cross-sections derived from control group rats with the vasospasm model (M1). Examining average values, the vessel lumen and vessel thickness were found to be 364.47 units and 9.123 units at the ends, respectively.

On the vessel cross sections obtained from the rats of the experimental group with the vasospasm model (M1) treated with Ginkgo biloba extract displayed decrease in lumen diameter and increase in wall thickness. Examining average values, the vessel lumen and vessel thickness were found to be 4292.60 units and 5.52 units at the ends, respectively which were similar to the results obtained from the non-operated control group (Table 1).

Table 1. Results from lumen area and wall thickness calculations

	Lumen area		Wall thickness	
	Average	SD	Average	SD
Control M_1	364.47	293.2	9.123	1.174
Control M_2	5682.50	447.9	3.43	0.962
Control non-operated	5172.70	757.62	3.786	0.848
EGb 761 M_1	4292.60	661.48	5.52	1.115
EGb 761 M_2	5735.30	580.87	2.82	0.82
EGb 761 non-operated	5263.70	738.27	2.92	0.736

Regarding lumen area and wall thickness, the difference between the control groups M1, M2 and non opere group and the experimental group EGb 761 M1 was found to be statistically significant ($p<0.0001$).

Treating rats of the vasospasm model with *Gingko biloba* was found to be effective on both wall thickness and lumen diameter. The effects on lumen diameter and wall thickness are correlating.

DISCUSSION

It was demonstrated in experimental subarachnoid hemorrhage (SAH) models that maximum vasospasm was reached at the end of the seventh day when rat femoral arteries are exposed to whole blood and a washed erythrocyte suspension. These changes of the vessel wall resembled the changes of the cerebral artery vasospasm occurring after subarachnoid hemorrhage. Both clinical and experimental knowledge indicate that vasospasm is related to blood volume in subarachnoid distance and blood exposure time of vessel walls (6).

Subarachnoid hemorrhage is the most commonly reason of cerebral vasospasm (7). Despite experimental and clinical research, the pathophysiology of cerebral vasospasm has not been understood yet. But vasoactive and potentially spasmogenic compounds was detected in blood and BOS of patients with SAH (8,9).

Advances in immunology revealed that the vessel endothelium acts like a dynamic immune organ. The regulation of vascular tonus is controlled by the endothelium. Endothelium not only synthesizes mediators but also being affected by the proactive mediators secreted. The greatest change in endothelium is seen in inflammation caused by

various causes. An increase in the productions of endotelin-1 (ET-1) and platelet-derived growth factor beta (PDGF) causes vasoconstriction while the induction of the synthesis of nitric oxide (NO) and prostaglandin I2 results in vasodilatation. The endothelium participates in the vasospasm process not only through the production of endothelium-derived relaxing factor (EDRF) but also through the release of calcium from smooth muscles (9).

A long living tall tree, Ginkgo biloba has been utilized by Chinese conventional medicine for 5000 years. Ginkgo glycosides increase hypoxia tolerance in the brain, preventing traumatic or toxic brain edema, and increase memory performance and learning capacity and cerebral microcirculatory blood flow by inhibiting the age-related reduction of muscarinergic choline receptors and a-2 adrenoreceptors by increasing choline uptake in the hippocampus.

In-vitro studies showed that Ginkgo biloba prevents thrombocyte aggregation by facilitating the synthesis of endogenous inhibitors (EDRF and PGI2) and the inhibition of ADP, epinephrin, collagen and thrombin. In addition, it has scavenging properties. (2,10,11). During cerebral ischemia, free radicals affect lipid bound enzymes, increase endothelium damage and contribute to irreversible cell damage. Studies revealed that EGb761 has a dose-dependent inhibitory effect on the production of lipid peroxides (12).

Our study has shown that significant differences occur in groups treated with Ginkgo biloba extract concerning both vessel lumen size and vessel wall thickness and the vasospasm effect is diminished. However, ongoing studies on Gingko biloba investigate the ingredients of this extract and try to reveal the chemistry of the actual interaction (13). In studies of SAH rat models, Sun et al. demonstrated that Ginkgo biloba extracts increase the microcirculatory blood flow and prevent the increase of the endotelin amount in plasma and brain tissue (4). Bilobalide found in Ginkgo biloba extracts has neuroprotective effects. Despite its yet unknown mechanism of action, it was found that γ- aminobutyric acid interacts with neural transmission by glutamate and glicyn. Under ischemic conditions it significantly decreases the glycine secretion induced by ischemia; however, it does not interact with glycine receptors (9,10).

NO is released during the conversion of L-arginine to L-citrulline by the nitric oxide synthase (NOS) enzyme. NOS1 enzyme is found in neurons while NOS3 in vessel endothelium. The structural NOS in endothelium cells is the main determinant of vascular tonus. Kobuchi et al. demonstrated that the NO synthase (iNOS) enzyme activity of cytosolic preparations from activated RAW 264.7 cells was inhibited by treatment with EGb 761. EGb 761 may act as a potent inhibitor of NO production under tissue-damaging inflammatory conditions. It was able to scavenge O-2 and OH- , inhibit lipid peroxidation of microsomes, and protect SH-SY5Y cells against H2O2 induced oxidative damage (14).

Various studies demonstrated that drugs described as antioxidants or free radical scavengers– nicotinamide, mannitol, deferoxamine, vitamin E, dexamethasone, methyl prednisolone, ticlopidine, leukotrien antagonists, thromboxane synthase inhibitors, nizofenone- contribute to the prevention of post-SAH vasospasm and its complications (7).

CONCLUSION

In a rat femoral artery model, we demonstrated the effect of EGb 761 on chronic morphological vasospasm through morphometric measurements. There have been statistically significant differences concerning lumen area and wall thickness. Bilobalide is one of many active constituents from Gingko biloba. Whilst there is good, sound evidence that bilobalide exhibits neuroprotective actions in a variety of model systems, there is currently no consensus on its mechanism of action.

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