Protective Role of EGb 761 on Cerebral Ischemic Damage, Comparison with Mannitol and U74389F

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Özet

EGb 761'in Mannitol ve U74389F ile karşılaştırmalı olarak serebral iskemi üzerine koruyucu rolü

Amaç: Mannitol, EGb 761 (Ginkgo biloba extract) ve U74389F (lazaroid) 'in antiödematöz etksini karşılaştırmak amacıyla bu çalışma dizayn edildi. **Gereç ve yöntem:** Herbirinde 10'ar rat olmak üzere 4 grup oluşturuldu. İzotonik NaCl uygulanan kontrol grubu, EGb uygulanan grup, U74389F uygulanan grup ve Mannitol uygulanan grup. Tüm gruplarda bilateral arteria karotis kommunislerin oklüzyonu ile serebral iskemi oluşturuldu. Her gruba ilgili olduğu ilaç uygulandı. Beyinler çıkartılarak beyin su miktarları belirlendi. **Bulgular:** Beyin su miktarı EGb 761, mannitol, ve U74389F uygulanan gruplarda kontrol grubuna göre anlamlı olarak azaldı ($p<0.001$). Diğer yandan su miktarı U74389F uygulanan grupta EGb 761 ve mannitol uygulanan gruplara göre daha fazlaydı. **Sonuç:** EGb 761, U74389F ve mannitol ratlarda oluşturulan deneysel beyin iskemisinde etkili tedavi edici ajanlardır. Bunların içinde EGb 761 ile mannitol en iyi ve aynı etkiyi göstermiştir.

Anahtar kelimeler: Beyin iskemisi, EGb 761, Gingko biloba, lazaroid, Mannitol, U74389F

Abstract

Aim: We designed an experimental study to compare the antiedematous effect of three different kind drugs which were Mannitol, EGb 761 (Ginkgo biloba extract) and U74389F (lazaroid). **Materials and Methods:** Forty rats were assigned into four groups (n=10, for each) as following: Control (saline) group, EGb 761 treatment group, U74389F treatment group, and mannitol treatment group. In all groups, cerebral ischemia was induced by bilateral common carotid artery occlusion. The agents were applied to appropriate groups. The brains were removed and brain water content was determined. **Results:** The brain water content significantly decreased in EGb 761, mannitol, and U74389F treated groups when compared to control group ($p<0.001$). On the other hand, the water content was higher in U74389F treated group, comparing with EGb 761 and mannitol groups. **Conclusion:** These results indicate that EGb 761, U74389F and mannitol are effective treatment agents in the experimental brain ischemia in rats. Among these agents EGb 761 and mannitol showed the best and the same effect.

Key words: Brain ischemia, EGb 761, Gingko biloba, lazaroid, Mannitol, U74389F

Introduction

Brain blood vessels exert a rigid control of solute and water exchange between the intraluminal and the interstitial space due to the presence of the blood-brain barrier (BBB). An increase of BBB permeability is the mechanism underlying development of vasogenic brain edema (1). Vasogenic brain edema is a threatening complication in many clinical situations such as brain tumour, incomplete ischemia, reperfusion following ischemia, traumatic brain injury or inflammation. The extent of brain damage is partly determined by the direct effects of the primary insult on brain parenchyma and vasculature. In addition, it may also trigger release and/or activation of mediator compounds which then may give rise to BBB dysfunction and development of secondary brain damage (1,2).

Transient brain ischemia in animal produces brain edema. It is known that severe ischemia is accompanied by a rapid decrease in extracellular

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sodium and chloride ion contents (3), and this is generally interpreted as a reflection of cellular influx of ions. This ion shift is accompanied by influx of water causing cellular edema. A lot of agents such as mannitol, glycerol and urea, acetazolamide, some steroids, calcium antagonists, antihistaminics, and free radical scavengers have been used, to prevent experimentally formed brain edema secondary to ischemia (4-10). The aim of this study was to explore protective role of Ginkgo biloba extract (EGb 761) on experimental postischemic brain edema and to compare its efficacy with Mannitol and U74389F (lazaroid).

Although the constituents of EGb 761 have not yet been completely defined, the main, and possibly clinically relevant, constituents of EGb 761 have been identified and quantitated (11). Nevertheless, when it is shown that a given constituent of EGb 761 is biologically active only in vitro, then its bio-availability after oral or parenteral administration is to be considered. In this study, pharmacological mechanisms of action, or concepts of action, for the in vivo and clinically anti-edema effects of EGb 761 was conclusively tried to be explained.

Materials and Methods

Subjects

In conducting the research described in this report, the investigators adhered to The Guide for Care and Use of Laboratory Animals (12), as prepared by the committee on the Guide for Laboratory Animal Resources. A total of 40 male Wistar rats were used, and assigned into four groups as following: Group A (n=10); control, normal saline group, group B (n=10); EGb 761 treatment group, group C (n=10); U74389F treatment group, and group D (n=10); mannitol treatment group.

EGb 761 was provided by the Laboratories of Schwabe in German, and U74389F was obtained from The Upjohn Company, MI, USA.

Surgical Procedure

All surgical procedures were performed under anaesthesia with ketamine hydroclorur (35 mg/kg) and xylazine (10 mg/kg) intraperitoneally. The neck was shaved and the skin was cleansed with povidone-iodine solution. Saline (1 cc), EGb 761 (100 mg/kg) (13,14), U74389F (3 mg/kg in 0.5 ml of citrate buffer solution (pH 3.0), and 20 % mannitol (0.4 g/kg) were administered intraperitoneally to the rats 30 minutes before ischemia (13-15). Since its half life is 22 hours, EGb 761 was administered only once (11). However, U74389F was administered 5 times during the experiment because its half time was 2 hours (16). On the other hand, mannitol was given in 30th minute, 3rd, 6th, and 9th hours of ischemia. Using a binocular loop and bipolar cautery, the neck was dissected to allow the simultaneous occlusion of both carotid arteries with aneurysm clips (Disposable vascular clip, Arosurgical Instruments, USA, Closing force: 60 g). Exactly 7 minutes after bilateral carotid artery occlusion (BCAO), the aneurysm clips were removed and cerebral circulation was restored. Twelve hours after ischemia, the brains were removed by using high speed drill under anaesthesia.

Estimation of Brain Water Content

For determination of brain swelling, the tissues were weighed after the removal of brains. Hemispheres were then dried for 48 hours at 100 ºC and weighed again. Cerebral water content was calculated as the difference of hemispheric wet and dry weight.

Statistical Analyses

Statistical comparison was carried out using the Kruskall Wallis and Mann Whitney U tests in the Statistical Social Pocket System (SSPS). P value of less than 0.05 was considered significant.

Results

The mean ± SD of the weights of rats were 190.1 ± 24.8, 181.9 ± 20.6, 181.3 ± 19.4, and 185.4 ± 24.7 g for group A, B, C, and D, respectively. The comparison of the weights of groups was insignificant ($p<0.05$, Kruskall Wallis).

Three of the rats died during and after the operation. One died for carotids rupture and the others could not tolerate the anaesthesia. The rest tolerated the operation well. The results are shown in Table 1.

Table 1 . Brain water content after bilateral common carotid artery occlusion in all groups (%).

N	Control (Group A)	EGB 761 (Group B)	Lazaroid (Group C)	Mannitol (Group D)
1	72.2	74.5	87.3	80.1
85.6	79.6	2	75.2	72.8
3	76.3	74.1	85.4	77.8
-	78.3	4	75.7	73.6
5	75.4	75.1	86.6	77.5
88.9	79.2	6	-	74.5
7	73.8	-	84.7	81.2
83.9	76.8	8	75.6	75.2
9	74.5	73.7	85.5	78.3
88.3	78.4	10	75.3	74.8
Mean ± SD	86.2±1.66	74.9±1.24	78.7±1.31	74.3±0.78

There was a significant difference in water content

of brain among the groups ($p>0.001$, Kruskall Wallis). The brain water content was significantly decreased in the treatment groups (Group B,C, and D) compared to the control group ($p<0.001$, for group B, C, and D, Bonferroni corrected Mann Whitney U). While significant differences were found between group B and C, and between group C and D ($p<0.001$, for both, Bonferroni Corrected Mann Whitney U), no difference was detected between group B and D ($p>0.05$, Bonferroni Corrected Mann Whitney U).

Discussion

In gerbils, forebrain ischemia for 5 minutes leads to ischemic cell death (17). Recently, two metabolic events (free radical formation and excitatory amino acid release) have been proposed to explain this phenomenon. Several pathophysiological mechanisms, such as increased excitatory input to the neurones (18), and intracellular calcium overload (19) have been proposed for this phenomenon. However, the precise mechanism involved during ischemia and recirculation remains to be clarified. Oxygen free radicals have been shown to cause an increased output of excitatory amino acids and to trigger the delayed loss of neurones and brain swelling following transient ischemia (20). Ferric iron plays an important role in free radical-mediated injury since it catalyses the conversion of superoxide and hydrogen peroxide to the more toxic hydroxyl radical which has been identified as an important mediator of ischemic brain edema (21). It was shown that neuronal death and hemispheric swelling were decreased by using antioxidants previous to the ischemia (10). In our study, we investigated the effects of three different generation of free radical scavengers on brain water content and edema.

EGb 761 is an extract of an Asian tree, Gingo bloba. Its major classes of constituents are flavonoids, terpenoids such as gingolides and bilobalide, and organic acids such as 6-hydroxykynurenic acid, 4-hydroxybenzoic acid (11). Clinical effects of EGb 761 were mainly attributed to flavonoids and terpenoids. It is known that EGb 761 can scavenge free radicals, and it can also inhibit platelet activating factor (PAF) – induced platelet aggregation (22-24).

A lot of studies performed in rat brain and spinal cord have indicated that EGb 761 prevents oxidative damage (24-27). In another study, in laboratory animals with cerebral ischemia, EGb 761 was shown to improve the cerebral metabolism and protect the brain against hypoxic damage (11,25).

In our study, pretreatment of EGb 761 significantly reduced brain water content. Flavonoids, ginkgolides and bilobalide could be involved in the possible anti-ischemic action mechanism of EGb 761; i.e., flavonoidler via their free radical-scavenging and enzyme-inhibitor activities (28), ginkgolides via their anti-PAF activity (29) and bilobalide via its anti-edema activity (30). Of course, other EGb 761 constituents could also contribute to this anti-ischemic effect. 6-hydroxykynurenate, an organic acid ingredient of EGb 761, would act as a competitive and non-competitive antagonist at neuronal NMDA-type glutamate receptors and as a competitive antagonist at non-NMDA-type receptors and could contribute to anti-ischemic, neuroprotective action of EGb 761 (31,32).

A new family of compounds, the lazaroid, has been developed by substituting an amino group on carbon 21 of the steroid nucleus. The 21-amino steroids have been shown to inhibit lipid peroxidation (33-37) without glucocorticoid activity and to attenuate ischemic injuries in brain (38,39) and spinal cord (40). In our study, U74389F, a lazaroid, had a lower effect than other agents studied on brain water content after ischemia in rats. But, when compared to control group, it had significant attenuation effect, i.e. It was the third most potent among others.

Mannitol is frequently used to reduced elevated intracranial pressure often associated with brain edema (41-43). Two main mechanisms of action of mannitol on cerebral compliance are discussed. First, mannitol increases CBF by a transient hypervolemia and reduce blood viscosity. This induces a compensatory vasoconstriction, thus reducing cerebral blood volume (44). Second, osmotic dehydration of the brain is a widely accepted mechanism of action (43). Additional beneficial effects of mannitol, e.g. reduction of ischemic damage have been reported and partly attributed to a free radical scavenging action (42-47). It is suggested that mannitol interferes with other mechanisms, e.g. acts as a free radical scavenger (42), thus, specifically interfering with edema promoting mediators, or that mannitol reduces the growth of the primary brain tissue necrosis from the focal injury. Findings that mannitol reduces the area of infarction following middle cerebral artery occlusion support this hypothesis (42).

In conclusion, our study demonstrated that free radical scavengers, EGb 761, U74389F, and mannitol are helpfull in decreasing the experimental brain edema performed by BCAO in rats. Among these agents,

EGb 761 and mannitol showed the same and the best effect. Identification of the mediators which are the most important in edema formation after BCAO occlusion requires additional investigation.

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