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Original Article

Effect of ginkgolide B on brain metabolism and tissue oxygenation in severe haemorrhagic stroke

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Abstract: Ginkgolide B, a diterpene, is an herbal constituent isolated from the leaves of Ginkgo biloba tree. The present study demonstrates the effect of ginkgolide B in osmotherapy on brain metabolism and tissue oxygenation. Multimodality monitoring including intracranial pressure (ICP), cerebral perfusion pressure (CPP), partial pressure of brain tissue oxygen (PbtO2), lactate/pyruvate ratio (LPR) and microdialysis were employed to study the effect of ginkgolide B osmotherapy. The results demonstrated that administration of 15% solution of ginkgolide B to the comatose patients with raised ICP (> 20 mm Hg) and resistant to standard therapy led to a significant decrease in ICP. The cerebral microdialysis was used to compare mean arterial blood pressure (MAP), ICP, CPP, PbtO2, brain lactate, pyruvate and glucose level after hourly intervals starting 3 h before and up to 4 h after hyperosmolar therapy. There was a decrease in ICP in 45 min from 23 ± 14 mm Hg ($P < 0.001$) to 18 ± 24 mm Hg and increase in CPP after 1 h of gingkolide B infusion from 74 ± 18 to 85 ± 22 mm Hg ($P < 0.002$). However there was no significant effect on MAP but PbtO2 was maintained in the range of 22-26. The peak lactate/pyruvate ratio was recorded at the time of initiation of osmotherapy (44 ± 20) with an 18% decrease over 2 h following gingkolide B therapy. Also the brain glucose remained unaffected.

Keywords: Osmotherapy, microdialysis, infusion, intracranial pressure, perfusion pressure

Introduction

Stroke, the third leading cause of death in United States has only intravenous tissue plasminogen activator (tPA) as the Food and Drug Administration (FDA)-approved treatment so far [1]. Two major problems of stroke therapy are: limited use of tPA due to its narrow therapeutic time window (3 hours) and despite promise in animal studies no neuroprotective drug has proved effective in phase III human studies. Therefore, a new strategy for stroke therapy would be a significant achievement.

The metabolic state of almost any tissue including brain energy metabolism during neurointensive care is monitored by microdialysis [2-4]. The common metabolites measured are glucose, lactate, pyruvate, glycerol, and glutamate. The ratio of lactate: pyruvate (LPR), a marker of the cellular redox state is an indication of mitochondrial function, where a value above 25 indicates anaerobic metabolism and ischemia in the brain [5-7].

Increased intracranial pressure (ICP) along with low cerebral perfusion pressure (CPP) episodes lead to severe brain injury resulting in increased morbidity and mortality [8-11]. Early recognition of such critical episodes using multimodal neuro-monitoring can be a useful strategy to provide insights into treatment efficacy. Osmotherapy is currently used when standard modes of therapy do not work [12, 13]. There are reports that mannitol reduces ICP and improves CPP [14-17]. Osmotherapeutics including mannitol act by dehydrating brain that causes decrease in intracellular volume [16]. Recently a transient increase in extracellular metabolites was observed in mannitol study therapy which supports this hypothesis [18].

The extract of Ginkgo biloba L. (Ginkgoaceae) has been used in the treatment of neural and vascular damage [19, 20]. The effect of EGb-

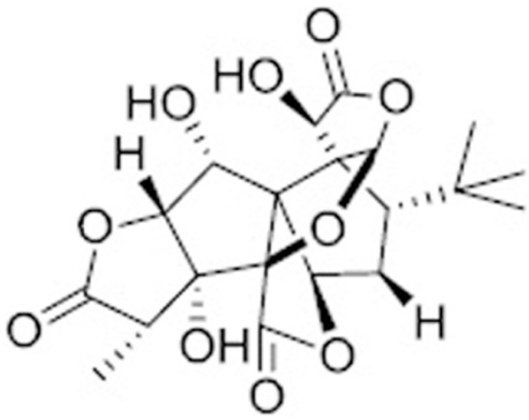

Figure 1. Structure of ginkgolide B.

761, a patented leaf extract has been investigated in cerebral insufficiency, dementia, particularly Alzheimer's disease, cerebral ischemia, and traumatic brain injury [21-28]. The flavonoids present in the extract may serve as free radical scavengers and neutralize ferryl ion-induced peroxidation [29, 30]. On the other hand terpenoid fraction containing bilobalide and ginkgolide possess a marked anti-apoptotic property [31-33] and act as selective antagonists of platelet activating factor respectively [34, 35].

Ginkgolide B (GB) (**Figure 1**) is the most potent antagonist of platelet activating factor [36-41]. GB exhibits a marked neuroprotective property against ischemia-induced impairment in vivo and in vitro [19, 20, 42-48]. Taking this into consideration we investigated the effect of GB in osmotherapy on brain metabolism and tissue oxygenation.

Methods

Patients

A total of 50 patients with non-traumatic severe acute haemorrhagic stroke were admitted to the neurological ICU at our hospital between January 2012 and June 2014. The patients underwent brain multimodality monitoring according to our institutional protocol. All the patients had a Glasgow Coma Scale ≤ 8 at the time monitoring was initiated. Median Hunt-Hess grade of 5 (IQR, 4-5) of aneurysmal subarachnoid haemorrhage (SAH) was observed in the patients at the time of admission. Among the 50 monitored patients, 15 with intracranial hypertension greater than 20 mm Hg and availability of multimodality data were selected for ginkgolide B treatment analysis.

Intracranial monitoring

The patients were subjected to multimodality monitoring including at least ICP, microdialysis and partial pressure of oxygen in brain tissue (PbtO2). Only the patients possessing survival probability for next 48 h and likely to remain unconscious for 48 h were subjected to multimodality monitoring. A CMA 70 microdialysis (MD) catheter (CMA/Microdialysis, North Chelmsford, Massachusetts) with a membrane cut-off of 20 kDa was used for microdialysis recording. A perfusion pump (CMA 106; CMA/Microdialysis) was connected to catheter for pumping perfusion fluid (147 mmol/L NaCl + 1.2 mmol/L $CaCl_2$ + 0.9 mmol/L $MgCl_2$ + 2.7 mmol/L KCl) at a flow rate of 0.3 µl/min through the system. The CMA 70 microdialysis catheter (CMA/Microdialysis, Stockholm, Sweden) was inserted through frontal burr-hole, triple-lumen bolt into the brain parenchyma. In to the hemisphere at great risk for secondary injury or in the right frontal lobe in patients with diffuse injury were placed the probes. In white matter CT scan was used to confirm the location immediately after the procedure. The samples were analysed (CMA 600; CMA/Microdialysis) after 1 h intervals for ECF glucose, pyruvate and lactate concentrations. Initially analyser was calibrated automatically and there after every 6 h standard calibration solutions were used. Quality controls were performed daily. A flexible polarographic Licox Clark-type probe (Licox GMBHTM, Kiel, Germany; Integra Neurosciences, Plainsborough, New Jersey) was used to measure PbtO2. ICP monitoring was performed using a parenchymal ICP monitoring device (Integra Neurosciences).

Clinical management

In order to have CPP ≥ 60 mmHg and ICP < 20 mmHg by haemodynamic and fluid management a stepwise management strategy [49] was employed. The strategy includes: a) combination of benzodiazepine, propofol and fentanyl or remifentanyl for sedation and analgesia; b) ventricular catheter for cerebrospinal fluid drainage, if needed; c) PCO_2 mild hyperventilation at 30-34 mm Hg; and d) administration of ginkgolide B to decrease the elevated ICP. Treatment effectiveness was measured in

Table 1. Baseline characteristics

Age (years)	47 (37-56)
Female	10 (59)
Admission Glasgow Coma Scale	11 (6-10)
Admission Acute Physiology And Chronic Health Evaluation 2	23 (17-29)
Admission diagnosis	
Subarachnoid haemorrhage	7 (76)
Admission radiographic findings	
Modified Fisher scale	5 (4-6)
SAH sum score	20 (15-28)
Intraventricular haemorrhage sum score	4 (4-8)
Hydrocephalus	9 (100)
Haematoma	5 (57)
Global cerebral oedema	7 (78)
Aneurysm size >10 mm	3 (35)
Intracerebral haemorrhage	3 (23)
Length of stay in hospital (days)	30 (15-44)
Mortality	6 (44)

In acute physiology and chronic health evaluation 2 four physiological variables: a) arterio-alveolar gradient of >125 mm Hg, HCO_3 of < 20 mmol/l, glucose of 9.9 mmol/l, mean arterial pressure of < 70 or >130 mm Hg (range 0-8) are included; subarachnoid haemorrhage (SAH) sum score grades the amount of blood in 10 basal cisterns and fissures (0 = no SAH, 1 = small SAH, 2 = moderate SAH, 3 = completely filled with SAH) by adding each of the 10 individual cistern scores (range 0 = 30); intraventricular haemorrhage sum score grades the amount of blood in the right and left lateral, third and fourth ventricle (0 = no blood, 1 = sedimentation, 2 = partly filled, 3 = completely filled) by adding each of the four individual ventricle scores (range 0-12). All the values are presented as median (IQR) or number (%).

terms of decrease in ICP below 20 mm Hg in a time dependent manner. While comparing pre and post ginkgolide B treatment CT scans only greater than 1 cm midline shift differences were considered significant.

Biochemical data

For sodium and serum osmolality, the first analysis was performed after 12 h and the second analysis after 24 h of the bolus before osmotherapy.

Data acquisition

A high-resolution data-acquisition system (BedmasterEX, Excel Medical Electronics, Jupiter, Florida) acquired automatically vital data from all the patient monitoring devices in the NICU. The data of brain metabolism and LICOX were incorporated into the data-acquisition system and plugged into a serial-to-TCP/IP interface device (Equinox ESP-8, Avocent, Sunrise, Florida). The physiological variables were monitored in all the patients continuously. The equation used for CPP calculation is:

{CPP = mean arterial pressure (MAP)-intracranial pressure (ICP)}.

Statistical analysis

The Student *t* test for continuous variables and χ^2 test for categorical variables were used in statistical analysis of pooled data. Multivariable general linear model was employed for time-series data analyses. SPSS 16 software (SPSS) was used for all statistical analyses. A *P* value of < 0.05 was considered statistically significant.

Results

General characteristics and outcome

Baseline characteristics are described in **Table 1**. The median patient age was 47 (37-56) years and 10 were females. Neuromonitoring was initiated at 48 h after ictus (median, IQR 1-3) and maintained for 8 days (median, IQR 5-13).

Gingkolide B osmotherapy and treatment effectiveness

The patients were administered 1200 mg/kg body weight mean dosage of gingkolide B. There was a decrease in ICP below 20 mm Hg in all the gingkolide B administrations. The mean time of effectiveness in this cohort was 3 h. After 3 h the ICP again began to rise and was above 20 mm Hg after 4 h of gingkolide B boli infusion (**Figure 2A**). Initially we used different dosages of gingkolide B and found that the decrease in ICP was sufficient at 1200 mg/kg of body weight (**Figure 2B**). Pre- and post gingkolide B CT scans for 20 boli revealed a decrease in midline shift in all the boli.

Gingkolide B osmotherapy and brain metabolism

The effect of gingkolide B on brain metabolism was studied by microdialysis. The multimodal

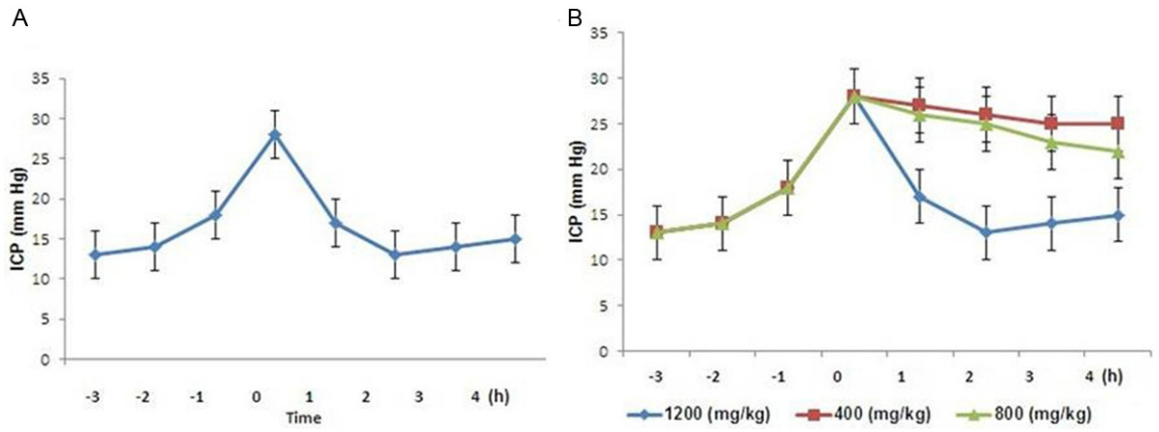

Figure 2. A. Mean time course of intracranial pressure (ICP) 3 h before and 4 h after gingkolide B infusion of n = 20 individual trials. Lines present mean values (± SE). B. Mean time course of intracranial pressure (ICP) 3 h before and 4 h after treatment with different gingkolide B concentrations.

Table 2. Systemic and cerebral physiological measurements before and after gingkolide B infusion (N = 20)

	Time (min)									
	-30	-15	Osmotherapy	15	30	45	60	120	180	
ICP (mm Hg)	18 (5)	25 (8)	28 (14)	27 (28)	21 (14)	15 (18)	15 (13)	13 (9)	15 (7)	18 (7)
CPP (mm Hg)	77 (14)	79 (21)	72 (18)	71 (29)	80 (24)	88 (18)	86 (20)	82 (19)	79 (19)	78 (18)
MAP (mm Hg)	96 (14)	98 (14)	97 (14)	98 (18)	93 (17)	97 (20)	97 (18)	95 (19)	93 (18)	90 (17)
PbtO2 (mm Hg)	23 (13)	27 (19)	26 (15)	27 (14)	24 (13)	23 (15)	28 (14)	30 (13)	27 (18)	31 (16)

Values are given as mean (SD).

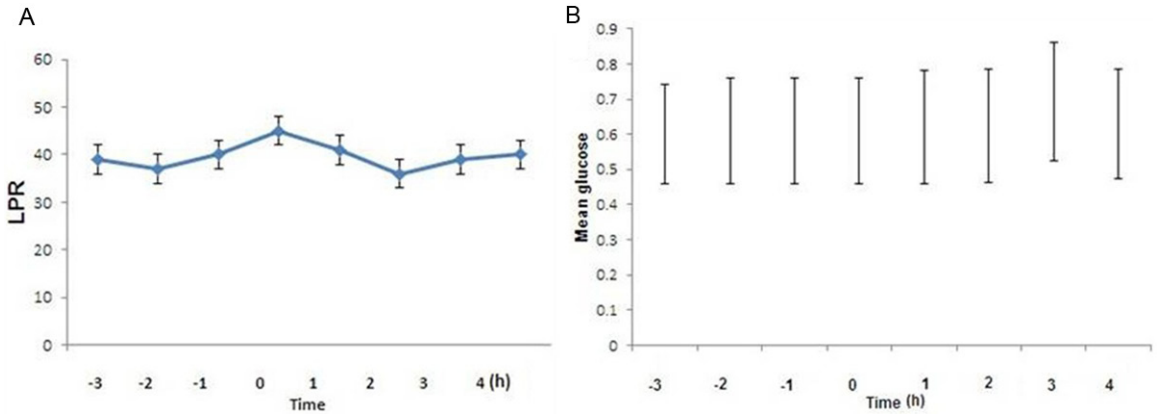

Figure 3. A. Mean time course of lactate-pyruvate ratio (LPR) 3 h before and 4 h after gingkolide B infusion of n = 20 individual trials. B. Mean time course of microdialysis of glucose after gingkolide B infusion. Error bars represent means and 1 SE of n = 22 individual trials.

cerebral monitoring was performed after 1 h intervals before, during and after infusion of gingkolide B (**Table 2**). We observed highest ratio of lactate/pyruvate at the time of gingkolide B infusion. Administration of gingkolide B caused a decrease in the ratio of lactate/pyruvate by 17% over 3 h to a mean level of 32 ± 21 (P = 0.002) (**Figure 3A**). The decrease in the level of lactate and pyruvate was highest, 4.6 ± 3.2 mmol/L, and 103 ± 39 mmol/L, respectively after 1 h of the treatment (**Table 2**). The concentrations of extracellular fluid glucose remained unaffected during the treatment (**Figure 3B**).

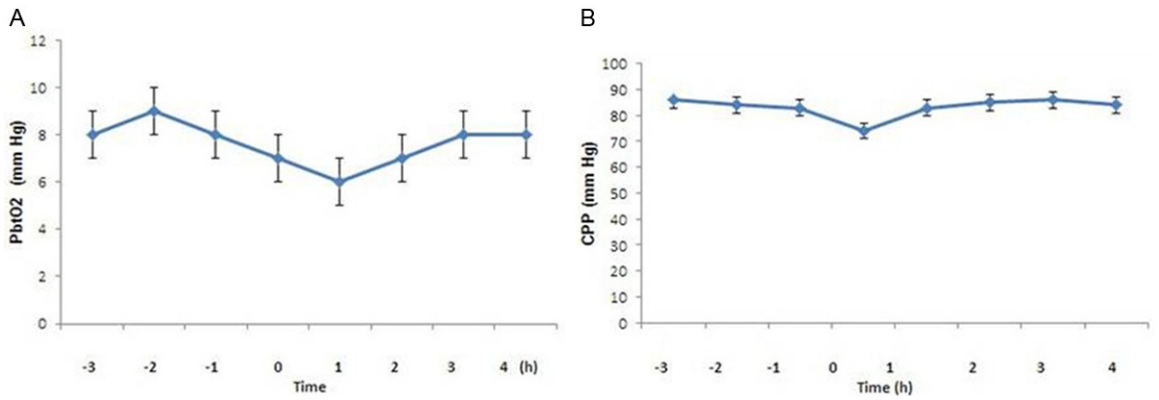

Figure 4. Mean time course of brain tissue oxygen tension (A) and CPP level (B) 3 h before and 4 h after gingkolide (B) infusion of n = 20 individual trials.

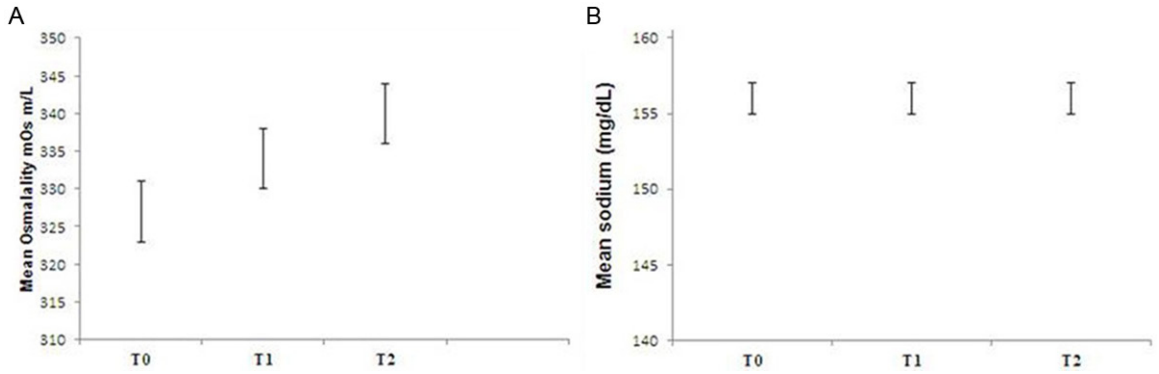

Figure 5. Mean time course of (A) serum osmolality and (B) serum sodium after gingkolide (B) osmotherapy. Error bars represent means and 1 SE of individual trials. The *P* value is given for significant differences (< 0.05). The x-axis represents time intervals to bolus: T0 = 3.0 ± 2.0 h preceding osmotherapy; T1 = 5.0 ± 2.0 h following osmotherapy; T2 = 30 ± 2.9 h after osmotherapy.

Gingkolide B osmotherapy and ICP, CPP, MAP and PbtO2

We studied the effect of gingkolide B osmotherapy at a dosage of 1200 mg/kg body weight on ICP, CPP, MAP and PbtO2. The administration of gingkolide B bolus resulted in a significant decrease in ICP at 45 min after osmotherapy from 23 ± 14 mm Hg ($P < 0.001$) to 18 ± 24 mm Hg. The effect lasted for 240 min ($P < 0.001$ for all time points). Administration of gingkolide B boluses, however, had no effect on MAP whereas the brain-tissue oxygen tension was maintained in the range of 22-26 after gingkolide B osmotherapy for 2 h (**Figure 4A**). The level of brain-tissue oxygen tension was 23 ± 19 mm Hg at the time of gingkolide B administration. The CPP was decreased to 74 ± 18 mm Hg at the time of ICP crisis (mean level 30 min before, 78 ± 17 mm Hg, $P = 0.02$). However the infusion of gingkolide B increased the level of CPP to 85 ± 22 mm Hg at 1 h after the infusion ($P = 0.001$) and remained elevated for 180 min ($P < 0.03$) (**Figure 4B**).

Gingkolide B osmotherapy and serum biochemistry

The measurement of serum biochemistry prior to and after osmotherapy demonstrated that infusion of gingkolide B increased serum osmolality. There was an increase in serum osmolality by 7 and 14 mOsm/kg within first 5 h (325 ± 21 mOsm/kg, $P = 0.005$) and 30 h (331 ± 24 mOsm/kg, $P = 0.04$) after gingkolide B infusion (**Figure 5A**). However there was no effect of gingkolide B infusion on the level of serum sodium which was stable at 152 ± 10 mg/dl (**Figure 5B**). The osmolar gap was 0 ± 10 before and 4 ± 9 mOsm/kg after mannitol infusion.

Discussion

The present study demonstrates that gingkolide B treatment significantly decreases ICP

and improves CPP in addition to decrease in LPR in severely brain-injured patients. However there was no significant effect on MD glucose and pyruvate levels. The PbtO2 after gingkolide B administration was maintained in the normal range. The possible explanation may be that extracellular MD glucose and brain tissue oxygen tension represent the net product of delivery, transportation and consumption. Raised ICP may increase the demand, leading to unchanged levels, despite improved delivery after gingkolide B administration. Treatment efficacy depends on the osmotic load and the rate of infusion [14, 15]. In this study, gingkolide B was given as infusion over 15-25 min. A slow rate of gingkolide B infusion may be effective for > 2 h, whereas a 5 min bolus administration shows an earlier ICP rebound.

Improvement of brain metabolism is essential as both the duration of brain metabolic crisis and the numbers of episodes in metabolic crisis have been associated with poor outcome [5, 50]. Gingkolide B infusion exerted a clear effect on ICP and CPP as reported in case of mannitol [7-10]. We did not observe a significant decrease in MAP but PbtO2 was maintained in the range of 22-26 after gingkolide B osmotherapy for 2 h. The measurement of serum biochemistry prior to and after osmotherapy demonstrated that there was an increase in serum osmolality by 7 and 14 mOsm/kg within first 5 h (325 ± 21 mOsm/kg, $P = 0.005$) and 30 h (331 ± 24 mOsm/kg, $P = 0.04$) after gingkolide B infusion. However there was no effect of gingkolide B infusion on the level of serum sodium which was stable at 152 ± 10 mg/dl.

In conclusion, the results of the study demonstrate that gingkolide B infusion effectively reduces ICP and increases CPP. Therefore gingkolide B appears to benefit brain metabolism as measured by the lactate-pyruvate ratio.

Disclosure of conflict of interest

None.

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