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Research Paper

The orally combined neuroprotective effects of sodium ferulate and borneol against transient global ischaemia in C57 BL/6J mice

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Abstract

Objectives This study aimed to investigate the possible modification of the neuroprotective effect of sodium ferulate, when orally co-administered with borneol, in transient global cerebral ischaemia-induced functional, histological and cellular alterations in mice.

Methods The bilateral common carotid artery occlusion was conducted in C57 BL/6J mice for 25 min. The mice were then subjected to a water maze test over an extended recovery period, followed by an assessment of neuronal loss in the CA1 region of the hippocampus (haematoxylin and eosin staining). The blood–brain barrier permeability (Evans blue tracing), brain oedema and oxidative stress were assayed and histological sections were also immunostained for gliofibrillar acid protein (GFAP) expression.

Key findings The ischaemia reperfused mice were associated with long-lasting spatial learning deficits in the absence of other behavioural impairments and with neurodegeneration in the hippocampal CA1 region. However, the histological injuries were significantly attenuated by oral co-administration of sodium ferulate and borneol. Furthermore, combined treatment with sodium ferulate and borneol resulted in a significant reduction in brain oedema, GFAP-positive cells, malonaldialdehyde levels and blood–brain barrier permeability, but an increase in superoxide dismutase activity.

Conclusions Borneol may have benefits for the neuroprotective effect of sodium ferulate against injury induced in the brain by ischaemia/reperfusion.

Keywords blood–brain barrier; borneol; neuroprotective effects; sodium ferulate; transient global ischaemia

Introduction

In recent years, there have been several important advancements in the development of therapies against ischaemia/reperfusion (I/R) induced brain injuries.[1,2] Many drugs and other natural substances derived from a wide variety of chemical and pharmacological classes have been shown to prevent postischaemic neuronal degeneration.[3] However, in clinical use the application of these drugs is limited by their poor bioavailability, caused by low metabolic stability and high rates of clearance by the liver.[4] The targeting of these drugs to the central nervous system (CNS) remains a formidable undertaking because the uptake of any compound is strictly regulated by the blood–brain barrier (BBB).[5] Strategies to promote the delivery of active pharmacological compounds to the brain are therefore under intensive investigation, including combined administration of drugs to produce greater access of the neuroprotective agent to injured tissue.[6]

Ferulic acid (FA), an effective component extracted from Chinese medicinal herbs, such as *Angelica sinensis*, has potent antioxidant and anti-inflammatory activities. FA is customarily used in the form of sodium ferulate (SF, Figure 1) to increase its solubility. The possible use of SF in the therapy of free radical-related syndromes such as neurodegenerative disorders, cancer and hepatic fibrosis has been well documented.[7,8] It has been reported that

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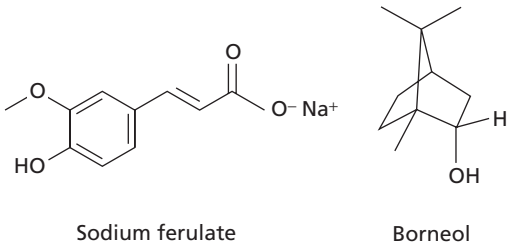

Figure 1 Chemical structures of sodium ferulate and borneol.

long-term administration of SF protected mice against learning and memory deficits induced by centrally administered β-amyloid.[9] SF (100 mg/kg, i.v.) administered at the beginning of middle cerebral artery occlusion (MCAO) or 30 min after MCAO may provide neuroprotection against oxidative stress-related apoptosis in rats.[10,11] Unfortunately, SF undergoes marked first-pass effects that limit its bioavailability and it is quickly metabolised in the liver.[12] In rats, with oral administration, the peak plasma concentration is reached after 15–30 min.[13] Furthermore, studies in rodents have clearly shown that following intravenous administration, SF accumulates in the liver, kidneys and lungs, whereas the concentration reached in other tissues, such as the brain or heart, is negligible.[14] Clearly, although SF has been proven to be an effective neuroprotectant, its pharmacokinetic properties impede its clinical application.

Borneol (Figure 1) is a common ingredient in many traditional Chinese herbal formulas and has the current Chinese name *Bing-Pian*. It possesses analgesic, anti-inflammatory, antibacterial and penetration enhancing effects.[15,16] An acute toxicity study showed that the LD50 of borneol is 13.68 g/kg p.o. in mice.[17] Borneol is a highly lipid-soluble chemical, which can be absorbed rapidly by the gastrointestinal tract and is able to penetrate the BBB. Thus borneol is often used as a 'guiding herb' – one that can guide other drug(s) to the target tissue or organ – in traditional Chinese medicine. Although the efficacy of borneol is weak when used alone, it strengthens the therapeutic functions of other herbs when it is used as an adjuvant and guiding drug.[18,19] The mechanism by which borneol opens the BBB is to loosen the intercellular tight junctions in the BBB, accelerate the transportation of substances through the intercellular passages and also to increase the number and volume of pinocytosis vesicles in the BBB cells, thus accelerating the transport of substances by way of cell pinocytosis. Studies also showed that borneol can accelerate the opening of the BBB, but this was a physiological opening and did not damage the brain tissue and the BBB.[20]

Our previous study showed that borneol can increase the concentrations of SF in plasma and in part of the brain.[21] However, to the best of our knowledge, the neuroprotective potential of orally combined SF and borneol has not been evaluated. In our study, SF (100–400 mg/kg) and borneol (10 mg/kg) were intragastrically administered at 30 min before and after I/R respectively. The effect of the combination of these two drugs against I/R in the brains of mice after transient cerebral ischaemia was assessed by consideration of behavioural and histological changes as well as brain oedema, BBB permeability, antioxidant status and lipid peroxidation.

Material and Methods

Animals and treatment

The study was approved by the Research Ethics Committee of Sun Yat-sen University and carried out in accordance with the Guide for the Care and Use of Laboratory Animals. C57 BL/6J mice (male, weighing 18–20 g, clean grade, certification no. 04057) were supplied by the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China), and were fed with a standard diet and maintained in a 12 h light–dark cycle. The animals were randomly divided into the following groups: sham-operated, vehicle, SF, borneol, SF + borneol.

The doses of SF and borneol (all from Sigma, USA) were chosen on the basis of previous in-vivo data and our preliminary dose-finding experiment.[21] SF (100–400 mg/kg) and borneol (10 mg/kg) were dissolved in 10% ethanol and were intragastrically administered by a tube to mice (p.o.) at 30 min before and after I/R, respectively. Mice were also treated with SF plus borneol to investigate the combination protective potential against I/R. Mice were randomly divided into the following groups ($n = 6$): sham-treated, which was administered 10% ethanol, I/R, SF 100 mg/kg per day, SF 400 mg/kg per day, borneol 10 mg/kg per day, SF 100 mg/kg per day + borneol, SF 400 mg/kg per day + borneol. Therapy was initiated 30 min before or after I/R. To evaluate the treatment effects, the tissue of the mice was collected for brain oedema analysis after 24 h of I/R, for BBB permeability detection after 48 h of I/R and for the Morris water maze (MWM) test after 4 days of I/R.

Surgical operation

The bilateral common carotid artery occlusion (2VO) was conducted in C57 BL/6J mice. Briefly, mice were anaesthetised with chloral hydrate (300 mg/kg, i.p.) and allowed spontaneous respiration throughout the surgical procedure. Through a midline cervical incision, the bilateral common carotid arteries were exposed and clipped with two vascular clamps simultaneously for 25 min in the I/R group.[22] Sham-operated animals received the same surgical procedures except that the carotid arteries were not clipped. After surgery, the mice were placed on a heating pad until they had recovered from anaesthesia.

Examination of brain oedema

Six mice chosen randomly from each group were sacrificed and cerebral oedema was measured with the dry–wet weight method[23] and represented by the water content of the brain tissues. Briefly, at 24 h after reperfusion, mice were anaesthetised and decapitated. The brain was rapidly removed, a 2-mm slice was made through the centre of the contusion and the caudal brain section was immediately weighed (wet weight). Brain sections were then placed in an oven, dehydrated at 110°C for 48 h and reweighed (dry weight). Brain oedema was estimated as the difference in percentage of brain water (wet – dry) ÷ wet weight between the left (injured) and right (uninjured) hemispheres. The percentage of water was calculated as follows:

$$\text{water content}\,(\%) = (\text{wet weight} - \text{dry weight})/\text{wet weight} \times 100\%$$

Blood–brain barrier permeability measurement using Evans blue

The integrity of the BBB was detected, as previously described, by quantitative measurement of Evans blue (EB) content at 48 h after I/R or sham surgery in six animals per group.[24] Briefly, 2% EB (Sigma, USA) solution was administered intravenously at a dosage of 0.1 ml per animal. Thirty minutes after injection, mice were perfused with saline to remove intravascular EB dye. Each brain sample was weighed and then homogenised with 2.5 ml phosphate-buffered saline (PBS) and mixed with 2.5 ml 60% trichloro-acetic acid to precipitate protein. After centrifugation, the supernatants were measured at 610 nm using a spectro-photometer. EB content was expressed as micrograms per gram of brain tissue against a standard curve.

Morris water maze test

Four days after surgery, the MWM test[25] was performed. The pool was filled with water (maintained at 23.0 ± 1.0°C), which covered a 10 cm circular platform. The invisible platform was submerged 1.0 cm below the water surface and placed in the centre of the northeast quadrant. The swimming activity of each mouse was monitored via a camera mounted overhead. The day before the experiment was dedicated to swimming training for 60 s in the absence of a platform. Each mouse was given four trials per day for four consecutive days to find the hidden platform. The daily order of the entries into individual quadrants was randomised so that all four quadrants were used once every day. In each trial, the mouse was allowed to swim for a maximum of 60 s to find the platform. When the mouse located the platform, it was permitted to remain on it for 10 s. If the mouse did not find the platform within 60 s, it was placed on the platform for 10 s. The time interval between each trial session was 30 min. Latency to escape from the water maze (finding the submerged platform) was recorded by computer.

The day after the last training trial, the mice were subjected to a probe test in which the platform was removed from the pool. Mice were allowed to swim for 60 s to search for the original platform. A record was kept of the swimming time in the pool quadrant where the platform had been previously placed.

Histological evaluation

After the MWM test, six mice chosen randomly from each group were anaesthetised with chloral hydrate (300 mg/kg, i.p.) and then perfused transcardially with normal saline followed by 4% paraformaldehyde. All brains were postfixed in the same fixative at 4°C, dehydrated and then embedded in paraffin blocks. Coronal sections of 5 μm were stained with haematoxylin and eosin (HE). Images were taken by Nikon microscopy (Nikon Eclipse 80*i*, Japan).

Immunohistochemistry of gliofibrillar acid protein

The dewaxed and rehydrated brain sections were treated with 3% H_2O_2 to block endogenous peroxidase. After rinsing for 15 min in PBS, the sections were incubated with polyclonal antigliofibrillar acid protein (GFAP) antibody (diluted 1 : 200, Dako Corp., USA) for 1 h at room temperature. Sections were washed in PBS for 15 min and incubated with an Envision detection system (Dako Corp., USA) at room temperature for 30 min, followed by rinsing in PBS for 15 min. Immuno reactivity was detected using 3,3′-diaminobenzidine tetrahydrochloride as the chromogen. The sections were counterstained with haematoxylin. Images were taken by Nikon microscopy and analysed using the Nikon image system (NIS-Elements D 3.1). Total GFAP intensity values were summed based on the frequency of positive pixels that occurred within an intensity spectrum ranging from 0 (none detected) to 256 (highest intensity). For GFAP quantification, two CA1 fields per section were selected for analyses because the CA1 is more damaged than other regions in this model.[26]

Measurement of superoxide dismutase activity and malonaldialdehyde level

Following the MWM test, eight animals from each group were decapitated under anaesthesia. The brains were removed, and the cortex and hippocampus were dissected on ice. The activity of superoxide dismutase (SOD) in brain tissue was measured by the method previously reported, using nicotinamide adenine dinucleotide reduced form as a substrate.[27] The SOD activity was expressed as units per milligram of protein. One unit of the enzyme was the amount required to inhibit the rate of chromogen formation by 50%. As a measure of lipid peroxidation, malonaldialdehyde (MDA) levels in brain tissue were estimated by measuring thiobarbituric acid reactive substances following the standard protocol, using an MDA detection kit (SBS Inc., China).

Statistical analysis

Data were expressed as mean ± SEM and analysed by a two-way analysis of variance. The comparisons between I/R and sham groups were performed with Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

Morris water maze test

The effect of I/R on the spatial learning and memory performance of mice was evaluated using the MWM test. As shown in Table 1, the mice suffering from transient global ischaemia exhibited longer escape latencies throughout the training than the sham-operated mice ($P < 0.05$). A low dosage of SF (100 mg/kg) treatment did not significantly improve memory impairment induced by I/R. However, borneol (10 mg/kg) and a high dosage of SF (400 mg/kg) did so slightly, while the combination of both drugs led to a significant improvement in the escape latency compared to the I/R injury group (Table 1).

In the probe trials, the swimming time in the target quadrant (Q3) was used to evaluate retention performance. Observation of the typical swimming tracks indicated that ischaemic mice often searched for the platform in an inappropriate way, resulting in longer latency to locate the platform. The mice in the sham-operated group swam longer in Q3 than those in the ischaemia group (Table 1). SF and borneol did not increase the shortened swimming time

Table 1 Effect of combination treatment with sodium ferulate and borneol on memory impairment in mice induced by ischaemia/reperfusion

Groups	Mean latency to find the hidden platform (s)					Swimming time spent in Q3 quadrant (s)
	Training day 1	Training day 2	Training day 3	Training day 4	Training day 5	
Sham	45.01 ± 9.35	37.69 ± 11.49	22.75 ± 8.23	14.29 ± 6.52	11.24 ± 8.37	48.87 ± 8.76
I/R	75.48 ± 13.75*	61.80 ± 10.55*	41.49 ± 12.16*	33.02 ± 9.48*	25.50 ± 8.96*	20.87 ± 3.49*
SF 100 mg/kg	67.19 ± 11.57	42.35 ± 9.76	41.05 ± 9.31	32.15 ± 7.24	28.62 ± 9.45	21.65 ± 9.45
SF 400 mg/kg	42.44 ± 13.34#	31.28 ± 8.51#	32.35 ± 13.34	24.00 ± 6.84	26.08 ± 6.33	27.37 ± 7.27
Bor	51.84 ± 9.70#	37.67 ± 12.36	33.46 ± 10.69	31.99 ± 6.93	16.60 ± 10.16	17.06 ± 11.72
SF 100 mg/kg + Bor	38.83 ± 11.87#	26.00 ± 10.77#	14.56 ± 6.89#	11.56 ± 7.75#	8.10 ± 4.15#	30.77 ± 3.67#
SF 400 mg/kg + Bor	58.91 ± 12.88	32.42 ± 13.96#	29.93 ± 11.46	18.28 ± 11.40#	13.21 ± 4.06#	37.06 ± 9.41#

Effect of combination treatment with sodium ferulate (SF) and borneol (Bor, 10 mg/kg) on memory impairment in mice induced by ischaemia/reperfusion (I/R) was investigated by the Morris water maze test. Data are presented as mean ± SEM, *$P < 0.05$ vs sham-operated group (Sham), #$P < 0.05$ vs I/R group, $n = 12$.

induced by I/R, while the combination treatment did so significantly ($P < 0.05$).

Histological examination

HE was used to stain the brain tissues in order to observe pathological alterations in the hippocampus of mice submitted to bilateral common carotid artery ligation and reperfusion (Figure 2). In the sham-operated group, no pathological changes were observed, while marked morphological changes were observed in the I/R group: neuronal cell loss, glial proliferation, nuclei shrinkage and dark staining of neurons, especially in the CA1 region. Although SF alone did not ameliorate the pathologic changes induced by I/R in our research, it markedly decreased the insults on neurons in the CA1 region in the combination-treated mice.

Brain oedema

To evaluate the effects of brain oedema following focal ischaemia, the water content of the brain was assayed at 24 h after reperfusion (Table 2). The water content of the brain hemisphere increased markedly in the I/R mice compared with those in the sham-operated mice ($P < 0.05$). Interestingly, the brain water content was significantly alleviated by combination treatment with borneol and SF ($P < 0.05$).

Blood–brain barrier integrity

The concentrations of extravasated EB dye (expressed as micrograms per gram of brain tissue) are shown in Table 2. In sham-operated mice, the baseline level of EB was 2.00 ± 0.64. I/R induced a significant increase in the content of EB in the brain (from 2.0 ± 0.64 to 8.8 ± 1.8, $n = 6$, $P < 0.05$). Borneol or SF (400 mg/kg) alone only slightly decreased EB extravasation ($P < 0.05$, compared to the I/R group), while a low dosage of SF (100 mg/kg) had no effect. Interestingly, SF (100 mg/kg) could significantly ameliorate the impairment of BBB permeability induced by I/R when combined with borneol ($P < 0.05$, compared to the I/R group).

Malondialdehyde level and superoxide dismutase activity in the hippocampus and cortex

Four days after transient cerebral ischaemia insult, the MDA level and SOD activity were measured. In the hippocampus, there was a significant increase (~71%) in MDA level and a significant decrease (~17%) in SOD activity compared to that of the sham-operated group ($P < 0.05$), while in the cortex MDA was increased by 36% and SOD decreased by 32%. SF (100 mg/kg) combined with borneol significantly reversed the brain MDA level (Table 3) and increased SOD activity (Table 3) in the brain of I/R mice ($P < 0.05$). Although SF (400 mg/kg) alone also increased the SOD activity in IR mice, it did not decrease the MDA level (Table 3).

GFAP expression

As shown in Figure 3, in the sham-operated group, only a few GFAP-positive cells were detected in the hippocampus. Following I/R insult, the number of GFAP-positive cells with dark-brown staining increased. The GFAP-positive cells were shaped irregularly, with several middle-length dendrites diffused in the hippocampus. In the mice treated with borneol or SF alone, GFAP expression was slightly decreased. However, in the group treated with SF plus borneol, the GFAP-positive cells were obviously fewer than those in the I/R group, which suggests that the combination treatment has a potential inhibitory effect against the activation of glia and thus protects neurons against transient global cerebral ischaemia.

Discussion

Despite recent advances in research on the mechanisms contributing to I/R-induced neuropathology and continuous findings of novel neuroprotective agents, the therapeutic record in clinic settings is still disappointing. Since the brain is one of the least accessible organs for the delivery of active pharmacological compounds, it is not surprising that many neuroprotective candidates fail to produce satisfying outcomes *in vivo*, although they may be quite effective *in vitro*.[28] Poor oral bioavailability or low metabolic stability can also compromise their efficacy in patients.[4,5] Recently, a hypothesis has been put forward, and repeatedly confirmed in animal stroke models, that combinations of neuroprotective agents may exert additive or multiplicative beneficial effects.[29–31] The present study for the first time demonstrates that the oral combination of SF and borneol exerts enhanced protection against brain injury following transient global brain ischaemia in C57 BL/6 mice through

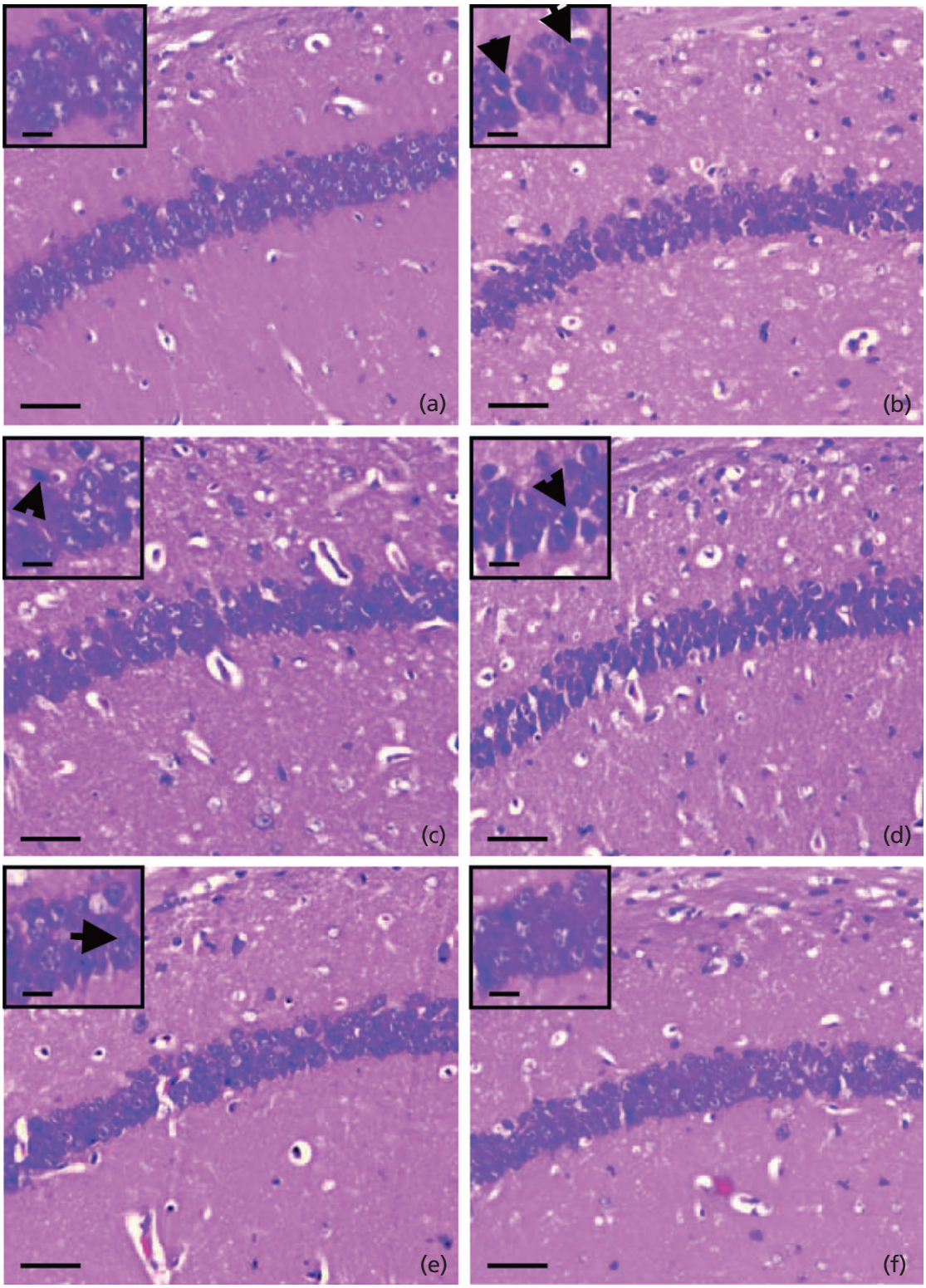

Figure 2 Effects of combination treatment with sodium ferulate and borneol on morphologic changes in brain induced by ischaemia/reperfusion. C57 BL/6J mice were submitted to bilateral common carotid arteries ligation for 25 min. At 30 min before surgery and 30 min after reperfusion, mice were treated by oral administration with sodium ferulate (SF) or borneol (Bor, 10 mg/kg). Following a water maze test, the hippocampal sections were stained with haematoxylin and eosin. Representative photomicrographs from six mice in each group are shown: (a), sham-operated; (b), ischaemia/reperfusion (I/R) model; (c), I/R + SF (100 mg/kg); (d), I/R + SF (400 mg/kg); (e), I/R + Bor (10 mg/kg); (f), I/R + SF (100 mg/kg) + Bor (10 mg/kg). The insets highlight the morphologic changes. Scale bars: 100 μm and 25 μm (inset).

Table 2 Effect of combination treatment with sodium ferulate and borneol on brain oedema and blood–brain barrier integrity in mice after ischaemia/reperfusion

Group	Water content (%)	EB (μg/g brain)
Sham	78.98 ± 0.13	2.00 ± 0.64
I/R	80.43 ± 0.40*	8.84 ± 1.79*
SF 100 mg/kg	79.49 ± 0.42	7.71 ± 1.00
SF 400 mg/kg	79.53 ± 0.53	4.84 ± 0.35#
Bor	79.33 ± 0.18#	4.57 ± 0.88#
SF 100 mg/kg + Bor	79.23 ± 0.20#	3.96 ± 0.85#

Effect of combination treatment with sodium ferulate (SF) and borneol (Bor, 10 mg/kg) on brain oedema and blood–brain barrier integrity in mice after ischaemia/reperfusion (I/R) EB, Evans Blue. Data are presented as mean ± SEM, $^{*}P < 0.05$ vs sham-operated group (Sham), $^{\#}P < 0.05$ vs I/R group, $n = 6$.

amelioration of brain oedema, recovery of BBB permeability, reduction of GFAP-positive cells, increase of SOD activity and reduction in MDA levels.

The treatment of ischaemia-related cerebral diseases with SF alone is compromised by its poor oral bioavailability and an insufficient accumulation in the brain.[12–14] Previously, we reported that borneol can increase the concentration of SF in plasma as well as in parts of the brain, and that increasing the dose of SF alone did not increase the concentration of SF in the brain.[21] In mice, the plasma half-life of SF was also prolonged when SF was administered in combination with borneol.

Borneol is a promising promoter for oral delivery of brain-targeting drugs.[16] Researchers have proved that borneol can accelerate the absorption of gastrodin in the gastrointestinal tract and promote its distribution to mouse brains.[32] Borneol can also increase the oral absorption of tetramethylpyrazine phosphate (TMPP) and the concentration of TMPP in brain tissue, without changing the behaviour *in vivo* of TMPP.[33] In this study, we investigated whether or not borneol could promote the neuroprotective effect of SF in the mouse 2VO model.

In the 2VO model, bilateral carotid occlusion causes a severe reduction in blood flow to the forebrain while the hindbrain remains well perfused by delivery of blood from the vertebrobasilar system.[34,35] Although the severity of damage in 2VO is typically mild, producing an average of 40% dead CA1 neurons with minimal animal mortality,[36] the model is reliable in providing sufficient hippocampal injury to allow potential manipulation of genotype to either improve or worsen damage in CA1.[37] With its long-lasting cognitive deficits accompanied by progressive neuronal damage, this model provides an opportunity to routinely allow factors such as delayed neuronal necrosis to be accounted for when estimating the final histological outcomes. In the present study, we observed that in the 2VO model group the mice exhibited learning and memory impairment (see Table 1), which is consistent with previous reports.[38] Our experiments proved that the spatial learning and memory performance of mice submitted to I/R in the MWM test was not improved by a low dosage of SF alone (see Table 1). However, when used in combination with borneol, SF can significantly ameliorate these cognitive impairments (see Table 1). In contrast, single administration of borneol has only a slight effect on spatial learning but none on memory performance (see Table 1). These results indicate that the combination treatment may produce enhanced beneficial effects on memory-related task performance.

Mounting evidence has suggested that I/R can cause neuronal injury in the hippocampal and dentate gyrus regions, which result in progressive cognitive impairment.[39,40] Thus the combination therapy with SF and borneol may be used at many ischaemic statuses that are involved in the development of mild cognitive impairment.

Accumulated experimental and clinical evidence indicates that BBB dysfunctions are associated with a number of serious CNS diseases, including stroke, because the BBB plays an important role in the homeostatic regulation of the brain microenvironment. Preserving the integrity of the BBB is a strategy to prevent brain ischaemia.[41] The present study shows that, in mice, transient cerebral ischaemia markedly increased BBB permeability, as indicated by EB extravasation, along with significant brain oedema. The combination treatment with borneol and SF ameliorated the reduction of BBB permeability induced by I/R and reduced the brain's water content (see Table 2).

It is well known that the BBB can be disrupted by a variety of pathological conditions, including ischaemic stroke, traumatic brain injury and Alzheimer's disease.[42] While the aetiology of the damage varies between these pathologies, increased permeability uniformly results in brain oedema and

Table 3 Effect of combination treatment with sodium ferulate and borneol on malonaldialdehyde levels and superoxide dismutase activity in mice brains after ischaemia/reperfusion

Groups	MDA level (nmol/mg protein)		SOD activity (U/mg protein)	
	Cortex	Hippocampus	Cortex	Hippocampus
Sham	148.42 ± 24.06	145.53 ± 36.74	323.50 ± 18.23	412.01 ± 22.18
I/R	201.97 ± 14.49*	248.60 ± 30.21*	221.29 ± 20.21*	344.04 ± 17.83*
SF 100 mg/kg	195.08 ± 37.99	212.13 ± 33.29	214.82 ± 18.24	311.17 ± 32.91
SF 400 mg/kg	200.07 ± 18.94	226.11 ± 24.70	284.87 ± 19.70#	394.52 ± 10.75#
Bor	202.86 ± 27.94	194.14 ± 17.13	276.71 ± 43.89	375.31 ± 30.11
SF 100 mg/kg + Bor	152.16 ± 15.66#	140.67 ± 18.37#	303.49 ± 22.68#	420.21 ± 19.21#

Effect of combination treatment with sodium ferulate (SF) and borneol (Bor, 10 mg/kg) on malonaldialdehyde (MDA) level and superoxide dismutase (SOD) activity in mice brains after ischemia/reperfusion (I/R). Data are presented as mean ± SEM, $^{*}P < 0.05$ vs sham-operated group (Sham), $^{\#}P < 0.05$ vs I/R group, $n = 6$.

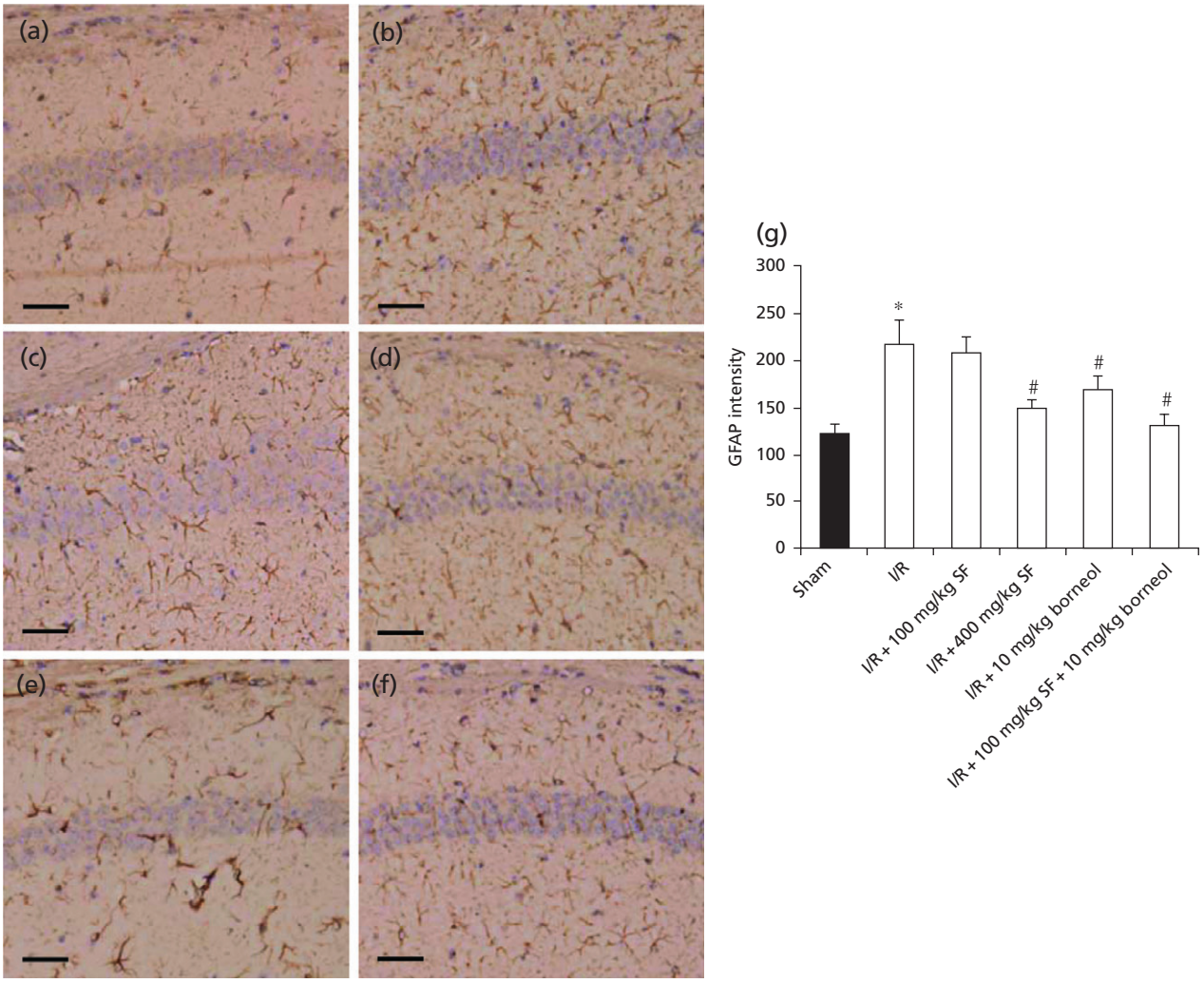

Figure 3 Effects of combination treatment with sodium ferulate and borneol on gliofibrillar acid protein expression in brain induced by ischaemia/reperfusion. C57 BL/6J mice were submitted to bilateral common carotid arteries ligation for 25 min. At 30 min before surgery and 30 min after reperfusion, mice were treated by oral administration with sodium ferulate (SF) or borneol (Bor, 10 mg/kg). Following the water maze test, the expression of gliofibrillar acid protein (GFAP) in the hippocampus was detected by immunohistochemistry. Representative photomicrographs from three mice in each group are shown: (a), sham-operated; (b), ischaemia/reperfusion (I/R) model; (c), I/R + SF (100 mg/kg); (d), I/R + SF (400 mg/kg); (e), I/R + Bor (10 mg/kg); (f), I/R + SF (100 mg/kg) + Bor (10 mg/kg). Scale bars: 100 μm. (g), the quantification of GFAP intensity in each group. $^{*}P < 0.05$, compared to sham group; $^{\#}P < 0.05$ compared to I/R group; $n = 3$.

may lead to secondary damage of the CNS.[43] Therefore, prevention of the disruption of the BBB by pharmacological intervention is expected to minimise tissue damage following pathological insults to the CNS.[44] It is particularly interesting that borneol can maintain BBB integrity but increase drug penetration into the brain. This 'selecting penetration' mechanism is still unclear. Recently, borneol has been reported to accelerate the opening of the BBB, but this was a physiological opening and did not damage brain tissue and the BBB.[20] Combining our data, we hypothesise that borneol can bidirectionly modulate BBB permeability or act as a medium to help small molecular drugs to penetrate the brain. In all, our findings suggested the potential application of SF plus borneol in CNS diseases such as brain ischaemia and Alzheimer's disease.

In this study, we also assayed MDA levels as a marker of lipid peroxidation, and SOD enzyme activity as a free radical scavenging agent in the cortex and hippocampus.[45] The results indicate that cerebral I/R leads to a significant increase in MDA levels and a significant decrease in SOD activities in both regions. Neither SF nor borneol treatment was found to significantly alter MDA levels and SOD enzyme activities in either the cortex or the hippocampus compared with the I/R model group. However, the combination SF and borneol treatment showed an obvious decrease in MDA levels, while there was a significant increase in SOD enzyme activity (see Table 3). These data suggest that the combination treatment potently inhibited the oxidative stress induced by I/R in the brains of mice. Although the mechanisms of brain I/R injury are still obscure, there is

some evidence that the cellular damage induced by cerebral ischaemia is due to oxidative damage caused by free radicals and lipid peroxidation.[46] In experimental studies of I/R injury, reperfusion increases the hazardous effect of early ischaemic injury by the release of reactive oxygen species (ROS).[47] ROS cause lipid peroxidation of cellular membranes, oxidation of protein and, finally, disruption of the structural integrity and functions of cells.[48] Although the present study indicates the neuroprotective effect of combination therapy was at least partially attributed to its antioxidative effects following I/R, further studies are still needed to ascertain the underlying mechanism.

A previous study has proven that FA administered intravenously at doses of 80 mg/kg and 100 mg/kg at the beginning of MCAO effectively reduces cerebral infarct areas and neurological deficits. Moreover, 100 mg/kg of FA administered 30 min after ischaemia still considerably prevents the progression of cerebral insult and improves neurological outcome.[10] In our study, SF and borneol were administered orally at 30 min before and after I/R respectively. Considering the need for patient adherence and the chronic progression of most cerebrovascular diseases, oral administration of drugs is more viable and convenient in a clinical setting.

Our results must be confirmed in other animal brain ischaemic models and the optimal therapeutic time window should also be determined in future studies. Besides these, a full dose–response experiment is still needed to determine the most effective dosing regimen of SF and borneol.

Conclusions

In this study we reported for the first time that SF in combination with borneol significantly enhances neuroprotective effects in brain I/R mice, which may in part be due to maintenance of the integrity of the BBB and a restoration of the redox system. Our findings suggest a potential application of SF combined with borneol for CNS diseases that is not only a potential therapy for neuroprotection, but also represents a new paradigm in multidrug development, which is thought to be a promising approach in the treatment of I/R-related cerebral diseases.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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