http://dx.doi.org/10.4314/ajtcam.v11i1.25

STUDY ON THE ANTI-CEREBRAL ISCHEMIA EFFECT OF BORNEOL AND ITS MECHANISM

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Abstract

Background: Borneol is the processed item from resin of *Dryobalanops aromatica Gaertn.* f. It can enhance the activity of antioxidant enzymes in brain tissue and reduce inflammatory response by improving the energy metabolism of ischemic brain regions, and thereby reduces brain tissue damage. The objective of this paper was to study the anti-cerebral ischemia effect of borneol and its mechanism.

Materials and Methods: The anti-cerebral ischemia effect of borneol was studied by ligation of bilateral common carotid arteries (CCA), and vagus nerves in mice and the acute cerebral ischemia-reperfusion experiment in rats.

Results: Compared with the blank and solvent control groups, the borneol low-; medium-; and high-dose groups can significantly prolong the gasping time of mice after decapitation, and extend the survival time of mice after ligation of bilateral CCA, and vagus nerves.

Conclusion: Compared with the Xueshuantong injection group, the prolongation of survival time of mice after ligation of bilateral CCA, and vagus nerves was more apparent in the high-dose borneol experimental group; each experimental group can significantly reduce the number of leukocyte infiltration, the number of ICAM-1-positive vessels, as well as the number of TNF-α-positive cells.

Conclusion: Borneol has an anti-cerebral ischemia effect.

Key words: borneol; cerebral ischemia-reperfusion; IL-1β, TNF-α; ICAM-1

Introduction

Borneol is the processed item from resin of *Dryobalanops aromatica Gaertn*. f. of family Dipterocarpaceae, or the crystalline substance extracted from leaves of *Blumea balsamifera* (L.), DC, of family Compositae, or the chemically synthesized product of camphor and turpentine (Wang et al., 1994). Borneol is acrid in taste, bitter and cool in nature It enters the heart, spleen and lung meridians; has the effects of inducing resuscitation, dissipating fire stagnation, improving eyesight, and relieving swelling and pain. The main constituent of Dryobalanops aromatica borneol is d-borneol, the main constituent of Blumea balsamifera borneol is L-borneol, while the synthetic borneol contains large amounts of isoborneol. Borneol can enhance the activity of antioxidant enzymes in brain tissues and reduce inflammatory response by improving the energy metabolism of ischemic brain regions, and thereby reduces brain tissue damage (He et al., 2006; Zha et al., 2006). Many anti-cerebral ischemia compounded Chinese medicinal prescriptions contain borneol (Liu et al., 2009; Wang et al., 2011), the research of anti-cerebral ischemia effect of borneol and its mechanism will broaden the application range of borneol, improve the use value of borneol, and lay the foundation for the development and industrialization of novel borneol drugs.

Materials and methods

Drugs, reagents and animals

Kunming mice, half male and half female, weighing between 18~22g, WiSTAR rats, male, weighing between 200~300g, were purchased from the Laboratory Animal Center of China Pharmaceutical University; TTC; Xueshuantong injection. This research was approved by the Shandong Animal Research Ethics Committee.

Gasping after decapitation experiment in mice

60, Kunming mice were randomly divided into six groups. Mice in the experimental groups were injected with 1.0, 2.0, 3.0mg·kg-1, borneol from tail vein respectively; mice in the positive control group were intravenously injected with 50mg·kg-1, Xueshuantong injection; and the mice in the blank group were intravenously injected with equivalent amount of sodium chloride injection. 30min., after administration, mice

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were decapitated quickly from bilateral post auricular neck areas, and the maintenance time of gasping after decapitation in mice was recorded immediately.

Bilateral CCA and vagus nerve ligation experiment in mice

Mouse grouping and administration were performed as in 1.2.1. 30min., after administration, the mice were lightly anesthetized with ether, bilateral CCA and vagus nerves were separated, and ligated simultaneously with surgical sutures, the survival time of mice was recorded immediately.

Acute cerebral ischemia-reperfusion experiment in rats (Longa et al., 1989)

Model establishment

After anesthesia, the right common carotid artery, internal carotid artery and external carotid artery of rats were separated; pterygopalatine artery was separated downward along the internal carotid artery, but without ligation. The proximal end of right common carotid artery and the distal end of external carotid artery were ligated, suture line was prepared in the proximal end of internal carotid artery, and arterial clamp was placed in the distal end; the right common carotid artery was incised, fishing line was inserted, and the arterial clamp was released, the fishing line was inserted about 20mm., to the internal carotid artery, until some resistance was felt, suture line was tightened, and the skin was sutured. Rats in the normal group did not go through any surgical treatment. 22hrs, after reperfusion, behavioral scoring was performed on survived rats in each group. Scoring standards: 0 point: no neurological deficit symptoms; 1 point: minor neurological deficits, cannot fully extend the right forepaw; 2, points: moderate focal neurological deficits, circling to the right; 3, points: severe focal neurological deficits, falling to the right; 4, points: cannot walk spontaneously, decreased level of consciousness. Upon completion of ischemia reperfusion, rats in each group were excessively anesthetized, and decapitated, right cerebral hemispheres were collected, medulla oblongata, cerebellum and lower brainstem were removed, followed by fixation, coronal sections were made in the MCA, perforating territory (frontal lobe, caudate putamen and hippocampus) in the right cerebral hemisphere. 4, sections were taken within the range of 1, mm, of which 1 section was routine H-E stained, and the remaining 3, sections were stained by S-P immunohistochemical method, the IL-1β, TNF-α and ICAM-1 were detected, respectively.

Results

Effect on gasping time of mice after decapitation

The results showed that compared with the blank and solvent control groups, the borneol low-; medium-; and high-dose groups, can significantly prolong the gasping time of mice after decapitation, as shown in Table 1.

Tab. 1: Effect of borneol on gasping time of mice after decapitation ($\bar{x}\pm s$, n=10)

Group	Dose ($mg \cdot kg^{-1}$)	Gasping time after decapitation (s)
Blank group	-	19.2±2.4
Borneol group	3.0	23.4±1.7**
Borneol group	2.0	21.9±2.5*
Borneol group	1.0	22.9±1.9*
Xueshuantong injection group	60.0	23.1±2.7**

* Comparison with the blank group P<0.05, ** Comparison with the blank group P<0.01

Effect on survival time of mice after ligation of bilateral CCA and vagus nerves

The experimental results are shown in Tab. 2. Compared with the blank control group, each experimental group can significantly prolong the survival time of mice after ligation of bilateral CCA, and vagus nerves; the prolongation of survival time of mice after ligation of bilateral CCA, and vagus nerves was more apparent in the high-dose borneol experimental group compared with the Xueshuantong injection group.

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As can be seen from Fig. 2, compared with the model control group, borneol experimental groups (1.0, 2.0, 3.0 mg·kg^{-1}), can significantly reduce the number of leukocyte infiltration, as well as the number of ICAM-1-positive vessels and the number of TNF-α positive cells. A trend of decrease in IL-1β expression was noted in the borneol experimental groups (1.0, 2.0, 3.0 mg·kg^{-1}), which was, however, not statistically significant (p>0.05).

Table 2: Effect on survival time of mice after ligation of bilateral CCA and vagus nerves ($\bar{x}\pm s$, n=10)

Group	Dose (mg·kg^{-1})	Survival time (s)
Blank group	-	152.5±42.8
Borneol group	3.0	225.8±68.1**
Borneol group	2.0	191.7±38.7*
Borneol group	1.0	180.5±61.2*
Xueshuantong injection group	60.0	204.1±49.6**

* Comparison with the blank group P<0.05, ** Comparison with the blank group P<0.01

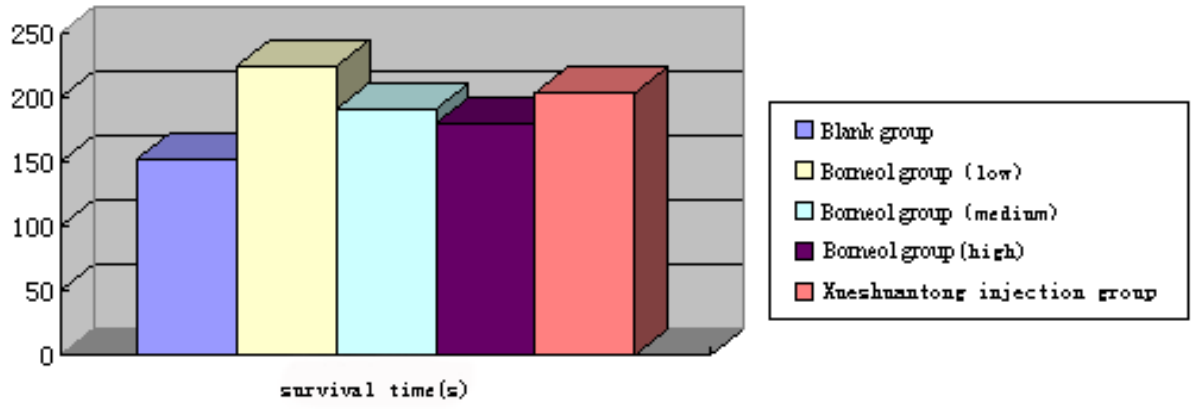

Figure 1: Comparison of survival time of mice after ligation of bilateral CCA and vagus nerves among each experimental group

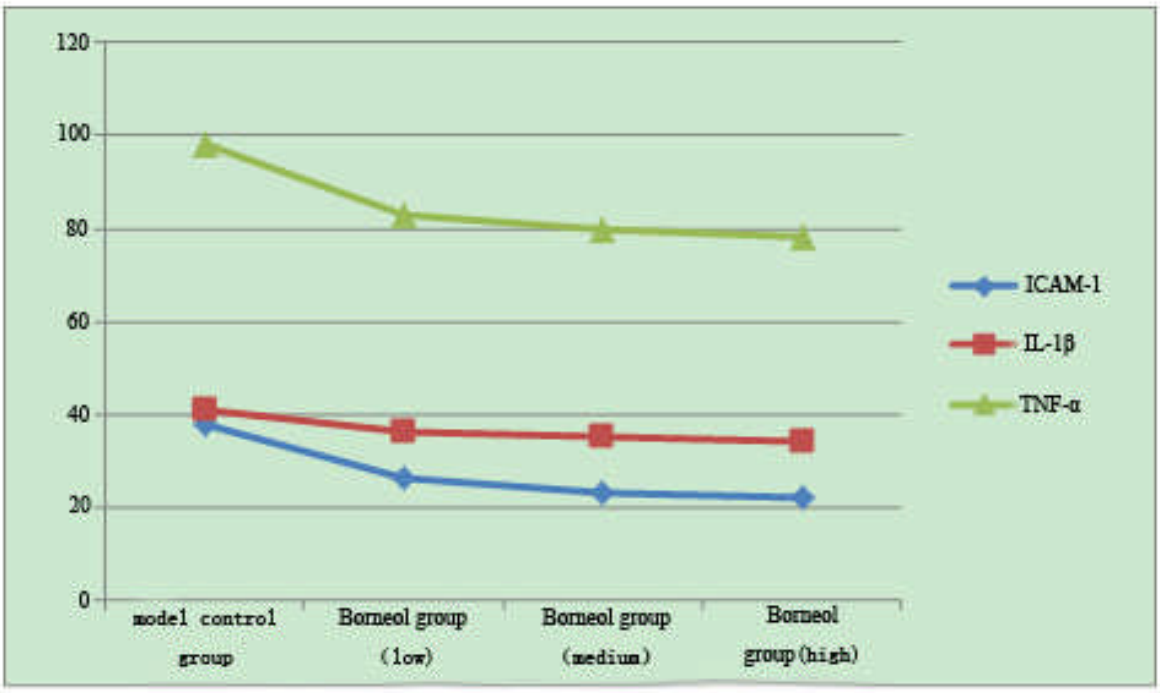

Figure 2: Effect on intracerebral inflammatory response after focal ischemia reperfusion in rats in each experimental group

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Discussion

Ischemic cerebrovascular disease is a central nervous system disease characterized by decrease in cerebral blood flow; cerebro-vascular diseases have high disability rate, high morbidity, high mortality and high recurrence rate, which not only can substantially reduce the quality of life of patients, but also can increase the economic burden of patients.Blood-brain barrier is a barrier system existing between blood and brain tissue, which consists of a three-layer structure: cerebral micro-vascular endothelial cells, basilar membranes and foot processes of stellate cells, its structural basis is cerebral micro-vascular endothelial cells (Sun et al., 2003). The destruction of blood-brain barrier is an important basis of cerebral ischemia-reperfusion injury, the inflammatory response after cerebral ischemia-reperfusion causes serious damage to the blood-brain barrier (Chen et al., 2004; Jieli et al., 2001). Leukocyte and vascular endothelial cell adhesion is a necessary condition and an important indicator of the inflammatory response after cerebral ischemia, the adhesion of leukocytes and endothelial cells is achieved through the action of adhesion molecules. In the early phase of ischemia-reperfusion, up-regulation of pro-inflammatory cytokines leads to neutrophil adsorption on the vessel walls, followed by monocyte and macrophage infiltration, thereby damaging the cerebral vascular endothelial cells, and inducing vasogenic cerebral edema and hemorrhage (Liu et al., 1994).

Metabolic acidosis, intracellular calcium overload, increased free radical production, inflammation and energy metabolic disturbance are the pathogenesis of cerebral ischemic injury, theoretically, blocking of any of the above-mentioned pathophysiological changes involved in the secondary injury of neurocytes after cerebral ischemia can all protect neurons against more serious damage, increase cell viability, and promote nerve recovery. The results of this study showed that borneol can prolong the gasping time of mice after decapitation, survival time of mice after acute cerebral ischemia induced by bilateral CCA, and vagus nerve ligation, and significantly reduce the number of leukocyte infiltration, the number ICAM-1-positive vessels, as well as the number of TNF-α-positive cells.

At present, there is lack of effective management means for the diagnosis, and treatment of cerebrovascular diseases in modern medicine. Various cerebral protective agents have single action, with huge side effects; these side effects includes, hemorrhage and reperfusion injury, whose clinical efficacy is not satisfactory. Borneol and other resuscitation-inducing aromatic Chinese medicines are widely used in the nervous system diseases, with good efficacy (Liu et al., 2007), our experiment demonstrated that they can reduce the injury of blood brain barrier endothelial cells after ischemia-reperfusion, and have certain cerebral protective effect.

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