Cardiovascular Pharmacology

Astragaloside IV attenuates cerebral ischemia–reperfusion-induced increase in permeability of the blood-brain barrier in rats

You Zhi Qua,1, Min Lia,1, Yan Ling Zhaob, Zhen Wei Za, Xiao Yan Weic, Jin Ping Liuc, Guo Dong Gaob

1 Department of Neurosurgery, Tangdu Hospital and Institute for Functional Brain Disorders, PR China
b Department of Traditional Chinese Medicine of Xijing Hospital, PR China
c Institute of Neurosciences, The Fourth Military Medical University, Xi’an 710032, PR China

ARTICLE INFO

Article history:
Received 18 August 2008
Accepted 9 January 2009
Available online 25 January 2009

Keywords:
Cerebral ischemia and reperfusion
Blood-brain barrier
Tight junction
Occludin
ZO-1
Astragaloside IV

ABSTRACT

Astragalus membranaceus is widely used to treat stroke and chronic debilitating diseases in China, but the mechanism has not been fully demonstrated to date. In the present study, we, using astragaloside IV, a purified extract from astragalus membranaceus, to a focal cerebral ischemia/reperfusion rat model, aimed to investigate the effect of astragaloside IV on the permeability of the blood-brain barrier since disruption of blood-brain barrier induced by ischemia/reperfusion leads to serious brain injuries. We found that astragaloside IV (10, 20 mg/kg) significantly attenuated the permeability of blood-brain barrier in comparison with vehicle group after ischemia/reperfusion assessed via Evans blue leakage (P < 0.05). This was further confirmed by examination of blood-brain barrier permeability under the electron microscope, using lanthanum as a tracer of blood vessel permeability. Lanthanum was usually found within the blood vessel in sham group, rather than in perivascular tissues as shown in vehicle group. In drug groups, lanthanum stain was mainly restricted within the cerebral capillary, indicating the potential protective effect of astragaloside IV on the integrity of blood-brain barrier in ischemia/reperfusion rats. Furthermore, we found that expression of occludin and zona occludens-1 (ZO-1), the tight junction proteins, was decreased in endothelial cells in vehicle group, which, however, could be reversed by astragaloside IV administration. We propose that regulation of tight junctional proteins in the endothelial cells may be one mechanism astragaloside IV-mediated in attribution to blood-brain barrier protection in the ischemia/reperfusion rats.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Cerebral ischemia provokes an irreversible neurodegenerative disorder that may lead clinically to progressive dementia and global cognitive deterioration. Increased vascular permeability and disruption of the blood-brain barrier could be the initiating factors for the development of cerebral infarctions (Date et al., 2006). It is well known that blood-brain barrier is composed of a continuous layer of brain capillary endothelial cells together with pericytes, a basal lamina, and astrocyte. Tight junctions between endothelial cells form a metabolic and physical barrier restricting the movement of macro-molecules between the blood and brain to maintain cerebral homostasis (Kago et al., 2006). Therefore, protection of blood-brain barrier in brain tissues may be potentially beneficial for neuronal recovery from ischemic/reperfusion injury.

Astragalus membranaceus, has been routinely used in China for patients with stroke or chronic debilitating diseases, and even for normal subjects who wish to further improve their vital functions (Luo et al., 2004). A pilot clinical investigation suggested that astragalus membranaceus is safe and may be beneficial for the treatment of acute cerebral infarction (Cai et al., 1994). Astragaloside IV, 3-0-beta-D-xylopyranosyl-6-0-beta-D-glucopyranos-ylcycloastragenol (Fig. 1), a purified small molecular saponin (MW 784), is one of the major and active components of the astragalus membranaceus. Pharmacological studies have demonstrated that astragaloside IV expresses a series of protective effects, such as anti-hypertension(Zhang et al., 2006), positive inotropic action(Li and Cao, 2002), anti-inflammation(Zhang et al., 2003b), antinociception(Yang et al., 2001), anti-infarction(Luo et al., 2004), and anti-viral activity (Lu et al., 1999). Although astragaloside IV can exert multipotent activities under pathophysiological conditions, the effects of astragaloside IV on the ischemia–reperfusion-induced disruption of the blood-brain barrier have not yet been clarified. The present study was undertaken to investigate whether there exist a potential protective effect of astragaloside IV on the permeability of the blood-brain barrier, via using astragaloside IV to an ischemia/reperfusion murine model.
2. Materials and methods

2.1. Animals and surgical preparation

All experiments were carried out on adult male Sprague–Dawley rats (250–300 g) obtained from the Animal Center in the Fourth Military Medical University. Protocols were approved by the Northwest China Committee of Experimental Animal Care, and their regulations were in accordance with NIH guidelines. All efforts were made to minimize animal suffering and the number of animals used. Transient focal cerebral ischemia (1.5 h duration) was induced as previously described (Chang et al., 2007). In brief, the rats were anesthetized by intraperitoneal injection of chloral hydrate (dissolved in distilled water) at a dose of 400 mg/kg. Rectal temperature was maintained at 37 ± 0.5 °C throughout the surgical procedure and up to 2 h after reperfusion. A midline neck incision was made. The right common carotid artery, external carotid artery, and internal carotid artery were exposed under an operative microscope. A 5.0 monofilament nylon thread, with its tip rounded by heating briefly near a flame, was inserted into the external carotid artery and then advanced into the lumen of the internal carotid artery to occlude the origin of the middle cerebral artery. The nylon filament remained in the lumen for 1.5 h, and was then withdrawn to allow the initiation of reperfusion. The animals were returned to cages after awakening from anesthesia. The sham-operated rats were treated identically, except that the middle cerebral arteries were not occluded after the neck incision. The animals that could not normally intake food and water in the second day after surgery or that developed seizures after surgery were excluded from the protocol.

2.2. Drug preparation and treatment schedule

The astragaloside IV used in this study was of high purity (99%) as determined by HPLC analysis, which was provided from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Animals were randomly separated into 4 groups: sham group, vehicle group, and drug group (astragaloside IV, 10 mg/kg), drug group (astragaloside IV, 20 mg/kg). Astragaloside IV was dissolved in dehydrated alcohol, and was diluted with physiological saline to the concentration of 1.0 mg/ml as a store solution, containing 5% alcohol (5:95, v/v). The store solution was diluted with physiological saline into astragaloside IV solutions according to the group respectively. Animals were randomly separated into 4 groups: sham group, vehicle group, and drug group (astragaloside IV, 10 mg/kg, i.v.) and transcardially perfused with 0.9% saline via a catheter through the artery, followed by perfusion with the fixative with the fixative with 2% glutaraldehyde and 2% lanthanum nitrate in 0.1 M sodium cacodylate (pH 7.4–7.5) at room temperature, as previously described (Czarnowska and Karwatowska-Prokopczuk, 1995; Wang et al., 2007). At the end of brain perfusion, brains were excised and brain tissues encompassing ischemic infarction were chosen as samples. The samples were isolated and divided into about 1 mm³ pieces. Then, they were postfixed with 0.5% osmium tetroxide in 0.1 M sodium cacodylate (pH 7.4–7.5) at room temperature, as previously described. After polymerization, three blocks were randomly selected from each brain sample. Ultrathin sections were cut with an Ultratome (Nova, IKB, Bromma, Sweden), and mounted on mesh grids (six to eight sections/grid). After counterstained with uranyl acetate and lead citrate, sections were observed under the JEM-100SX electron microscope (JEOL Ltd., Tokyo, Japan).

2.5. Immunohistochemistry

Six animals from each group were used for immunohistochemistry assay for occludin and zonae occludens-1 (ZO-1). Animals were each perfused transcardially with 150 ml warm 0.9% saline followed by 500 ml ice cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed and postfixed in the same fixative for 2 h at 4 °C. They were then cryoprotected in 30% sucrose in 0.1 M phosphate buffer overnight at 4 °C. Alternate sets of serial coronal sections of brain were cut at 14μm thickness on a cryostat (CM1900; Leica, Heidelberg, Germany) and mounted on gelatin-coated slides for occludin and ZO-1 immunohistochemistry, respectively. Slides were blocked in PBS (pH 7.4) for 3 h, containing 5% bovine serum albumin (BSA), 5% normal goat serum (NGS), and 1% Triton X-100. Then, slides were incubated overnight with monoclonal mouse anti-occludin or polyclonal rabbit ZO-1 antibodies (Zymed, San Francisco, CA, USA) at room temperature. After rinsing in PBS, slides were incubated with fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG or goat anti rabbit IgG, respectively (Molecular Probes, Eugene, Oregon). Fluorescence was detected under an Olympus fluorescence microscope (BX-51, Olympus, Tokyo, Japan) equipped with a mercury arc lamp. The number of occludin- or ZO-1-positive vessels in the penumbra region was counted in five sections per animal and the mean of occludin- or ZO-1-positive vessels per 6 animals in each group was calculated. The microscopic observations were carried out.
2.6. Statistical analysis

The results are expressed as mean±standard deviation (S.D.) for six animals in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using the SPSS software package for Windows. Post hoc testing was performed for inter-group comparisons using the Bonferroni’s test; significance at P-values <0.05 has been given respective symbols in the figures.

3. Result

3.1. Effect of astragaloside IV on blood-brain barrier integrity after ischemia/reperfusion

To measure the effect of astragaloside IV on blood-brain barrier integrity after focal cerebral ischemia, we detected tissue contents of Evans blue dye. The contents of Evans blue dye were markedly increased in vehicle group in comparison with sham group (P<0.01). In animals treated with astragaloside IV, the increase in Evans blue content induced by ischemia/reperfusion was significantly attenuated in a dose dependent manner (Fig. 2).

3.2. Electron microscopic examination of effect of astragaloside IV on blood-brain barrier integrity

Lanthanum nitrate has been proven lacking the ability to penetrate the blood-brain barrier and thus widely used as a marker to examine the integrity of blood-brain barrier by transmission electronic microscope (Bradbury and Deane, 1993; Sundstrom et al., 1985). Under the electron microscope, cerebrovascular vessel in sham animals exhibited capillary integrity with normal endothelial cell and basal lamina. Lanthanum stain was exclusively located in the lumen of cerebral capillary (Fig. 3A1, A2). In vehicle group, the endothelial cell and its nucleus was swollen and deformed, and the lumen of capillary was collapsed (Fig. 3B1). The integrity of blood-brain barrier was destroyed, presenting perivascular edema, vacuolation, and membrane damage, which resulted in the leakage of capillary lanthanum stain to the perivascular parenchyma (Fig. 3B2). In drug groups, the structure of endothelial cell was preserved and lanthanum stain was mainly restricted within the cerebral capillary (Fig. 3C1, C2, D1, D2).

3.3. Immunohistochemical analysis of tight junctions-associated accessory proteins occludin and ZO-1

In the penumbra region, the number of tight junctional protein-positive vessels was examined. The number of occludin- and ZO-1-positive vessels was decreased in the vehicle group, forming a
significant difference with that in sham group (Fig. 4A2, B2) \( (P < 0.01) \). Astragaloside IV treatment markedly up-regulated the expression of occludin- and ZO-1 in contrast to the vehicle group (Fig. 4A3, B3, A4, B4) \( (P < 0.05) \), though the up-regulation of the proteins couldn’t attain the control level, as showed in sham group (Fig. 4).

4. Discussion

Stroke is the most common cause of death in China. The proportion of cerebral infarction varied from 45.5% to 75.9% from population to population (Zhang et al., 2003a). Precious studies have demonstrated that astragaloside IV could offer neuroprotective effect against the formation of cerebral infarction by its antioxidant properties (Cai et al., 1994; Luo et al., 2004).

Recent research has implicated blood-brain barrier integrity as a leading factor in the clinical outcome of stroke (Hom et al., 2007). Middle cerebral artery occlusion is able to cause disruption of the blood-brain barrier (Gao et al., 2008; Chi et al., 2008), leading to the development of brain injuries after ischemia/reperfusion. Indeed, it has been proposed that the majority of stroke-induced brain injuries are related to disruption of the blood-brain barrier, resulting in the secondary damage to neurons (Asahi et al., 2001). Therefore, it has become an important objective to maintain the integrity of the blood-brain barrier to protect brain from the secondary injuries.

We found that astragaloside IV maintained the integrity of blood-brain barrier shown by reducing the contents of Evans blue dye in the brain parenchyma after ischemia/reperfusion. Under the electron microscope, astragaloside IV markedly prevented endothelial cells death and attenuated the leakage of lanthanum. The present study provided the first evidence of astragaloside IV in protection of the blood-brain barrier against ischemia–reperfusion-induced increase in the blood-brain barrier permeability.

Tight junctions are vital to the structure and function of blood-brain barrier. The tight junctional barrier of blood-brain barrier is formed by the interaction of membrane-associated accessory proteins, including occludin and claudin, with the plasma membranes of adjacent cells. ZO-1, zonae occludens-3 (ZO-3), and cingulin, as cytoplasmic tight junctional accessory proteins, provide the linkage of tight junctions with the actin cytoskeleton (Matter and Balda, 2003). It has been reported that the expression of occludin and ZO-1 in the endothelial cells were decreased at the early period of embolism, and the decrease in occludin- or ZO-1-positive vessels was correlated with the increase in blood vessel permeability (Date et al., 2006). On the contrary, hepatocyte growth factor has been found to attenuate...
the increase in permeability of blood vessel induced by cerebral ischemia through preserving expression of occludin and ZO-1 proteins (Date et al., 2006). Additionally, recombinant human erythropoietin has also been found to reduce the Evans blue leakage induced by focal ischemia by maintaining the expression of occludin (Li et al., 2007). In the present study, the finding of the higher expression of occludin and ZO-1 in the endothelial cells in drug groups than that in vehicle group may imply the potential mechanism that astragaloside IV protects the blood-brain barrier against ischemia-reperfusion-induced disruption by up-regulating the expression of tight junctional proteins.

Previous in vivo and in vitro studies have found that astragaloside IV exerted its anti-inflammatory effects by decreasing the expression of adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 and inhibiting the production and release of the inflammatory mediators such as interleukin-1 and tumor necrosis factor-alpha (Zhang et al., 2003; Wang et al., 2002). Furthermore, inflammation occurs with arterial occlusion, leading to compromise of blood-brain barrier and further cerebral infarction (Huang et al., 2006). Therefore, astragaloside IV may offer significant protection against the dysfunction of blood-brain barrier via regulating inflammatory cytokines on the endothelial cells. Nevertheless, future studies are needed to further address this hypothesis.

In conclusion, we demonstrated for the first time that astragaloside IV has the ability to maintain the integrity of the blood-brain barrier after ischemia/reperfusion mediated by up-regulating the expression of tight junctional proteins in the endothelial cells.

Acknowledgements

We wish to thank Professor Ying-ying Liu (Institute of Neurosciences, the Fourth Military Medical University) for her help in electron microscopy and the language of the article. We also thank Master Tao Ke (Department of Epidemiology, the Fourth Military Medical University) for his help in statistical analysis. This work was supported by grants of National Scientific Foundation of China (No.C03050201).

References


