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Original article

Effect of salvianolic acid B on TNF-α induced cerebral microcirculatory changes in a micro-invasive mouse model

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ABSTRACT

Purpose: To investigate the effects of salvianolic acid B (SAB) on tumor necrosis factor α (TNF-α) induced alterations of cerebral microcirculation with a bone-abrading model.
Methods: The influences of craniotomy model and bone-abrading model on cerebral microcirculation were compared. The bone-abrading method was used to detect the effects of intracerebroventricular application of 40 μg/kg·bw TNF-α on cerebral venular leakage of fluorescein isothiocyanate (FITC)-albumin and the rolling and adhesion of leukocytes on venules with fluorescence tracer rhodamine 6G. The therapeutical effects of SAB on TNF-α induced microcirculatory alteration were observed, with continuous intravenous injection of 5 mg/kg·h SAB starting at 20 min before or 20 min after TNF-α administration, respectively. The expressions of CD11b/CD18 and CD62L in leukocytes were measured with flow cytometry. Immunohistochemical staining was also used to detect E-selectin and ICAM-1 expression in endothelial cells.
Results: Compared with craniotomy method, the bone-abrading method preserved a higher erythrocyte velocity in cerebral venules and more opening capillaries. TNF-α intervention only caused responses of vascular hyperpermeability and leukocyte rolling on venular walls, without leukocyte adhesion and other hemodynamic changes. Pre- or post-SAB treatment attenuated those responses and suppressed the enhanced expressions of CD11b/CD18 and CD62L in leukocytes and E-selectin and ICAM-1 in endothelial cells induced by TNF-α.
Conclusions: The pre- and post-applications of SAB during TNF-α stimulation could suppress adhesive molecular expression and subsequently attenuate the increase of cerebral vascular permeability and leukocyte rolling.

Introduction

For the observation of brain microcirculation, craniotomy is often used by stripping off the parietal bone to expose microvessels on pia mater. In order to protect the local microcirculation from the impact of the changed physical conditions, coverslip and resin glue are used to close the local observation window, catheters and infusion pumps to produce an artificial thermostatic cerebral fluid circulation.[1,2] However, the unavoidable change of intracranial pressure after craniotomy disturbs the hemodynamic function of cerebral microcirculation, even under so-called normal or quiescent condition, possibly leading to the misinterpretation of microcirculatory functional alteration. The primary purpose of this study was to establish a microinvasion model to preserve an intact cerebral microcirculation.

As a typical pro-inflammatory mediator, tumor necrosis factor α (TNF-α) can trigger a serious inflammatory response, including the activation of leukocytes and endothelial cells, adhesion of leukocytes on endothelium and the increase of vascular permeability, etc.[1] It is also suggested that TNF-α induced inflammation can play a crucial role in secondary insult of traumatic brain injury.[3,4] In this

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study, the early-phase change of brain microcirculation induced by TNF-α was observed in a microinvasion model and compared with that in craniotomy.

Salvia miltiorrhiza (SM) has been popularly used for the treatment of a variety of cardiovascular disorders such as coronary heart disease, hyperlipidemia and cerebrovascular disease. The major water soluble components of SM are dihydroxylphenyl lactic acid and salvianolic acid B (SAB).[5–7] SAB is found to suppress neutrophil-endothelial adhesion and inhibit the expression of intercellular adhesion molecule 1 (ICAM-1) induced by TNF-α in human umbilical venous endothelial cells.[8] Using the microinvasive bone-abrading method, this study aimed to observe the effect of SAB on TNF-α-induced alteration of leukocyte rheology and permeability in cerebral venules. The expressions of adhesive molecules in leukocytes and endothelial cells were detected to explore the mechanisms of rheological and permeability changes in cerebral venules.

Methods

Reagents

SAB was purchased from the Feng-Shan-Jian Medical Company, Kunming, China. Fluorescein isothiocyanate (FITC)-labeled rat anti-mouse monoclonal antibodies against CD11b, CD18 and CD62L were purchased from BD Biosciences Pharmingen, USA. Mouse TNF-α, FITC-albumin and Rhodamine 6G was from Sigma–Aldrich, Inc., USA. Goat anti-mouse antibodies against E-selectin and ICAM-1 were purchased from Santa Cruz Biotechnology, Inc., USA. Biotin-free horseradish peroxidase (HRP)-labeled anti-goat secondary antibody and 3, 3′-diaminobenzidine tetrahydrochloride (DAB) substrate kit were from Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China. All other chemicals used were of the highest grade available commercially.

Animals

C57BL mice weighing 18–22 g were purchased from the Animal Center, Health Science Center, Peking University (Beijing, certificate No. SCXK 2006-0025). The mice were housed individually in cages at 22 °C and the humidity of 40% under a 12-h light/dark cycle with free access to tap water and pellet food. The animals were fasted for 12 h before experiment but allowed free access to water. All animals were handled according to the guidelines of Peking University Animal Research Committee.

At 20 min after being anesthetized with urethane (2.0 g/kg body weight, intraperitoneal route), the mice were tracheotomized and mechanically ventilated with small animal ventilator (ALC-V8, Alcott Biotech Co., Shanghai, China) in room air. A left side incision was made on the neck, and a custom-designed occluder (a small noose consisting of a fine polyethylene catheter in a plastic tube) was implanted in left lateral common carotid artery for the detection of mean artery pressure.[9] To evaluate the possible influence of systemic circulation on cerebral venular rheology and permeability, mean arterial pressure (MAP) was measured by left common carotid artery cannulation. The femoral vein was cannulated for intravenous infusion of drugs and intraperitoneal administration of FITC-albumin or rhodamine 6G.

The animal head was secured in a stereotactic frame and the skull was exposed through a midline incision. In the craniotomy model, a high-speed portable dental drill (Strong-90, Saeshin, Korea) was used to cut open a 3 mm × 3 mm cranial window in the left parietal bone and the dura mater was exposed and kept moist by dripping 37 °C normal saline. In the bone-abrading model, the dental drill was used to abrade the left parietal bone until the thickness of the bone was about 0.1 mm and there was no obvious bump on the surface. The target vessel could be clearly seen while the bone was not broken and the dura mater was not exposed. Saline at 37 °C was intermittently dripped to maintain the moisture of parietal bone surface and to avoid grinding-induced local high temperature.

Intracerebroventricular injection of TNF-α to C57BL mice

A small hole was drilled with a dental drill 2 mm from right side of the cranial raphe on a line drawn through the anterior base of the ears. A microinjector with 26-gauge needle was inserted 2 mm perpendicular to the skull and TNF-α was slowly injected at the dose of 40 μg/kg·bw (0.2 mg/ml, 4 μl for a 20 g mouse) in 30 s and then the skull pinhole was closed with bone wax. For ascertaining the areas in ventricular system of the brain where the drug was given, 1% (w/v) Evans Blue (4 μl per mice) was injected into the cerebroventricle, and the brain was taken out to observe the spread of Evan Blue under a stereomicroscope. At 20 min after injection, the whole brain surface was stained mildly. At 2 h after injection, the staining in right periventricle was reduced and the whole brain surface was further stained (images not shown). This indicated that intraventricular injection method used in the experiment could ensure the direct role of TNF-α on the pial surface.

Application of SAB before or after TNF-α intervention

The mice were randomly divided into 4 groups: control group with intravenous infusion of saline (6 ml/kg·h), beginning at 20 min before controlled intracerebro-ventricular injection of saline (200 μl/kg·bw) and lasting for 120 min without any TNF-α or SAB intervention, TNF-α treated group with 120 min continuing intravenous infusion of saline and intracerebroventricular injection of 40 μg/kg·bw TNF-α at 20 min, SAB + TNF-α treated group with 120 min continuing intravenous infusion of SAB (0.83 mg/ml, 5 mg/kg·h), beginning at 20 min before TNF-α administration, and TNF-α + SAB treated group with 120 min continuing intravenous infusion of SAB at 20 min after TNF-α administration.

Erythrocyte velocity in venules

The cerebral microcirculation was observed using a fluorescence microscope (Leica DM-LFS; Leica Microsystems, Wetzlar, Germany) equipped with a color monitor (TCL J2118A; TCL, Huizhou, China), a video timer (VTG-55B; FOR-A, Tokyo, Japan) and a DVD recorder (DVR-R25; Malata, Xiamen, China). Venules ranging from 30 to 50 μm in diameter and 200 μm in length were selected for the study. The microvessel images were recorded through a high-speed video camera system at a rate of 1000 frames per second (FASTCAM-ultima APX; Photron, San Diego, USA), and the recordings were replayed at a rate of 25 frames per second from the stored images.[5,10] The erythrocyte velocity was measured with Image-Pro Plus 5.0 software (Media Cybernetics, USA) before (baseline) and at 0, 20, 40, 60, 80, 100, and 120 min after TNF-α treatment, respectively.

Leukocyte rolling and adhesion in venules

The fluorescence tracer rhodamine 6G was administrated (5 mg/kg·bw) to the animal via the femoral vein 10 min before TNF-α administration. The cerebral cortex venules were observed using a camera (USS-301, UNIQ, USA) mounted to an intravital microscope (BX51WI, Olympus, Japan) illuminated with a mercury lamp and fluorescence videos were stored and processed. The rolling leukocytes were identified as cells rolling obviously slower than erythrocytes in the venules. The adherent leukocytes were identified as cells attached to the venular wall for more than 30 s. The number of rolling and adherent leukocytes was counted in a selected venule of