Acta Anaesthesiologica Taiwanica 53 (2015) 12–15

Contents lists available at ScienceDirect

Acta Anaesthesiologica Taiwanica

journal homepage: www.e-aat.com

Original Article

Platonin preserves blood–brain barrier integrity in septic rats

Chia-Tse Yeh [1], Ming-Chang Kao [2, 3], Cay-Huyen Chen [2, 3 †], Chun-Jen Huang [2, 3 * †]

[1] Department of Family Medicine, Taipei Tzu Chi Hospital, Taipei, Taiwan
[2] Department of Anesthesiology, Taipei Tzu Chi Hospital, Taipei, Taiwan
[3] School of Medicine, Tzu Chi University, Hualien, Taiwan

ARTICLE INFO

Article history:
Received 6 January 2015
Accepted 9 February 2015

Key words:
cecal ligation and puncture;
Evans blue dye;
inflammation;
rat;
tight junction

ABSTRACT

Objectives: Platonin possesses potent anti-inflammatory and antioxidative capacities. Because systemic inflammation and oxidative stress are crucial in mediating sepsis-induced blood–brain barrier (BBB) integrity loss, this study elucidated the effects of platonin on preserving BBB integrity in septic rats.
Methods: A total of 72 adult male rats (200–250 g) were randomized to receive cecal ligation and puncture (CLP), CLP plus platonin, sham operation, or sham operation plus platonin ($n = 18$ in each group). Systemic inflammation and oxidation levels and BBB integrity in the surviving rats were determined after 24-hour monitoring.
Results: Plasma levels of interleukin-6 (IL-6) and malondialdehyde (MDA)—markers of systemic inflammation and oxidation—and the grading of Evans blue staining of the brains, BBB permeability to Evans blue dye, and brain edema levels—markers of BBB integrity—in rats that received CLP were significantly higher than rats that received sham operation (all $p < 0.001$). By contrast, the plasma levels of IL-6 ($p < 0.001$) and MDA ($p < 0.001$), and the grading of Evans blue staining ($p = 0.015$), BBB permeability to Evans blue dye ($p = 0.043$), and brain edema levels ($p = 0.034$) in rats that received CLP plus platonin were significantly lower than rats that received CLP. Experimental data further revealed that the concentration of tight junction protein claudin-5, a major structural component of BBB, in rats that received CLP was significantly lower than rats that received CLP plus platonin ($p = 0.023$).
Conclusion: Platonin could attenuate sepsis-induced BBB integrity loss in rats.

1. Introduction

Septic patients, although without direct brain infection, could develop brain dysfunction.[1,2] The etiology largely remains to be established.[3–5] However, cumulative data indicate that blood–brain barrier (BBB) integrity loss may contribute to the development of brain dysfunction in these patients.[3–5] Previous data further indicate that systemic inflammation and oxidative stress are crucial in mediating BBB integrity loss in septic patients.[3–5] Experimental data that therapies aiming at decreasing systemic inflammation and/or oxidative stress could preserve BBB integrity in septic animals[6,7] further support this fact.

Platonin, a cyanine photosensitizing dye and a potent antioxidant, possesses potent anti-inflammatory capacity.[8–10] Previous data revealed that platonin could improve circulatory failure and mortality in septic rats.[8] Previous *in vitro* and *in vivo* data also demonstrated that platonin could inhibit endotoxin-induced inflammatory response.[9,10] However, the question of whether platonin can exert significant effects on preserving BBB integrity in septic animals remains unanswered. To elucidate further, we thus conducted this septic rodent study with the hypothesis that platonin can preserve BBB integrity in septic rats.

2. Materials and methods

This study was approved by the Animal Use and Care Committee of Taipei Tzu Chi Hospital, Taipei, Taiwan (100-IACUC-013). Care and handling of the animals were in accordance with National

Conflicts of interest: The authors state that there are no financial and nonfinancial conflicts of interest upon the publication of this study.

* Corresponding author. Department of Anesthesiology, Taipei Tzu Chi Hospital, Number 289, Jianguo Road, Xindian District, New Taipei City 231, Taiwan.
E-mail address: huangcj1112@gmail.com (C.-J. Huang).

† These two authors contributed equally to this study.

http://dx.doi.org/10.1016/j.aat.2015.02.001

Institutes of Health guidelines. A total of 72 adult male Sprague-Dawley rats (BioLASCO, Taipei, Taiwan; 200–250 g) were used for the experiments.

2.1. Sepsis model

This study employed the widely used cecal ligation and puncture (CLP) polymicrobial sepsis model for investigation. All rats were anesthetized by an intraperitoneal injection of a ketamine/xylazine mixture (110/10 mg/kg body weight), following which a transverse laparotomy (1 cm in length) was performed at the right lower quarter of the abdominal wall under sterile conditions. In 36 rats, the cecum was ligated and two 0.5-cm blade incisions were made.[11] Then the abdominal wall wound was closed with a 4-0 silk suture. Rats that received CLP were designated as "CLP". To control the effects of operational procedures, the other 36 rats received laparotomy, cecal identification, and wound closure, but not CLP (i.e., sham operation). Rats that received sham operations were designated as "sham". After recovery, all rats were closely monitored for 24 hours without restraint.

2.2. Experimental protocol

According to the aforementioned procedures, rats were then divided into the following four groups ($n = 18$ in each group): the CLP group, the CLP plus platonin (CLP + platonin) group, the sham group, and the sham plus platonin (sham + platonin) group. Platonin (100 μg/kg; Kankohsha Co., Osaka, Japan; dissolved in a mixture of 0.5 mL normal saline) was injected via the tail vein immediately after CLP. The dosage of platonin was determined according to a previous report.[8] To control for the effects of vehicle, rats in the CLP and sham groups also received an injection of 0.5 mL normal saline via the tail vein.

After closely monitoring for 24 hours, the surviving rats received anesthesia (a ketamine/xylazine mixture, 110/10 mg/kg body weight; administered intraperitoneally) followed by tracheostomy and cannulation of the femoral artery and the femoral vein. Rats were mechanically ventilated with a small animal ventilator (SAR-830/P ventilator; CWE, Ardmore, PA, USA) using a protocol of 10-mL tidal volume with room air at a frequency of 60 breaths/min. Rats were allowed to acclimatize to the stress of surgery for at least 20 minutes prior to blood sample collection (1 mL of blood drawn from the femoral vein by cannulation), and BBB integrity evaluation was performed. Then, all rats were killed with a high-dose pentobarbital (100 mg/kg, intraperitoneally).

2.3. Systemic inflammation and oxidative stress markers assay

The blood sample was centrifuged (1500 × g) to separate the plasma content. The plasma level of cytokine interleukin-6 (IL-6; the systemic inflammation marker) was then analyzed using the enzyme-linked immunosorbent assay (ELISA kit for IL-6; R&D Systems, Inc., Minneapolis, MN, USA). The level of malondialdehyde (MDA; systemic oxidation marker) was also measured according to a previously published protocol.[12]

2.4. BBB integrity evaluation: BBB permeability assay

BBB permeability was investigated using the Evans blue extravasation methods, including Evans blue staining of the brains and quantification of BBB permeability to Evans blue dye.[13,14] One third of the surviving rats from each group were randomly chosen to receive intravenous injection of Evans blue dye (4 mL/kg; 2%, (Sigma-Aldrich Corp., St. Louis, MO, USA)). The dye was allowed to circulate for 60 minutes. Then, sternotomy was performed followed by cannulation of the left ventricle and the right atrium. We then perfused the heart with normal saline through the left ventricle at a pressure of 110 mmHg, until colorless fluid was obtained from the right atrium. The brain was then removed. Evans blue staining of the brain was determined according to the gross appearance of the brain using the following criteria: Grade 0, no staining on the surface; Grade 1, faint and localized staining; Grade 2, moderate blue staining; and Grade 3, extensive dark staining.[13]

The collected brain was then divided. Each hemisphere was weighed and homogenized in phosphate-buffered saline (3.5 mL; Sigma-Aldrich) followed by vortex mixing for 2 minutes with 60% trichloroacetic acid (2.5 mL; Sigma-Aldrich) to precipitate protein. After cooling and centrifuging (30 minutes at 1000 × g), the absorbance of the supernatants for Evans blue dye was measured at 610 nm with a spectrophotometer.[14]

2.5. BBB integrity evaluation: Brain edema assay

The levels of brain edema were measured using the wet-to-dry weight ratio according to a previous report.[15] One third of the surviving rats from each group were randomly chosen for this assay. In brief, the brain was removed immediately after killing and divided into hemispheres. Each hemisphere was weighed (the wet weight) and then dried at 110°C in an oven for 24 hours and weighed again (the dry weight). The water content in the hemisphere was then calculated as follows:

$$\text{water content (\%)} = (\text{wet weight} - \text{dry weight})/\text{wet weight} \times 100. \quad (1)$$

2.6. Tight junction protein claudin-5 expression assay

Tight junction protein claudin-5 in endothelial cells is a major structural component of BBB.[16] We chose to evaluate claudin-5 expression using immunoblotting assay. One third of the surviving rats from each group were randomly chosen for this assay. In brief, snap-frozen brain tissue samples were processed according to a previous report.[17] After electrophoresis and transfer, the nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA) were incubated with the primary antibody solution of claudin-5 (1:200 dilution, polyclonal claudin-5 antibody; Zymed Laboratories, San Francisco, CA, USA) or actin (the internal standard, 1:500 dilution; polyclonal actin antibody; Millipore Corporation; Burlington, MA, USA) followed by incubation with the secondary antibody (i.e., horseradish peroxidase-conjugated antirat immunoglobulin G antibody; Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA). Bound antibody was detected by chemiluminescence (ECL plus kit; Amersham Pharmacia Biotech), and densitometric analysis was performed to quantify the protein-band densities.

2.7. Statistical analysis

One-way analysis of variance was used to test the differences among these groups. The Student–Newman–Keuls test was used for *post hoc* analysis. All data were presented as means ± standard deviations. The significance level was set as 0.05. A statistical software package (SPSS 11.5 for Windows; SPSS Science, Chicago, IL, USA) was used for data processing and analyses.

3. Results

At 24 hours, 18 rats survived in the sham group, 18 in the sham + platonin group, 15 in the CLP group, and 16 in the CLP + platonin group.