Original Article

Platonin preserves blood–brain barrier integrity in septic rats

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A B S T R A C T

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.

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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, further support this fact.

Platonin, a cyanine photosensitizing dye and a potent antioxid-

ant, possesses potent anti-inflammatory capacity. 6–10 Previous data revealed that platonin could improve circulatory failure and mortality in septic rats. 11 Previous in vitro and in vivo data also demonstrated that platonin could inhibit endotoxin-induced inflammatory response. 12, 13 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
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. 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. 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 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 acclimate 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. One 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.

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.

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. 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:

\[ \text{water content} (\%) = \frac{(\text{wet weight} – \text{dry weight})}{\text{wet weight}} \times 100 \]  

2.6. Tight junction protein claudin-5 expression assay

Tight junction protein claudin-5 in endothelial cells is a major structural component of BBB. 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. 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.
3.1. Systemic inflammation and oxidative stress

As expected, the plasma IL-6 and MDA concentrations of the sham and sham + platonin groups were low (Fig. 1). By contrast, the plasma IL-6 and MDA concentrations of the CLP group were significantly higher than those of the sham group (both \( p < 0.001 \); Fig. 1). Moreover, the plasma IL-6 and MDA concentrations of the CLP + platonin group were significantly lower than those of the CLP group (both \( p < 0.001 \); Fig. 1).

3.2. BBB permeability and brain edema

The grading of Evans blue staining of the brains, the BBB permeability to Evans blue dye, and the brain edema level of the sham and sham + platonin groups were significantly lower than that of the CLP group (all \( p < 0.001 \); Figs. 2 and 3). By contrast, the grading of Evans blue staining of the brains (\( p = 0.015 \); Fig. 2), the BBB permeability to Evans blue dye (\( p = 0.043 \); Fig. 2), and the brain edema level (\( p = 0.034 \); Fig. 3) of the CLP + platonin group were significantly lower than those of the CLP group.

3.3. Tight junction protein claudin-5

Claudin-5 concentrations in rat brains of the sham and sham + platonin groups were comparable (Fig. 4). However, the claudin-5 concentration of the CLP group was significantly lower than that of the sham group (\( p < 0.001 \); Fig. 4). By contrast, the claudin-5 concentration of the CLP + platonin group was significantly higher than that of the CLP group (\( p = 0.023 \); Fig. 4).

4. Discussion

Data from this study confirmed that polymicrobial sepsis induced by CLP could cause systemic inflammation and post-oxidative stress in animals. Data from this study further confirmed that CLP could cause significant BBB integrity loss. These data, together with those from previous studies,3–7 provide clear evidence to support the fact that systemic inflammation and oxidative stress play crucial roles in mediating BBB integrity loss in septic animals.

Of note, data from this study revealed that the systemic inflammation and oxidative stress imposed by CLP could be mitigated by platonin. These data confirmed the potent antioxidative and anti-inflammatory capacity of platonin.8–10 Moreover, our data demonstrate for the first time that BBB integrity loss induced by CLP could be mitigated by platonin. These data confirmed our hypothesis. Clear evidence to support the concept that therapies that can decrease systemic inflammation and oxidative stress can help preserve BBB integrity in septic animals was also provided by these data.5,7 As platonin is currently used clinically,3,18 the clinical implications of our data should be invaluable. Judging from our data, we further speculate that incorporating platonin as part of the therapies against sepsis should be a beneficial therapeutic strategy.

Data from this study also confirmed that CLP could induce significant alteration in tight junction protein expression. Because
tight junction proteins in endothelial cells are major components of BBB, the adverse effect of CLP on impairing the expression of tight junction proteins will certainly contribute to the development of BBB integrity loss in septic animals. This concept is supported by the present study, as our data revealed that interventions (e.g., platonin) that can restore the expression of tight junction proteins could preserve BBB integrity in septic animals. Moreover, considering the crucial roles of systemic inflammation and oxidative stress in this regard, we thus further speculate that these aforementioned mechanisms may also contribute to the adverse effects of CLP on impairing the expression of tight junction proteins.

Although this study confirmed the effects of platonin on preserving BBB integrity in septic rats, certain study limitations do exist and need to be addressed. First, this is a rodent study. Because different species might react differently, caution should be exercised if further data interpretation is intended. Second, only one dose of platonin was used in this study. Thus, the question of whether the therapeutic effects of platonin are dose dependent remains unstudied. Third, this study focused on the therapeutic effects of platonin. The safety of this therapy remains to be elucidated.

In conclusion, platonin could preserve BBB integrity in poly-microbial septic rats. The mechanisms may be associated with its effects on mitigating systemic inflammation and oxidative stress induced by CLP.

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