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Simvastatin attenuates sepsis-induced blood-brain barrier integrity loss

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ABSTRACT

Background: Systemic inflammation and oxidative stress are crucial in mediating blood-brain barrier (BBB) integrity loss during sepsis. Simvastatin possess potent anti-inflammation and antioxidation capacity. We sought to elucidate whether an acute bolus of simvastatin could mitigate BBB integrity loss in a rodent model of polymicrobial sepsis.

Methods: A total of 96 adult male rats (200–250 g) were randomized to receive cecal ligation and puncture (CLP), CLP plus simvastatin, sham operation, or sham operation plus simvastatin ($n = 24$ in each group). After maintaining for 24 h, BBB integrity in the surviving rats was determined.

Results: CLP significantly induced BBB integrity loss, as grading of Evans blue staining of the brains, BBB permeability to Evans blue dye, and brain edema levels in rats receiving CLP were significantly higher than those receiving sham operation. In contrast, grading of Evans blue staining ($P = 0.020$), BBB permeability to Evans blue dye ($P = 0.031$), and brain edema levels ($P = 0.009$) in rats receiving CLP plus simvastatin were significantly lower than those receiving CLP alone. Tight junction proteins claudin-3 and claudin-5 in endothelial cells are major structural components of BBB. Our data revealed that concentrations of claudin-3 and claudin-5 in rats receiving CLP were significantly lower than those receiving CLP plus simvastatin ($P = 0.010$ and 0.007). Immunohistochemistry further revealed significant fragmentation of claudin-3 and claudin-5 in rats receiving CLP. Moreover, levels of claudin-3 and claudin-5 fragmentation in rats receiving CLP plus simvastatin were significantly lower than those receiving CLP.

Conclusions: Simvastatin mitigates BBB integrity loss in a rodent model of polymicrobial sepsis.

1. Introduction

Septic encephalopathy refers to brain dysfunction observed in septic patients without direct brain infection [1–3]. The incidence varies between 8 and 70%, depending mainly on the used inclusion criteria [1–3]. Clinical data indicate that septic encephalopathy is an independent predictor of mortality in septic patients [3,4]. Though the etiology is not fully

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established, loss of blood-brain barrier (BBB) integrity has been shown to be one of the crucial mechanisms that underlie the development of septic encephalopathy [5–8]. Experiments demonstrate that robust systemic inflammation and oxidative stress play essential roles in mediating the development of loss of BBB integrity in septic encephalopathy [5–9]. This concept is supported by *in vivo* data in which therapies aimed at decreasing systemic inflammation or attenuating oxidative stress preserved BBB integrity in septic animals [10–12].

Simvastatin, one of the commonly used statins, reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase and is widely prescribed for hyperlipidemia to reduce the risk of atherosclerotic complications [13]. In addition, simvastatin has been shown to possess potent anti-inflammation and antioxidation capacity [14–18]. Clinical data reveal that simvastatin inhibits inflammation responses and improves survival of septic patients with multiple organ dysfunction syndrome [18]. Experimental data also indicate that simvastatin can attenuate organ injures induced by ischemia–reperfusion protocols [16,17]. However, the question of whether simvastatin can exert significant effects on preserving BBB integrity remains unstudied. We conducted this intact animal study to determine if an acute bolus of simvastatin could mitigate loss of BBB integrity in rats experiencing sepsis.

2. Materials and methods

This study was approved by the Animal Use and Care Committee of Taipei Tzu Chi Hospital (100-IACUC-013). Care and handling of the animals were in accordance with National Institutes of Health guidelines. A total of 96 adult male Sprague–Dawley rats (BioLASCO, Taipei, Taiwan) (200–250 g) were used for the experiments. The following factors were considered pertinent for sample size determination, including BBB integrity assays, sepsis model mortality, and power analysis of results. For BBB integrity assays, our design required four sets of assays that is, BBB permeability assay, brain edema assay, immunoblotting assay, and immunohistochemistry assay. The 24-h mortality of rodents induced by the cecal ligation and puncture (CLP) model of sepsis was reported to be around 30% [19]. In addition, power analysis based on previous data of systemic inflammatory mediators [20] revealed that a sample size of five rats in each group could achieve a power of 0.8 ($\alpha = 0.05$) for each assay. According to these previously mentioned considerations, we thus decided to include a total of 96 rats ($n = 24$ in each group) in this study.

2.1. Sepsis model

In this study, we used the rodent CLP polymicrobial model of sepsis. In brief, all rats were anesthetized with intraperitoneal (ip) injection of a ketamine and/or xylazine mixture (110/10 mg/kg body weight, respectively). After sterilization of the operation site, a transverse laparotomy of 1-cm length was performed at the right lower quarter of the abdominal wall. In 48 rats, the cecum was identified and ligated, and two 0.5-cm blade incisions were made to induce polymicrobial sepsis, according to a previously published protocol [19]. Then, the incision wound was closed with a 4-0 silk. Rats that received the cecal ligation and/or punctures were designated as "CLP." To control the effects of operational procedures, the other 48 rats received sham operations that is, laparotomy, cecum identification, and wound closure, but not CLPs. Rats that received sham operations were designated as "sham." To reduce postoperative pain, all wounds were locally infiltrated with 0.25% bupivacaine before closure. After wound closures and recovery from anesthesia, all rats were placed, without restraint, in cages and closely monitored.

2.2. Experimental protocol

The rats were divided into four groups ($n = 24$ in each group) as follows: the CLP, the CLP plus simvastatin (CLP + statin), the sham, and the sham plus simvastatin (sham + statin) groups. Simvastatin (10 mg/kg; Sigma–Aldrich, St. Louis, MO; dissolved in a mixture of 0.05 mL dimethyl sulfoxide and/or 0.45 mL normal saline) was injected via the tail vein immediately after CLP. The dosage of simvastatin was determined to match the dosage that could protect liver from ischemia–reperfusion injuries [21]. To control for the effects of vehicle, rats in the CLP and sham groups also received injection of a mixture of 0.05 mL dimethyl sulfoxide and/or 0.45 mL normal saline via the tail vein.

Previous data indicated that CLP could induce significant BBB integrity breakdown by 24 h after induction of sepsis [7]. We thus chose to determine the differences in the levels of BBB integrity among these four groups at 24 h after CLP induction. After closely monitoring for 24 h, the surviving rats were anesthetized again with injection of ketamine and/or xylazine mixture (110/10 mg/kg body weight, ip) followed by tracheostomy and cannulation of femoral artery and femoral vein. Rats were mechanically ventilated with a small animal ventilator (SAR-830/P ventilator; CWE, Ardmore, PA), using a 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 min before hemodynamic data collection. Hemodynamic data, including mean arterial pressure (MAP) and heart rate (HR), were continuously monitored with a polygraph (Model MP100; BIOPAC, Santa Barbara, CA). After the completion of hemodynamic data collection, blood sample collection, and BBB integrity evaluation, all rats were euthanized with a high-dose pentobarbital (100 mg/kg, ip). To eliminate experimenter bias, the persons who performed the assays were blinded to the treatment grouping of the samples.

2.3. Blood sample collection and measurements of systemic inflammation and oxidation markers

A total of 1 mL of blood was drawn from the femoral vein cannulation. The blood sample was centrifuged to separate plasma. The levels of systemic inflammation markers, cytokine interleukin-6 (IL-6) and chemokine macrophage inflammatory protein-2 (MIP-2), in plasma samples were analyzed using the enzyme-linked immunosorbent assay (ELISA Kits for IL-6 and MIP-2; R&D Systems, Inc, Minneapolis, MN). In addition, the level of systemic oxidation marker malondialdehyde (MDA) in plasma was measured, by our previously published protocol [22].