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Berberine alleviates postoperative cognitive dysfunction by suppressing neuroinflammation in aged mice

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ABSTRACT

Postoperative cognitive dysfunction (POCD) is a significant cause of morbidity after surgery, especially for the elderly. Accumulating evidence has demonstrated that neuroinflammation plays a key role in the pathogenesis of POCD. Thus, we hypothesized that berberine, an isoquinoline alkaloid with anti-inflammatory effects, could improve surgery-induced cognitive impairment. Twenty-month-old male C57BL/6 mice were subjected to exploratory laparotomy with isoflurane anesthesia to mimic the clinical human abdominal surgery. For the interventional studies, mice received berberine (10 mg/kg) or vehicle intraperitoneally. For the in vitro study, we examined the effects of berberine on lipopolysaccharide (LPS)-induced inflammatory mediators by cultured BV2 cells. Behavioral tests, expressions of IBA1, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 were performed at the indicated time points. In the present study, we showed that surgery impaired the contextual fear memory, as evidenced by the significantly decreased freezing time to the context. This behavioral change coincided with marked increases in IBA1, TNF-α, IL-1β, and IL-6 in the prefrontal cortex and hippocampus only at 24 h but not 7 d after surgery. In BV2 cells, LPS induced significantly increased TNF-α and IL-1β expressions. Notably, berberine treatment rescued surgery-induced cognitive impairment and inhibited the release of IBA1, IL-1β, and IL-6 in the hippocampus. In line with the in vivo study, berberine treatment suppressed LPS-stimulated production of TNF-α and IL-1β in BV2 cells. In conclusion, our study suggests that berberine could alleviate POCD by suppressing neuroinflammation in aged mice.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a common complication after surgery, which affects a wide variety of cognitive domains, including attention, memory, executive function, and speed of information processing [1–3]. The incidence of POCD varies extensively from 41 to 75% at 7 days to 18–45% at 3 months postoperatively, depending on the type of surgery studied and the composition of the test battery used for the definition of POCD [4]. The possibility of surgery-induced long-lasting cognitive impairment has gained ongoing public concerns over the past decades because it is associated with worse outcomes, including increased hospital stay, reduced quality of life, loss of social dependence, and increased mortality [1–4]. Although many pathological factors have been linked to POCD, accumulating evidence has suggested that neuroinflammation plays a substantial role in the pathogenesis of POCD [5]. Thus, inhibiting the production of inflammatory mediators may be a potential therapeutic strategy for POCD. Unfortunately, current *anti*-inflammatory drugs have many side effects, thus searching for alternative agents is urgently needed.

Berberine, an isoquinoline alkaloid purified from Chinese herbs, has multiple therapeutic effects for metabolic disorders, microbial infection, and inflammatory diseases [6]. Berberine has been extensively investigated and found to possess a wide variety of pharmacological and biological activities, including *anti*-inflammatory, *anti*-oxidant, *anti*-tumor, and cholesterol-lowering effects [7–11]. It has been demonstrated that berberine attenuates the production of inflammatory mediators through suppression of toll-like receptor 4/nuclear factor-κB signaling in an animal model of endotoxemia [12]. Since berberine readily crosses the blood-brain barrier (BBB) [13], it is interesting to examine whether berberine can suppress surgery-induced neuroinflammation and hence prevent the development of POCD. Although it has been shown that berberine exerts neuroprotection in cerebral ischemia [14] and Alzheimer's disease [15], the therapeutic effects of berberine on POCD has not been investigated. Therefore, the present study investigated whether systemic administration of berberine can improve surgery-induced cognitive impairment in a clinically relevant model of POCD. For the in vitro study, we examined whether berberine can suppress

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lipopolysaccharide (LPS)-induced inflammatory mediators by cultured BV2 microglia cells.

2. Material and methods

2.1. Animals

All experiments were approved by the Ethics Committee of Huai'an First People's Hospital and all procedures were performed in accordance with the Guideline for the Care and Use of Laboratory Animals from the National Institutes of Health (Bethesda, MD, USA). One hundred and eighteen 20-month-old male C57BL/6 mice were obtained from the Animal Center of Nanjing Medical University, Nanjing, China. Animals were housed 2–3 per cage in temperature (23 ± 1 °C) controlled rooms with standard rodent chow and water available *ad libitum*. The experiments were begun 2 weeks after the animals acclimating to the environment.

2.2. Anesthesia and surgery

Exploratory laparotomy was performed by aseptic procedures with isoflurane anesthesia according to one previous study [16]. The mice were anesthetized in a chamber prefilled with 1.5% isoflurane in 100% oxygen and an abdominal median incision about 1 cm was made, penetrating the peritoneal cavity. Then, the investigator explored the viscera, intestine and musculature. After that, sterile 4–0 chromic gut sutures were used to suture the peritoneal lining and skin. To prevent infection, the wound was dressed with polysporin. The surgical procedure was performed under isoflurane anesthesia and lasted for about 10 min. For the mice served as controls, neither anesthesia nor surgery was performed. The flow chart for the experimental protocol was summarized in Fig. 1A.

2.3. Experimental protocol

Berberine (Sigma, St. Louis, MO, USA) dissolved in 0.9% saline (0.2 ml) or a corresponding volume of vehicle (0.9% saline) was administered intraperitoneally after surgery. We chose the route of administration based on one previous study in which they showed that intraperitoneal injection of berberine improved neurological deficits in an animal model of traumatic brain injury [13]. To determine the optimal dosage, berberine at 2, 10, or 50 mg/kg was intraperitoneally administered immediately for one or three consecutive injection (s) post-surgery. Our study showed that repeated administration of berberine at the dose of 10 or 50 mg/kg was sufficient to reverse the cognitive impairment induced by surgery. Thus, berberine at the dose of 10 mg/kg was used in our subsequent experiment. Subsequently, animals were randomized into one of the four following groups: (1) control + vehicle; (2) control + berberine (10 mg/kg); (3) surgery + vehicle; and (4) surgery + berberine (10 mg/kg).

2.4. Cell culture

The murine BV2 cells were purchased from China Center for Type Culture Collection (CCTCC, Wuhan, China). BV2 Cells were maintained in DMEM/H-G containing 10% FBS and antibiotics (100 IU/ml penicillin and 100 mg/ml streptomycin) at 37 °C in 5% CO_2 incubator (Heal Force Development LTD, Hong Kong). At the time of harvest, the cells were washed with PBS, and trypsinized, then centrifuged (1000 g for 5 min) and resuspended in serum-free DMEM; then the cells (approximately 5×10^5 cells/ml) were seeded in plates. The cells were pretreated with various concentrations of berberine (0, 2.5, 5, 10 μmol) 30 min before LPS (Escherichia coliO55:B5, St. Louis, MO, USA, 0.5 μg/ml) treatment.

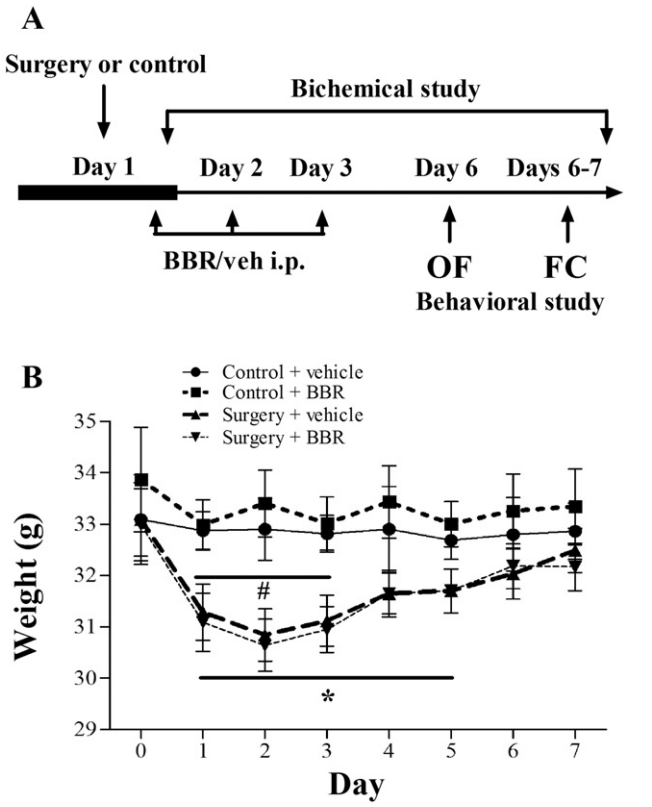

Fig. 1. Schematic timeline of the experimental procedure and effects of berberine on body weight in aged mice. For the behavioral study, the mice underwent anesthesia and surgery on day 1, and then they were treated with berberine at 10 mg/kg or the equal volume of vehicle i.p. immediately post-surgery. Open field test and fear conditioning training were performed on day 6. Fear conditioning tests were performed on day 7 (A). Berberine treatment did not affect body weight in aged mice (B). The data are presented as the mean ± SEM ($n = 12$). OF, open field; FC, fear conditioning; BBR, berberine; i.p., intraperitoneally. $^*P < 0.05$ compared with the control + vehicle group, $\#P < 0.05$ compared with the surgery + vehicle group.

2.5. Behavioral studies

Behavioral studies were performed as described previously [16]. The investigator was blinded to the animal grouping. The open field test was carried out in a white opaque plastic chamber (40 cm × 40 cm × 40 cm) at 6 d post-surgery to assess the exploratory and locomotor activities. The mice were placed in the center of the open field and their activity was monitored for 5 min. Its activity was automatically recorded with a video tracking system.

Two hours after the open field test, mice were trained for fear conditioning. Each mouse was placed into a conditioning chamber and allowed to explore for 3 min, and then a 30 s tone (70 db, 3 kHz) was delivered followed by a 2 s foot shock (0.7 mA). When it was finished, the mouse stayed in the chamber for another 30 s and then returned to the home cage. Twenty-four hours later, all mice were subjected to the contextual fear conditioning test. For the contextual fear conditioning, mice were placed into the same chamber in which they were trained, observed for 5 min without tone or foot shock, and scored for the freezing behavior. The auditory-cued fear test was performed 2 h after the contextual fear conditioning in a novel chamber. Mice were placed into the novel chamber and allowed to explore for 3 min. Then the training tone was delivered for an additional 3 min and the freezing behavior was scored. Freezing behavior was defined as the absence of all visible movement except for respiration, and it was recorded and expressed as the percentage of the observation period.