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The role of microglia activation in the development of sepsis-induced long-term cognitive impairment

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ABSTRACT

Oxidative stress and inflammation is likely to be a major step in the development of sepsis-associated encephalopathy (SAE) and long-term cognitive impairment. To date, it is not known whether brain inflammation and oxidative damage are a direct consequence of systemic inflammation or whether these events are driven by brain resident cells, such as microglia. Therefore, the aim of this study is to evaluate the effect of minocycline on behavioral and neuroinflammatory parameters in rats submitted to sepsis. Male Wistar rats were subjected to sepsis by cecal ligation and puncture (CLP). The animals were divided into sham-operated (Sham+control), sham-operated plus minocycline (sham+MIN), CLP (CLP+control) and CLP plus minocycline (CLP+MIN) (100 μg/kg, administered as a single intracerebroventricular (ICV) injection). Some animals were killed 24 h after surgery to assess the breakdown of the blood brain barrier, cytokine levels, oxidative damage to lipids (TBARS) and proteins in the hippocampus. Some animals were allowed to recover for 10 days when step-down inhibitory avoidance and open-field tasks were performed. Treatment with minocycline prevented an increase in markers of oxidative damage and inflammation in the hippocampus after sepsis. This was associated with an improvement in long-term cognitive performance. In conclusion, we demonstrated that the inhibition of the microglia by an ICV injection of minocycline was able to decrease acute brain oxidative damage and inflammation as well as long-term cognitive impairment in sepsis survivors.

1. Introduction

Despite its high prevalence, morbidity and mortality, sepsis-associated encephalopathy (SAE) is still poorly understood (Gofton and Young, 2010). Furthermore, survivors of sepsis have a prolonged form of cognitive dysfunction (Widmann and Heneka, 2014; Ywashyna et al., 2010), and it is supposed that acute brain dysfunction may be associated with long-term cognitive impairment (Gunther et al., 2012). In animal models of sepsis, acute encephalopathy is observed (Steckert et al., 2013; Schwalm et al., 2014), and survivors present long-term cognitive impairment (Mina et al., 2014). Thus, the use of relevant preclinical models has facilitated the understanding of the mechanisms associated with brain dysfunction. Some studies have demonstrated the involvement of oxidative stress and inflammation as a major step in the development of SAE and long-term cognitive impairment (Mina et al., 2014; Schwalm et al., 2014; Barichello et al., 2007; Hernandes et al., 2014). To date, it is not known whether brain inflammation and oxidative damage are a direct consequence of systemic inflammation or whether they are driven by brain resident cells, such as microglia or astrocytes (Michels et al., 2014).

Microglia are known for playing a key role in mediating inflammatory processes associated with various neurodegenerative diseases (Dehmer et al., 2000; Jamin et al., 2001). The inhibition of microglia using minocycline has emerged as a relevant

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pharmacological tool to study the role of microglia in different central nervous system (CNS) diseases (Wang et al., 2005). Minocycline is effective in animal models of global cerebral ischemia, (Yrjänheikki et al., 1998; Arvin et al., 2002), focal cerebral ischemia, (Yrjanheikki et al., 1999; Wang et al., 2003; Xu et al., 2004) and traumatic brain injury (TBI) (Sanchez Mejia et al., 2001). In addition, minocycline decreased brain inflammation induced by systemic and brain administration of LPS (Zhu et al., 2014; Yoon et al., 2012), but there are no reports about the effects of minocycline in a clinically relevant model of sepsis.

We hypothesized that sepsis-induced brain inflammation and oxidative damage is mediated by the activation of microglia, and microglia inhibition could improve long-term cognitive impairment observed in sepsis survivors.

2. Materials and methods

2.1. Drugs

Minocycline was purchased from Sigma–Aldrich, Saint Louis, USA and was prepared according to the method described previously (Shan et al., 2007).

2.2. Ethics

The experimental procedures involving animals were performed in accordance with the National Institutes of Health (Bethesda, MD, USA) Guide for Care and Use of Laboratory Animals and with the approval of our institutional ethics committee.

2.3. Sepsis induction – cecal ligation and perforation (CLP) model

Rats were subjected to CLP as previously described (Fink and Heard, 1990). Briefly, animals were anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under aseptic conditions, a 3 cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was ligated with a 3.0 silk suture at its base, below the ileocecal valve, and was perforated once with a 14-gauge needle. The cecum was then squeezed gently to extrude a small amount of feces through the perforation site. The cecum was then returned to the peritoneal cavity, and the laparotomy was closed with 4.0 silk sutures. Animals were resuscitated with regular saline (50 mL/kg) subcutaneously (s.c.) immediately after and 12 h after CLP. All animals received antibiotics (ceftriaxone at 30 mg/kg and clindamycin 25 mg/kg) every 6 h s.c. for a maximum of 3 days. In sham-operated group, rats were subjected to all surgical procedures, but the cecum was neither ligated nor perforated. To minimize variability between different experiments, the CLP procedure was always performed by the same investigator. We extensively characterized long-term cognitive impairment using this animal model (Barichello et al., 2005a,b; Tuon et al., 2008; Petronilho et al., 2012).

2.4. Minocycline treatment

Rats were subjected to CLP or sham-operated conditions and divided into sham-operated (sham+control), sham-operated plus minocycline (sham+MIN), CLP (CLP+control) and CLP plus minocycline (CLP+MIN) groups. Minocycline (100 μg/kg) was administered as a single intracerebroventricular (ICV) injection immediately after surgery.

2.5. Behavior test

The animals were subjected to inhibitory avoidance and open-field tasks 10 days after surgery. The behavioral procedures were conducted between 13:00 and 16:00 h in a sound-isolated room. A single animal performed only one behavioral test. All behavioral tests were performed by the same person who was blind to the animal group. For each behavioral task, a total of 12 animals per group was used.

2.6. Inhibitory avoidance

The inhibitory avoidance procedure was described in a previous report (Roesler et al., 1999). The apparatus was an acrylic box (50 × 25 × 25 cm) whose floor consisted of parallel-caliber stainless-steel bars (1-mm diameter) spaced 1 cm apart and a platform that was 7 cm wide and 2.5 cm high. Animals were placed on the platform, and their latency to step down on the grid with all four paws was measured with an automatic device. Training sessions were performed 10 days after surgery. Immediately after stepping down on the grid, animals received a foot shock of 0.3 mA for 2 s. In test sessions carried out 24 h after training, no foot shock was given, and the step-down latency (maximum of 180 s) was used as a measure of retention.

2.7. Open field test

Behavior was assessed in an open field apparatus to evaluate both locomotor and exploratory activities. The apparatus is a 40 cm × 60 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field is divided into nine rectangles by black lines. The animals were gently placed on the left rear quadrant and left alone to explore the arena for 5 min (training session). Immediately after this procedure, the animals were taken back to their home cage, and 24 h later, they were submitted again to a similar open-field session (test session). Every cross of the black lines and rearing performed in both sessions were counted for 5 min. The decrease in the number of crossings and rearings between the two sessions was taken as a measure of the retention of habituation memory (Vianna et al., 2000).

2.8. Blood brain barrier permeability

The integrity of the blood brain barrier (BBB) was investigated using Evans blue dye extravasation (Belayev et al., 1996) 24 h after the procedure. The dye was administered (2% wt/vol in phosphate-buffered saline (PBS)) intravenously (3 mL/kg) through the rat femoral vein 1.5 h after the animals were perfused using normal saline (250 mL) through the left ventricle at 110 mmHg pressure until colorless perfusion fluid was obtained from the right atrium. Quantitative evaluation of BBB permeability was achieved by measuring the content of Evans blue in the hippocampus by its fluorescence intensity (ng/mg of brain tissue) (Spectramax M2 microplate reader, Molecular Devices). A total of five animals for each group was used for the determination of BBB permeability.

2.9. Levels of cytokines

Concentrations of hippocampal TNF-α, IL-1β and IL-6 were determined by ELISA on a microplate reader using a commercial kit (Peprotech) 24 h after the surgery. A total of five animals for each group were used for the determination of cytokine levels.