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Inhibition of indoleamine 2,3-dioxygenase 1/2 prevented cognitive impairment and energetic metabolism changes in the hippocampus of adult rats subjected to polymicrobial sepsis

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ABSTRACT

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection that may affect the brain. We investigated the role of indoleamine 2,3-dioxygenase (IDO-1/2) inhibition on long-term memory and energetic metabolism after experimental sepsis by caecal ligation and perforation (CLP). Experimental sepsis increased the activity of complexes I, II-III and IV at 24 h after CLP, and IDO-1/2 inhibition normalized the activity of these complexes in the hippocampus. Wistar rats presented impairment of habituation and aversive memories 10 days after CLP. Adjuvant treatment with the IDO inhibitor prevented long-term cognitive impairment triggered by sepsis.

1. Introduction

Central nervous system (CNS) dysfunction secondary to sepsis can occur in 8–70% of septic patients and is associated with generation of pro- and anti-inflammatory cytokines, impaired cerebral microcirculation, and an imbalance of neurotransmitters.

During sepsis, the central nervous system (CNS) is one of the first organs affected, and this is clinically manifested as sepsis-associated encephalopathy (SAE) (Sprung et al., 1990). In animal models of sepsis, SAE is associated with an acute increase in the blood-brain barrier permeability, the generation of pro- and anti-inflammatory cytokines (Comim et al., 2011b), impairment of cerebral microcirculation (Taccone et al., 2010), oxidative stress (Barichello et al., 2006), apoptosis (Comim et al., 2013), long-term cognitive dysfunction (Tuon et al., 2008), and depressive-like behaviour (Comim et al., 2010). Evidence suggests that SAE is aggravated by neuroinflammation and excitotoxicity, which may contribute to neuronal dysfunction and degeneration.

When systemic inflammation secondary to an infection reaches the brain, it may up-regulate the production of pro-inflammatory mediators. In the brain, these pro-inflammatory mediators are responsible for the activation of a protein complex (nuclear factor kappa-light-chain-enhancer of activated B cells, NF-κB) that controls the transcription of cytokines (Konsman et al., 2002; Nadjar et al., 2003). Simultaneously, they also increase the mRNA expression of indoleamine 2,3-deoxygenase (IDO). IDO-1 and IDO-2 are intracellular heme-containing enzymes that together with tryptophan 2,3-dioxygenase (TDO) initiates the first step of tryptophan degradation along the kynurenine pathway (Moon et al., 2015; Too et al., 2016). IDO-1 is widely distributed throughout the body and its expression is linked to energy homeostasis and immune defence, which is relevant for neurological diseases (Moffett and Namboodiri, 2003). IDO-2 has been shown to be unimportant for adaptive immunity (Dal-Pizzol et al., 2014); however,

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its function in the CNS is unclear (Metz et al., 2014). TDO is expressed in a significant proportion of human tumours (Pilotte et al., 2012). The activation of IDO-1/2 results in the depletion of tryptophan and the accumulation of kynurenine and its metabolites. These metabolites can affect the function of neurons and inhibit the proliferation of T cells. Increasing evidence demonstrates that IDO is a potential therapeutic target in the treatment of neurological diseases.

Our central hypothesis is that activation of IDO by sepsis induces long-term cognitive impairment, which can be ameliorated through its inhibition. We evaluated the effect of IDO-1/2 inhibition on energetic metabolism by investigating changes in the activity of respiratory chain complexes (I, II, II-III and IV) in the prefrontal cortex, hippocampus, striatum and cortex during the first hours after sepsis induction and aversive and habituation memories 10 days after sepsis induction.

2. Material and methods

2.1. Animals

Male Wistar rats (60 days old, 250–300 g) were obtained from our breeding colony (UNESC). The animals were housed five to a cage with food and water available ad libitum and maintained on a 12 h light/dark cycle (lights on at 7:00 a.m.). All experimental procedures involving animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behaviour (SBNeC) recommendations for animal care. All procedures were approved by the Animal Care and Experimentation Committee of the UNISUL, Brazil (15.014.4.08.IV)

2.2. Experimental procedure

Rats were subjected to cecal ligation and perforation (CLP) (Comim et al., 2011a; Comim et al., 2008; Dal-Pizzol et al., 2013). The rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally (i.p.). A midline laparotomy (3 cm) was performed to expose the caecum with the adjoining intestine. The caecum was tightly ligated with a 3.0-silk suture at its base (below the ileocaecal valve) and perforated once with a 14-gauge needle. The caecum was then gently squeezed to extrude a small amount of faeces from the perforation site and then returned into the peritoneal cavity. The laparotomy was closed with 4.0-silk sutures. All animals were returned to their cages with free access to food and water. In the sham-operated group, the rats were subjected to all surgical procedures, but the caecum was neither ligated nor perforated. After the surgery, all groups received "basic support" and antibiotic therapy (30 mg/kg ceftriaxone and 25 mg/kg clindamycin subcutaneously (s. c.) every 6 h for 3 days).

2.3. Organization of the experimental groups

The investigation was conducted at two different time points. At the first time point, the animals were divided into 3 groups (6–7 rats per group): sham, CLP, and CLP treated with an IDO inhibitor. The IDO inhibitor (1-DL-methyltryptophan) was obtained from Sigma-Aldrich Co. (St. Louis, MO). The inhibitor was administered intracerebroventricularly (in Tris buffer, pH 7.4, 10 μg) at 0 and 6 h after the CLP surgery (Barichello et al., 2013). Furthermore, 24 h after sepsis induction, the animals were killed and the prefrontal cortex, hippocampus, striatum and cortex was immediately isolated on dry ice and stored at −80 °C for analysis of proteins involved in energetic metabolism.

For the second experiment, the rats were divided into 3 groups: sham, CLP, and CLP treated with an IDO inhibitor ($n = 10$ rats per group). The IDO inhibitor was administered intracerebroventricularly (in Tris buffer, pH 7.4, 10 μg) at 0 and 6 h after the CLP surgery (Barichello et al., 2013). The rats also received antibiotic therapy. Ten days after sepsis induction, the animals were free from infection. The animals were then subjected to behavioural testing. Survival was analyzed by Kaplan-Meier curves that included all infected animals from the time of CLP up to 10 days after infection, at which time the surviving animals were killed.

2.4. Mitochondrial respiratory chain activity

The samples were removed and homogenized (1:10, w/v) in SETH buffer (250 mM sucrose, 2 mM EDTA, 10 mM Tris base, and 50 IU/ml heparin, pH 7.4) to determine the activity of mitochondrial respiratory chain enzyme complexes (I, II, II–III and IV). NADH dehydrogenase (complex I) was evaluated by the rate of NADH-dependent ferricyanide reduction at 420 nm (Cassina and Radi, 1996). The activity of succinate-DCIP oxidoreductase (complex II) and succinate-cytochrome c oxidoreductase (complexes II–III) was determined according to the methods described by Fischer et al. (1985). Complex II activity was measured by the decrease in absorbance at 600 nm due to the reduction of 2,6-DCIP. The activity of complexes II–III was measured by cytochrome c reduction from succinate. The activity of cytochrome c oxidase (complex IV) was assayed by the decrease in absorbance at 550 nm due to oxidation of the previously reduced cytochrome c (Miro et al., 1998). The activities of the mitochondrial respiratory chain complexes were expressed as nmol/min/mg protein (Miro et al., 1998). The Lowry protein assay was used to normalize the results based on the protein concentration (Lowry et al., 1951).

2.5. Behavioural task

Ten days after sepsis induction, the rats were randomized and subjected to the step-down inhibitory avoidance and habituation to open field behavioural tests.

2.5.1. Habituation to open field test

For the habituation to open field test, the animals were gently placed on the left rear quadrant of the arena and allowed to explore the arena for 5 min (training session). Immediately following the training session, the animals were returned to their home cages. Twenty-four hours later, the animals underwent a similar open-field session (test session). The number of crossings of the black lines and rearing was counted in both sessions. A decrease in the number of crossings and rearing between the two sessions indicated retention of habituation (Vianna et al., 2000; Barichello et al., 2010).

2.5.2. Step-down inhibitory avoidance test

During the training trial of the step-down inhibitory avoidance test, the rats were placed on the platform and their latency to step down onto the grid with all 4 paws was measured using an automatic device. Immediately after stepping onto the grid, the animals received a 0.4-mA, 2.0-second foot shock and were returned to their home cages. The retention test was performed 24 h after the training trial (long-term memory). The procedure for the retention test was identical to the training trial, except that no foot shock was administered. The step-down latency during the retention test (maximum = 180 s) measured retention of the inhibitory avoidance memory (Quevedo et al., 1999; Barichello et al., 2010).

3. Statistical analyses

Data from the habituation to an open field test and the biochemical analyses are reported as the mean ± S.E.M and were analyzed using an ANOVA followed by Tukey's post hoc test. Data from the inhibitory avoidance test is reported as the median and interquartile ranges, and comparisons among the groups were performed using Mann–Whitney *U* tests. The Wilcoxon test was used for comparisons within individual groups. A $^{*}p < 0.05$ was considered statistically significant. The Kaplan-Meier test was used to analyze the survival rates. Statistically significant differences between groups were identified using the log-