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Low dose dexamethasone reverses depressive-like parameters and memory impairment in rats submitted to sepsis

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ABSTRACT

Sepsis is characterized by a systemic inflammatory response of the immune system against an infection, presenting with hypothalamic–pituitary–adrenal (HPA) axis dysfunction, behavior alterations, and high mortality. In this study, we aimed to evaluate the effects of dexamethasone on mortality, anhedonia, circulating corticosterone and adrenocorticotropin hormone (ACTH) levels, body and adrenal gland weight, and aversive memory in sepsis survivor rats. Male Wistar rats underwent sham operation or cecal ligation and perforation (CLP) procedure. Rats subjected to CLP were treated with "basic support" and dexamethasone (at 0.2 and 2 mg/kg daily for 7 days after CLP, intraperitonially) or saline. After 10 days of sepsis procedure, it was evaluated aversive memory, sweet food consumption, and body and adrenal gland weight. Serum and plasma were also obtained. It was observed that low dose dexamethasone reverted anhedonia, normalized adrenal gland and body weight, corticosterone and ACTH levels, and decreased mortality and avoidance memory impairment, demonstrating that low doses of dexamethasone for moderate periods may be beneficial for sepsis treatment and its sequelae—depressive-like parameters and memory impairment.

Sepsis is characterized by a systemic inflammatory response of the immune system against an infection, presenting with high mortality rates. Presently, it consists on the 10th main cause of death in intensive care units (ICU) [1]. Reports have attributed the increase in the transcription of pro- and anti-inflammatory molecules as important parts of sepsis physiopathology [10]. Septic encephalopathy (SE) is a brain dysfunction associated to sepsis reported to occur in 8–70% of septic patients, and its presence is strongly associated with higher mortality. SE is caused by systemic inflammation in the absence of direct brain infection and clinically characterized by slowing of mental processes, impaired attention, disorientation, delirium, or coma [40], and more recently, cognitive impairment [42].

Under physiologic conditions, the activation of the hypothalamic–pituitary–adrenal (HPA) axis plays a pivotal role in the body's response to stress, and results in a rise in blood cortisol concentration. Our group recently verified, in an animal model of cecal ligation and perforation (CLP), that sepsis survival rats presented with depressive-like parameter such as anhedonia, weight loss, increase in corticosterone and adrenocorticotropic hormone (ACTH) levels, and decrease in hippocampal weight and BDNF levels [13]. Such depressive-like symptoms have been seen in ICU patients who survived sepsis [38], and may imitate symptoms of cognitive impairment [20]. Some reports have shown that sepsis survivor rats also have symptoms of depression, demonstrated by the forced swimming task [42]. In addition, such depressive-like behavior, observed 10 days after the CLP, was reversed by classical antidepressant agent: imipramine [41].

In the search for effective drugs, the use of corticosteroids in the management of severe sepsis has been under investigation for many years. Several recent trials have shown that low replacement doses of steroids are beneficial in septic patients, improving hemodynamic status [6] and reducing mortality [2]. In this context, aversively motivated learning is influenced by neuromodulators and hormones related to emotional aspects of the training experience. Emotionally arousing events cause an increase in corticosterone, which is known to modulate memory [30]. In addition, Roozendaal showed, in an animal model, that a single administration of corticosteroids given shortly before or after a learning experiment enhances long-term storage of the newly acquired information [36]. Furthermore, dexamethasone, a

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synthetic corticosteroid, prevented long-lasting learning impairment following neonatal hypoxic-ischemic brain insult [23], and also after a combination of lipopolysaccharide (LPS) injection and hypoxia-ischemia in neonatal rats [21]. More recently, we demonstrated that dexamethasone in a single dose of 0.3 mg/kg reversed aversive impairment in the sepsis group 10 days after sepsis induction [43]. Thus, in this study, we aimed to evaluate the effects of dexamethasone on: (1) mortality; (2) anhedonia; (3) circulating corticosterone and ACTH levels; (4) body and adrenal gland weight; and (5) aversive memory in sepsis survivor rats.

To this purpose, we utilized one hundred male Wistar rats (3–4 months old, 300–350 g) obtained from our breeding colony (UNESC). The animals were housed 5 to a cage with food and water available *ad libitum,* and maintained on a 12-h light/dark cycle (lights on at 7:00am). 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 Behavior recommendations for animal care, and were approved by the Animal Care and Experimentation Committee of UNESC, Brazil.

The animals were subjected to CLP as previously described [42,13,41]. Briefly, the rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg) given intraperitoneally (i.p.). Then, a 3-cm midline laparotomy was performed allowing exposure of the cecum with the adjoining intestine. The cecum was tightly ligated with a 3.0-silk suture at its base, below the ileo-cecal valve, and perforated once with a 14-gauge needle. The cecum was then gently squeezed to extrude a small amount of feces from the perforation site and then returned into the peritoneal cavity, followed by the closure of the laparotomy 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 submitted to all surgical procedures, but the cecum was neither ligated nor perforated. After surgery, all groups received "basic support" for 3 days (50 mL/kg saline immediately and 12 h after CLP, plus 30 mg/kg ceftriaxone and 25 mg/kg clindamycin every 6 h) [42,13,41]. After induction, the rats ($n = 20$—ten animal for anhedonia and biochemical parameters and 10 animals for behavioral task) were randomly divided into four groups: Sham + Saline; Sepsis + Saline; Sepsis + Dexa0.2 mg/kg; and Sepsis + Dexa2 mg/kg. Dexamethasone, obtained from Ache Pharmaceutical Industry, was administered i.p. at 0.2 or 2 mg/kg immediately after CLP procedure, with a daily dose for 7 days (0–7th day), this dose represents low and high doses of dexamethasone in animal model [43,8,44,9,26,14,3]. All treatments were administered in a volume of 1 mL/kg of dexamethasone plus saline.

After 10 days of sepsis (10th day) procedure, the animals were free from infection signs. Blood cultures performed were all negative at this point (data not shown). Then, the animals were placed in a lightened rectangular box (40 cm × 15 cm × 20 cm) with a glass ceiling, floor, and side walls made of wood, divided into nine equal rectangles by black lines. Ten Froot Loops (Kellogg's® pellets of wheat, corn starch, and sucrose) were placed in one extremity of the box. The animals were subjected to five 3-min trials once daily for 5 days to become familiarized with the food (10–17th day). After habituated, the animals were exposed to two 3-min test sessions, when the number of ingested pellets was measured (16–17th days). This task was performed in light cycle and the evaluation was conducted by an observer blind to the groups. It was significant when the animal ate 1, 1/2, or 1/4 part of the Froot Loops, in accordance with a previous study [13]. The number of crossings of the black lines and rearings performed was measured in the first (10th day) session to determine locomotor activity [13].

After the rats were anesthetized, blood was withdrawn, and serum and plasma were utilized to evaluate corticosterone and ACTH levels, respectively [13]. Corticosterone levels were determined using enzyme immunoassay kits. Serum concentrations of ACTH were determined using commercially available radioimmunoassay kits (both from Diagnostic Products Corporation, LA, USA) for animals. Both analyses were performed by a commercial laboratory blind to the experimental conditions. Body weight was measured before CLP (0th day), 10 (10th day), and 17 days (17th day) after CLP. At the 17th day – after the consumption of sweet food – the rats were anesthetized i.p. with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), followed by death by decapitation, and the adrenal gland removed and weighted in an analytical scale [13].

The inhibitory avoidance task was performed to evaluate aversive memory (10th day) [42]. The apparatus consisted of an acrylic box (50 cm × 25 cm × 25 cm) with the floor 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. In the training trial, animals were placed on the platform and their latency to step-down on the grid with all four paws was measured with an automatic chronometer. Immediately after stepping down on the grid, the animals received a 2 s 0.4 mA foot shock and returned to their home cage. A retention test trial was performed 24 h after training (long-term memory). The retention test trial was procedurally identical to training, except that no foot shock was presented. The retention test step-down latency (maximum, 180 s) was used as a measure of inhibitory avoidance retention. The behavioral tests were performed by the same person blind to the experimental group. Finally, the mortality was assessed during the 17 days after the CLP procedure during the treatment with dexamethasone.

The Statistical Package for the Social Sciences (SPSS) 17.0 was utilized for statistical analyses. All data are presented as mean ±S.E.M. (data normally distributed). Differences among experimental groups were determined by one-way ANOVA, followed by *Tukey* as *post hoc* test for sweet food intake, locomotor activity, adrenal gland and hippocampus weight, circulating corticosterone and ACTH levels. By contrast, body weight was analyzed employing Student's *t*-test for paired data, when the data were normally distributed. Data of inhibitory avoidance are presented as median (interquartile range) of retention test latencies. Differences between training and test session latencies within each group were determined using the *Wilcoxon* test. Comparisons between groups were performed using *Mann–Whitney U* test. Mortality was assessed by Kaplan–Meier Survival Analysis and Log Rank test. Values of $p < 0.05$ were considered statistically significant.

Fig. 1 demonstrates anhedonia test, body and adrenal weight, and circulating corticosterone and ACTH levels. Sepsis decreased sucrose intake in Sepsis + Saline when compared to Sham + Saline group ($F_{(5-54)} = 19.94$; $p = 0.00$; Fig. 1A). Treatment with dexamethasone at 0.2 mg/kg ($F_{(5-54)} = 19.94$; $p = 0.00$; Fig. 1A) and 2 mg/kg ($F_{(5-54)} = 19.94$; $p = 0.00$; Fig. 1A) were able to reverse the reduction of sweet food consumption when compared to Sepsis + Sham group. In the locomotor activity evaluation, there were no alterations in the number of crossings ($F_{(3-40)} = 0.56$; $p = 0.64$) and rearings ($F_{(3-40)} = 1.17$; $p = 0.34$; between groups (data not shown). This showed that the results observed were not secondary to locomotor alterations and that dexametasone treatment did not modify the locomotor activity.

Septic animals treated with saline did not increase body weight ($t = -0.78$; df = 11; $p = 0.45$; Fig. 1B) as did control animals ($t = -6.52$; df = 11; $p = 0.00$; Fig. 1B) when compared to the weight before surgery. There was an increase in adrenal gland weight in Sepsis + Saline ($F_{(5-66)} = 10.46$; $p = 0.00$; Fig. 1C) in comparison to Sham + Saline group. Treatment with dexamethasone at 0.2 mg/kg ($t = -11.88$; df = 9; $p = 0.00$; Fig. 1B) and 2 mg/kg ($t = -10.64$; df = 9; $p = 0.00$; Fig. 1B) normalized body weight and the increase in adrenal gland weight (0.2 mg/kg: $F_{(5-66)} = 10.46$; $p = 0.00$; 2 mg/kg: $F_{(5-66)} = 10.46$; $p = 0.00$; Fig. 1C) when com-