Effect of mild sepsis on behavioral and biochemical changes on the stress-induced animal model of depression

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Abstract

The systemic inflammation that occurs during severe sepsis can be the cause of long-term consequences. Studies have demonstrated a possible correlation between the neuroinflammatory process and depression. However, the effects of mild sepsis as a tolerant factor for some depressive parameters have not been described. Thus, the objective of this study is to evaluate the effect of mild sepsis on behavioral and biochemical changes on the stress-induced animal model of depression. To this aim, adult male Wistar rats were submitted to cecal ligation and perforation and after 30 days it was submitted to the chronic mild stress protocol (CMS). The animals were submitted to stressors for 40 days. To evaluate the efficacy of CMS induction anhedonia was determined as the amount of sweet food consumption. The levels of neurotrophins and oxidative damage were also evaluated in the hippocampus. It is observed that mild sepsis improved depressive-like parameters after CMS protocol associated with an increase of neurotrophins levels and a decrease of oxidative damage in the hippocampus. In conclusion, our data suggest that a previous mild systemic inflammation caused by the induction of mild sepsis could decrease consequences in the central nervous system (CNS) induced by CMS protocol.

Introduction

Severe sepsis often affects the brain. At admission, cerebral dysfunction may develop in up to 71% of septic patients. This condition is caused by systemic inflammation without brain infection and clinically characterized by mental processes impairment, disorientation, delirium or coma [1-3]. Clinical and pre-clinical researches have shown that sepsis is associated to the generation of pro- and anti-inflammatory cytokines [4-6], increase in the blood-brain barrier (BBB) permeability [4], impairment of cerebral microcirculation [7], oxidative stress [8], hippocampal apoptosis [9] long-term cognitive impairment [10,11] and depressive-like behavior [12]. However, studies have shown that prior mild sepsis has some modality-specific effects on the behavioral response to subsequent immune challenge [13-15].

In this context, some studies have associated the processes of neuroinflammation and depression [16-19]. Clinical studies have reported an increase in the inflammatory response during the course of major depression evidenced by an increase in pro-inflammatory cytokine levels in the serum [20,21]. Patients with major depression who are healthy seem to have activated inflammatory pathways, with an increase of pro-inflammatory cytokines, chemokines, acute-phase proteins and adhesion molecules [22-24]. Pro-inflammatory cytokines can enter and alter the brain function, even though they are released peripherally, modifying neurotransmitter metabolism, neuroendocrine

function through hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, neuronal plasticity, decrease of neurotrophin levels as BDNF and greater activation of the glutamatergic pathways, influencing the synaptic behaviors [25].

Studies demonstrate that a vulnerable brain can be more easily affected by responses associated with stress as a result of systemic inflammation [26-28]. In this context, some authors have shown that prior history of an immune challenge may affect central and behavioral responses to subsequent immune challenge, either leading to exaggerated responses via priming mechanisms or lessened responses via endotoxin tolerance [29-31]. Given the persistent upregulation of neuroinflammation following severe sepsis and that association between depression and neuroinflammation, we set out to enquire whether prior mild sepsis would impact on depressive-like parameters

(anhedonia, oxidative damage and neurotrophins levels) induced by the chronic mild stress (CMS) response in the CNS. We used the chronic mild stress (CMS) paradigm as a model of depression and characterized its effects with to long-term outcomes after mild sepsis.

Methods

Animals

Male Wistar rats (60 days old, weighing 220-310g, n=50) were obtained from the University of Southern Santa Catarina (UNESC) breeding colony. Animals were caged in groups of 5 with water and food ad libitum and were maintained on a 12 h light-dark cycle (lights on at 7:00 a.m.), at a temperature of 23°C. All experimental procedures were performed by the approval of the Ethics Committee of Animals Use from UNESC (Protocol 051/2014) and conformed to international regulations.

Mild sepsis induction

Rats were subjected to cecal ligation and perforation (CLP) [31-33]. Briefly, animals were anesthetized using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), given intraperitoneally. Under the aseptic condition, a 3-cm midline laparotomy was performed to allow the exposure of the cecum with the adjoining intestine. The cecum was tightly ligated with a 3.0-silk suture at its base, below the ileocecal valve, and perforated once with a 20-gauge needle. The cecum was then gently squeezed to extrude a small number of feces from the perforation site, returned to the peritoneal cavity, followed by the closure of the laparotomy with 4.0-silk sutures. The rats were volemically resuscitated with normal saline (50 mL/kg subcutaneously) immediately and 12h after CLP. All animals were returned to their cages with free access to water and food. In the sham-operated group, the rats were submitted to all surgical procedures, but the cecum was neither ligated nor perforated. After surgery, both groups received 30 mg/kg ceftriaxone and 25 mg/kg clindamycin subcutaneously every 6h for a total of 3 days. In the groups where the animals underwent sepsis, there was a 33% mortality. Survivors rats were divided in four experimental groups: 1) sham + control (n=10); 2) sham + CMS (n=10); 3) CLP + control (n=10) and 4) CLP + CMS (n=10). After 30 days of induction, the CMS protocol was carried out as described below.

Chronic mild stress protocol

Chronic mild stress (CMS) protocol was adapted from [34]. Animals were divided in control (n=20) and CMS (n=20) groups. Controls were kept undisturbed in their home cages during the 40 days of the experiment, receiving only ordinary care with daily supports of food and water. The stressors used in this protocol were: (a) 24h of water deprivation; (b) 24h of food deprivation; (c) flashing light for 3 h; (d) isolation (1-3 days); (e) 2h of restraint and (f) 2h of restraint at 4°C. Animals were submitted to stressors for 40 days. Stress was applied at distinct periods every day and offered in a random order in to minimize its predictability.

To evaluate the efficacy of CMS induction anhedonia was determined as the amount of sweet food consumption. Briefly, rats were placed in a box (40x15x20 cm) divided into 9 equal rectangles. Ten Froot Loop(s) (Kellogg's®) were placed in one extremity of the box. In the training trials, rats were exposed to the environment for 3 min, once a day, during 5 consecutive days to become familiarized with the food. After, animals were exposed to 2 test sessions (3 min each, on consecutive days) during which the number of ingested Froot Loop(s) was measured. Anhedonia test took 7-days in total and it was realized

after the finish to the CMS [34,35]. Immediately after the behavioral tests, the rats were sacrificed by decapitation and the hippocampus dissected and stored at 80°C.

Measurement of neurotrophins levels

For analysis of the neurotrophic factors, the total hippocampus were homogenized in phosphate-buffered solution (PBS) with 1mM phenylmethylsulfonyl fluoride (PMSF) and 1mM ethylene glycol bis(2-aminoethylether)-N,N,N'N'-tetraaceticacid (EGTA). Homogenates were centrifuged at 10,000 g for 20 min. Then, supernatants were collected for quantification of neurotrophic factors levels. BDNF, NGF and GDNF levels in the hippocampus were measured by sandwich enzyme-linked immunosorbent assay using commercial kits according to the manufacturer's instructions (NGF and BDNF levels were assessed with a kit from Chemicon (USA) and GDNF levels was assessed with a kit from Biosensis (USA)). Total protein was measured by the BCA method using bovine serum albumin as a standard.

Oxidative damage

Oxidative damage in lipids was assessed by measuring the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, as previously described by Draper and Hadley (1990). Hippocampus samples were mixed with 1 mL of trichloroacetic acid 10% and 1 mL of thiobarbituric acid 0.67% and then heated for 30 min. TBARS levels were determined spectrophotometrically by measuring the absorbance at 532 nm. The Protein carbonyl was measured through determination of carbonyl groups content based on the reaction with dinitrophenylhidrazine (DNPH), as previously described by [35]. Proteins were precipitated by the addition of 20% trichloroacetic acid and were redissolved in DNPH. The absorbance was monitored spectrophotometrically at 370 nm. Protein content was measured by the BCA method using bovine serum albumin as a standard.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 18.0 and GraphPad Prism version 6.0 were utilized for statistical analyses. Data from brain oxidative damage, neurotrophins and anhedonia were expressed as mean and standard derivation, and analyzed by factorial analysis of variance (ANOVA), followed by Tukey post-hoc test. For all comparisons, $p < 0.05$ was considered statistically significant.

Results

The parameters of anhedonia are shown in Figure 1. The animals of sham + CMS and CLP + control group demonstrated significantly decreased in Froots Loops intake when compared to the animals in the Sham + Control group. The animals of the CLP + Control group showed a decrease of Froots Loops intake when compared to Sham + Control animals. However, there was a significant difference in food intake between CLP + CMS and CLP + control groups, demonstrating possible protection.

Figure 2 shows the levels of neurotrophins through analysis of BDNF (Figure 2A), NGF (Figure 2B) and GDNF (Figure 2C) in the hippocampus. Animals of sham + CMS and CLP + control groups showed a significant decrease in the BDNF, NGF e GDNF hippocampus levels when compared to the sham + control group. However, the animals of the CLP + CMS group showed an increase of BDNF, NGF e GDNF hippocampus levels when compared to CLP + control animals.

Figure 3 demonstrates the results of oxidative damage in the hippocampus evaluating the protein carbonylation (Figure 3A)
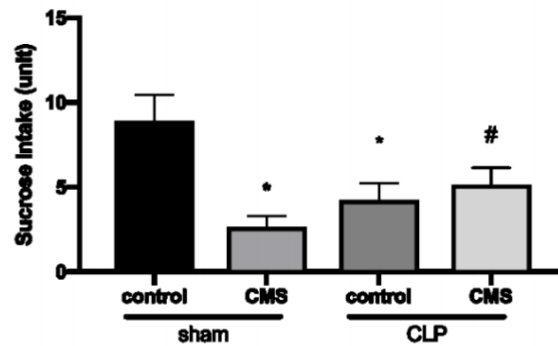
Figure 1. Anhedonia test. The data is expressed by mean and standard derivation. *p<0.05 versus sham + control group; #p<0.05 versus CLP + control
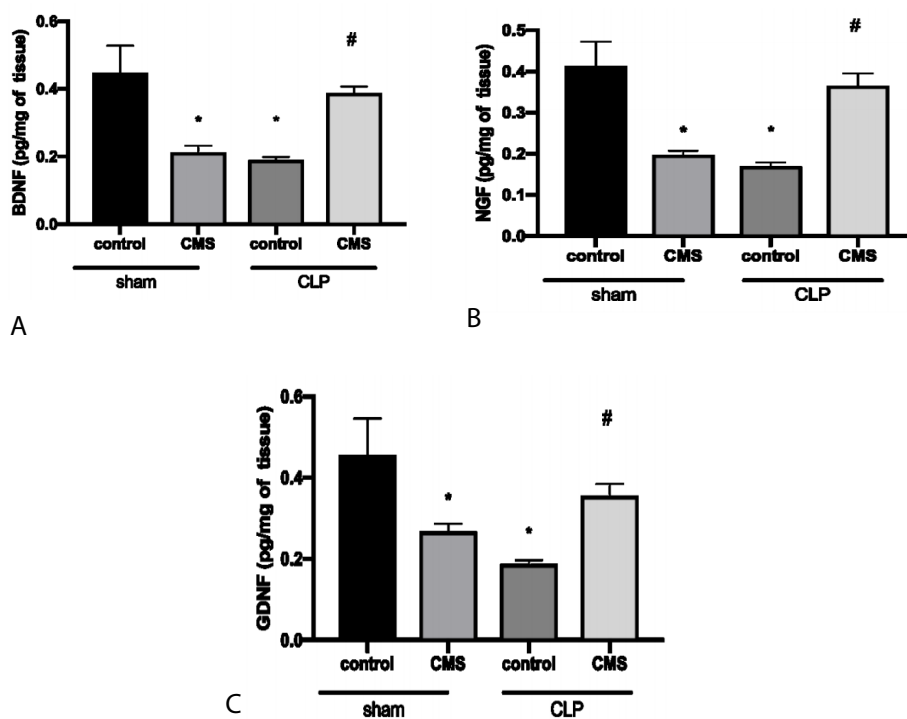

Figure 2. Neurotrophin levels in the hippocampus through evaluation of BDNF (2A), NGF (2B) and GDNF (3C). The data is expressed by mean and standard derivation. *p<0.05 versus sham + control group; #p<0.05 versus CLP + control
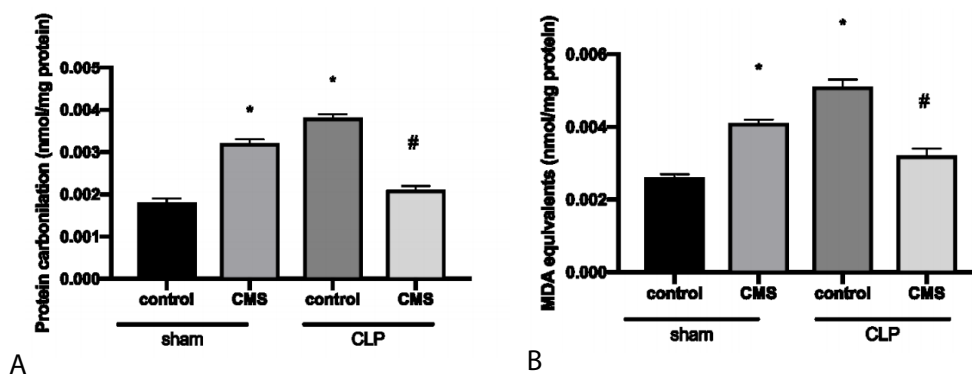

Figure 3. Oxidative damage in the hippocampus through the evaluation of protein carbonylation (3A) and lipidic peroxidation (3B). The data is expressed by mean and standard derivation. *p<0.05 versus sham + control group; #p<0.05 versus CLP + control

and lipid peroxidation (Figure 3B). There was an increase of protein carbonylation and lipid peroxidation in the hippocampus of the sham + CMS and the CLP + control groups when compared to the sham + control group. The animals the of CLP + CMS group showed a decrease of protein carbonylation and lipid peroxidation when compared to CLP + control animals.

Discussion

This study demonstrates that mild sepsis improved depressive-like behavior assessed by the anhedonia test after CMS protocol and this was related to an increase of the neurotrophins levels and a decrease in the protein carbonylation and lipid peroxidation in the hippocampus.

Studies have shown that oxidative stress can be associated with the sepsis immune alterations and that inhibitory methods to treat sepsis consequences can be a new study pathway of the possible resolution. In this context, endotoxin tolerance has been shown to develop an immune response that is protective of sepsis. After the tolerance induction, the organism becomes resistant [36-38] and this tolerance represents an immune adaptation response to a first stimulus that cannot be solved by the innate response [39]. The mechanisms of tolerance have been associated with protection against tissue damages in the hepatic and renal ischemia/reperfusion [40,41] and hemorrhagic shock [42] in animal models. In these studies, there was increased resistance to stressful situations showing that tolerance is far more than altered inflammatory responsiveness.

During the development of sepsis, it is generally believed that excessive inflammation leads to organ dysfunction. In this study, mild sepsis per se causes a depressive-like behavior characterized by the decrease of sucrose intake associated with the increase of oxidative damage and the decrease of neurotrophins. However, the depressive-like animal model caused by CMS protocol is also associated with increased oxidative damage [43] and a decreased of neurotrophins levels [44]. In other inflammatory diseases, such as meningitis it can be observed the correlation between cognitive impairment and low levels of BDNF [45]. The animals submitted to mild sepsis and exposed to CMS protocol after 30 days did not show depressive-like behavior, oxidative damage or altered levels of neurotrophins in the hippocampus. It is possible that this protection is due to tolerance caused by previous sepsis and prepare an animal to deal with further stressors such as CMS protocol.

In a recent study, it was demonstrated that the previous stress caused by CMS can protect the brain against the systemic acute and severe stress elicited by sepsis [46]. In this study we found the opposite: that mild sepsis can be associated with a possible modulation of neurotrophin levels and oxidative damage in the hippocampus. This modulation can be related to the prevention of depressive-like behavior after the CMS protocol. It is known that major depression is associated with worse outcomes in critically ill patients [47-49]. However, this outcome was not evaluated in this study.

It is important to note some limitations of this study [50,51]. We used a model of mild sepsis, which has provided clinically relevant information. However, it was not possible to determine if there is a "dose-dependent" effect. In a future study, other severity models should be used to detect more accurately a possible tolerance. Another important point is that this study did not fully explore a mechanism to elucidate the modulatory effects of systemic inflammation. It has however provided some evidence that this must include mechanisms associated with the control of oxidative stress and neurotrophins, but not inflammation [52]. Thus, it seems clear that somehow systemic inflammation related to mild sepsis modulates the brain response after

stress promoted by the CMS protocol, and this may have an impact on the long-term brain dysfunction [53-55]. In conclusion, our data suggest that a previous systemic inflammation caused by the induction of mild sepsis could decrease the long-term depressive-like behavior and hippocampal oxidative damage associated with an increase of neurotrophin levels induced by CMS protocol.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Ethics Committee of Animals Use – UNESC 051/2014.

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