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ORIGINAL ARTICLE

Dimethyl Fumarate Limits Neuroinflammation and Oxidative Stress and Improves Cognitive Impairment After Polymicrobial Sepsis

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Abstract

Sepsis is caused by a dysregulated host response to infection, often associated with acute central nervous system (CNS) dysfunction, which results in long-term cognitive impairment. Dimethyl fumarate (DMF) is an important agent against inflammatory response and reactive species in CNS disorders. Evaluate the effect of DMF on acute and long-term brain dysfunction after experimental sepsis in rats. Male Wistar rats were submitted to the cecal ligation and puncture (CLP) model. The groups were divided into sham (control) + vehicle, sham + NAC, sham + DMF, CLP + vehicle, CLP + NAC, and CLP + DMF. The animals were treated with DMF (15 mg/kg at 0 and 12 h after CLP, per gavage) and the administration of *n*-acetylcysteine (NAC) (20 mg/kg; 3, 6, and 12 h after CLP, subcutaneously) was used as positive control. Twenty-four hours after CLP, cytokines, myeloperoxidase (MPO), nitrite/nitrate (N/N), oxidative damage to lipids and proteins, and antioxidant enzymes were evaluated in the hippocampus, total cortex, and prefrontal cortex. At 10 days after sepsis induction, behavioral tests were performed to assess cognitive damage. We observed an increase in cytokine levels, MPO activity, N/N concentration, and oxidative damage, a reduction in SOD and GPx activity in the brain structures, and cognitive damage in CLP rats. DMF treatment was effective in reversing these parameters. DMF reduces sepsis-induced neuroinflammation, oxidative stress, and cognitive impairment in rats subjected to the CLP model.

Keywords Sepsis · Neuroinflammation · Cognitive impairment · Oxidative stress · Dimethyl fumarate

Introduction

Sepsis is the most frequent cause of death in intensive care units (ICU) (Mayr et al. 2014), and it is determined by a systemic inflammatory response associated with an infection (Singer et al. 2016). The central nervous system (CNS) is rapidly damaged during sepsis, causing sepsis-associated encephalopathy (SAE), which is clinically characterized by disorientation, *delirium*, or coma (Wassmer et al. 2006). Also, long-term memory disturbance and impaired learning ability frequently affect sepsis survivors (Iwashyna et al. 2010).

The pathophysiological mechanisms associated with SAE development comprise the production of proinflammatory mediators, oxidative stress, and mitochondrial dysfunction (Dal-Pizzol et al. 2014). Proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, activate target cells and stimulate the production of other cytokines, chemokines, reactive oxygen species (ROS), reactive nitrogen species (RNS), eicosanoids, and proteolytic enzymes (Doyle and O'Neill 2006). In fact, the production of inflammatory mediators is exacerbated in sepsis, leading to microcirculation dysfunction, tissue damage, and multiple organ failure (Comim et al. 2011b).

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Sepsis survivors usually display an increase in IL-6 levels and hippocampal hypotrophy (Wiersinga et al. 2014); similar findings are observed in preclinical studies using rodent model of sepsis, in which TNF-α, IL-1β, IL-6, and IL-8 levels in the CNS increase within a few hours after sepsis onset (Mina et al. 2014; Michels et al. 2015a). As the inflammatory scenario persists, several deleterious consequences take place, e.g., increased blood-brain barrier (BBB) permeability, oxidative damage, energy metabolism alterations, microglial activation and glutamate excitotoxicity, aggravating SAE, and contributing to neuronal dysfunction, degeneration, and cognitive impairment (Petronilho et al. 2012; Michels et al. 2014, 2015b; Mina et al. 2014).

Oxidative stress in sepsis favors systemic inflammation and multiple organ failure due to the excessive production of reactive species and the deterioration of antioxidant defenses (Chelkeba et al. 2015). In response, neutrophils are activated and recruited to the infectious site, thus stimulating ROS and RNS production (Andrades et al. 2011; Comim et al. 2011b; Lange et al. 2012; Zheng et al. 2015). At high concentrations, such species disrupt the oxidation-antioxidant axis and generate oxidative stress, i.e., a deleterious cellular process to membrane lipids, proteins, and DNA (Singer 2014).

Considering that neuroinflammation and oxidative stress lead to cerebral changes in sepsis, therapeutic alternatives are necessary to prevent SAE or minimize its consequences (Zampieri et al. 2011). In this sense, dimethyl fumarate (DMF), a fumaric acid ester, is an important target for CNS changes due to both processes (Albrecht et al. 2012; Parodi et al. 2017). Studies involving multiple sclerosis and psoriasis show that DMF suppresses the inflammatory response, oxidative stress, neuronal injury, and microglial activation (Wilms et al. 2010; Albrecht et al. 2012; Gill et al. 2014; Parodi et al. 2015).

Dimethyl fumarate acts in two main pathways: (i) it increases nuclear factor erythroid 2-related factor 2 (Nrf2) activation, which enhances the endogenous levels of antioxidant enzymes, such as glutathione peroxidase (GPx) (Kobayashi and Yamamoto 2006; Linker et al. 2011), and (ii) it controls the immunomodulatory response by inhibiting nuclear factor-κB (NF-κB). Studies showed an antiinflammatory effect of DMF, represented by a reduced cytokine production in response of NF-κB inhibition (Wierinckx et al. 2005; Scannevin et al. 2012).

In view of all these aspects, the lack of studies evaluating DMF on brain damage after sepsis and considering that neuroinflammation and oxidative stress are intrinsically related to this condition, we hypothesize that DMF may have protective effect against such changes. To test our hypothesis, we measured neutrophil infiltrate, TNF-α, IL-6, and cytokine-induced neutrophil chemoattractant-1 (CINC-1) levels; nitrite and nitrate concentration; oxidative damage to lipids and proteins; the antioxidant activity of SOD, CAT, and GPx; and long-term cognitive damage in the hippocampus, prefrontal cortex, and total cortex of rats submitted to polymicrobial sepsis.

Materials and Methods

Animals

Male *Wistar* rats (60 days, 250–300 g) were used in this study. Animals were housed in groups of four per cage with food and water ad libitum, on a 12-h light/dark cycle (lights on at 7:00 a.m.). All experimental procedures were approved by the Animal Care and Experimentation Committee of UNISUL (protocol number 15.009.4.03.IV), Brazil.

Sepsis Induction—CLP Model

Sepsis was induced by cecal ligation and puncture (CLP), as previously described (Hubbard et al. 2005). Briefly, animals were anesthetized intraperitoneally (i.p.) with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg). Under aseptic conditions, a 3-cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was tightly ligated with a 3.0 silk suture in the middle of its length (below the ileocecal valve), perforated once with a 14-gauge needle, squeezed gently to extrude a small amount of feces through the perforation site, and returned to the peritoneal cavity, and the laparotomy was closed with 4.0 silk sutures. Animals were resuscitated with regular saline (50 mL/kg) and ceftriaxone (30 mg/kg) subcutaneously (s.c.) immediately after CLP and 12 h after. 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. To minimize variability between different experiments, the same investigator always performed the CLP procedure.

Experimental Groups and Sample Obtention

Animals were randomly divided into six groups: sham + vehicle, sham + NAC, sham + DMF, CLP + vehicle, CLP + NAC, or CLP + DMF. Considering a 40% mortality rate, for biochemical analysis, 7 animals per sham group and 12 animals per CLP group were used, and for behavior analysis, 12 animals per sham group and 18 per CLP group were used (Carvalho et al. 2008). The animals were treated with DMF (15 mg/kg dissolved in 0.08% dimethylsulfoxide at 0 h and 12 h after CLP, per gavage) or *n*-acetylcysteine (NAC) (20 mg/kg, at 3, 6, and 12 h after CLP, s.c.) as control (Barichello et al. 2007; Reick et al. 2014). Twenty-four hours after sepsis induction, all animals designed for biochemical evaluations were subjected to a painless assisted death with

thiopental overdose (0.5 g/kg, i.p.) followed by decapitation. The remaining animals designed for behavioral evaluation were subjected to the same euthanasia procedure at 10 days after sepsis induction. The brain structures prefrontal cortex, hippocampus, and "total cortex" (the remaining tissue after harvesting both previous structures) were quickly isolated and stored at − 80 °C until further analysis. Behavioral tests were performed 10 days after surgery procedure.

Biochemical Analysis

Neutrophil Infiltrate

Neutrophil infiltrate in the three brain structures was measured by MPO activity [22]. Brain tissues were homogenized (50 mg/mL) in 0.5% hexadecyltrimethylammonium bromide and centrifuged at 15,000×*g* for 40 min. An aliquot of supernatant was mixed with a solution of 1.6 mM tetramethylbenzidine and 1 mM H_2O_2. Activity was measured spectrophotometrically as the change in absorbance at 650 nm at 37 °C. Data were expressed as milliunits per milligram of protein (De Young et al. 1989).

Cytokine Measurement

Interleukin-6, TNF-α, and CINC-1 concentrations in the three brain structures were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (R&D Systems, Minneapolis, MN) and were expressed as picograms per milliliter.

Nitrite/Nitrate Concentration

Nitrite/nitrate (N/N) concentration was measured in the three brain structures by Griess reaction, by adding 100 μL of Griess reagent [0.1% (*w*/*v*) naphthylethylendiamide dihydrochloride in H_2O and 1% (*w*/*v*) sulfanilamide in 5% (*v*/*v*) concentrated H_3PO_4], with vol. [1:1] to the 100 μL sample. After 1 h of incubation at room temperature, absorbance was recorded in a spectrophotometer at 550 nm (Green et al. 1982). Data were expressed as nanomoles of nitrite/nitrate concentration per milligram of protein.

Lipid Peroxidation

Lipid peroxidation was measured in the three brain structures by formation of thiobarbituric acid (TBA) reactive substances (Draper and Hadley 1990). After dissection, samples were washed with PBS, harvested, and lysed. Thiobarbituric reactive species, obtained by acid hydrolysis of 1,1,3,3-tetra-ethoxy-propane (TEP), were used as the standard for the quantification of TBARS. TBA 0.67% was added to each tube and vortexed. The reaction mixture was incubated at 100 °C for 20 min, and the reaction was stopped by placing samples on ice. The optical density of each solution was measured in a spectrophotometer at 535 nm. Data were expressed as nanomoles of malondialdehyde (MDA) equivalents per milligram of protein.

Carbonyl Protein Formation

Carbonyl protein content was measured in the three brain structures using 2,4-dinitrophenylhydrazine (DNPH) in a spectrophotometric assay (Levine et al. 1990). Briefly, sample tissues were sonicated in ice cold homogenization buffer containing phosphatase and protease inhibitors (200 nM calyculin, 10 μg/mL leupeptin, 2 μg/mL aprotinin, 1 mM sodium orthovanadate, and 1 μM microcystin-LR) and centrifuged at 1000×*g* for 15 min to sediment insoluble material. Three hundred-microliter aliquots of the supernatant containing 0.7–1.5 mg of protein were treated with 300 μL of 10 mM DNPH, dissolved in 2 M HCl, and compared with 2 M HCl alone (reagent blank). Samples were incubated for 1 h at room temperature in the dark, stirred every 10 min, precipitated with trichloroacetic acid (final concentration of 20%), and centrifuged at 16,000×*g* at 4 °C for 15 min. The pellet was washed three times with 1 mL of ethanol/ethyl acetate (1:1 *v*/*v*). Each time, the pellet was lightly vortexed and left exposed to the washing solution for 10 min before centrifugation (16,000×*g* for 5 min). The final pellet was dissolved in 1 mL of 6 M guanidine in 10 mM phosphate buffer-trifluoroacetic acid, pH 2.3, and the insoluble material was removed by centrifugation at 16,000×*g* for 5 min. Absorbance was recorded in a spectrophotometer at 370 nm for both DNPH-treated and HCl-treated samples. Carbonyl protein levels were expressed as nanomoles of carbonyl per milligram of protein.

Superoxide Dismutase Activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined in the three brain structures by a spectrophotometric assay based on superoxide-dependent oxidation of epinephrine to adrenochrome at 32 °C (Bannister and Calabrese 1987). Absorption was measured at 480 nm. SOD-specific activity was represented as milliunits per milligram of protein.

Catalase Activity

Catalase (CAT; EC 1.11.1.6) activity was determined in the three brain structures by the absorbance decrease at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100, 10 mM potassium phosphate buffer, pH 7.0, and the supernatants containing 0.1–0.3 mg protein. mL-1 (Aebi 1984). The specific activity was expressed as milliunits per milligram of protein.

Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was determined in the three brain structures spectrophotometrically in a system containing GSH/NADPH/GR by H_2O_2 dismutation. In this assay, the enzyme activity is indirectly measured by NADPH decay at 340 nm. The enzymatic activity was expressed as nanomoles per minute per milligram of protein (Wendel 1981).

Protein Determination

All biochemical measures were normalized to the protein content with bovine serum albumin as standard (Lowry et al. 1951).

Behavioral Analyses

Habituation to the Open Field Task

The habituation to the open field task evaluates motor performance in the training section and non-associative memory in the retention test session. Habituation to an open field was carried out in a 40 × 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 was divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear quadrant and left to explore the arena for 5 min (training session). Immediately following this, the animals were taken back to their home cage and 24 h later submitted again to a similar open-field session (test session). The number of times the animal crossed the black lines and rearing behavior in both sessions was counted. The decrease in the number of crossings and rearings between the two sessions was taken as a measure of the retention of habituation (Vianna et al. 2000; de Lima et al. 2005).

Novel Object Recognition Task

This task evaluates non-aversive and non-spatial memory. The apparatus and procedures for the object recognition task are the same used for open-field task. All animals were submitted to a habituation session when they could freely explore the open field for 5 min. No objects were placed in the box during the habituation trial. Crossings of the black lines and rearings performed in this session were evaluated as locomotors and exploratory activity, respectively. Twenty-four hours after habituation, training was conducted by placing individually a rat for 5 min in the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. The short-term recognition memory test was performed 1.5 h after training, when the rats explored the open-field for 5 min in the presence of one familiar (A) and one novel (B) object. The long-term recognition memory test was performed 24 h after training following the same protocol. All objects had similar textures, colors, and sizes, but distinctive shapes. A recognition index was calculated for each animal reported as the ratio TB/(TA + TB) (TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B). Exploration was defined as sniffing (exploring the object 3–5 cm away from it) or touching the object with the nose and/or forepaws (de Lima et al. 2005).

Data Analysis

Data was analyzed using Statistical Package for the Social Sciences software (SPSS, Chicago, IL, USA). Data is expressed as mean ± S.D., and differences among experimental groups were determined by one-way analysis of variance (ANOVA), followed by Tukey *post-hoc* for all inflammation and oxidative stress analysis. For behavior tests, data was expressed as median ± interquartile rage and differences among groups were determined by Mann-Whitney and Wilcoxon test. In all comparisons, statistical significance was set at $p < 0.05$.

Results

DMF Reduces Neuroinflammation in Sepsis

Figure 1 shows the results of MPO activity, i.e., indicative of activated neutrophil infiltrate, in the hippocampus, prefrontal cortex, and total cortex at 24 h after CLP procedure. CLP + vehicle animals presented higher levels of MPO, compared to sham + vehicle animals, in all brain structures. DMF treatment was effective in decreasing neutrophil infiltrate after sepsis in all brain structures.

Figure 2 shows the results of cytokines. CINC-1 levels were initially elevated (Fig. 2a) in all brain structures of CLP + vehicle group, while DMF treatment significantly decreased CINC-1 levels only in the total cortex. Regarding TNF-α levels (Fig. 2b), it was increased in the hippocampus and prefrontal cortex at 24 h after sepsis induction, and DMF treatment was effective in decreasing this elevation only in the hippocampus. Concerning IL-6 levels (Fig. 2c), CLP + vehicle animals displayed enhanced levels in all brain structures, while DMF treatment was effective in decreasing it only in the hippocampus.

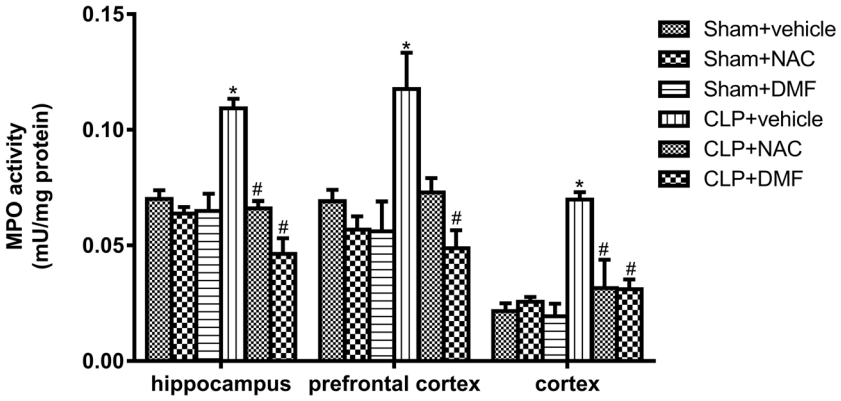

Fig. 1 MPO activity in the hippocampus, prefrontal cortex, and total cortex of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is expressed as mean ± standard deviation, analyzed by one-way ANOVA with Tukey *post-hoc* test. $^{*}p < 0.05$ compared to sham + vehicle group and $^{\#}p < 0.05$ compared to CLP + vehicle group

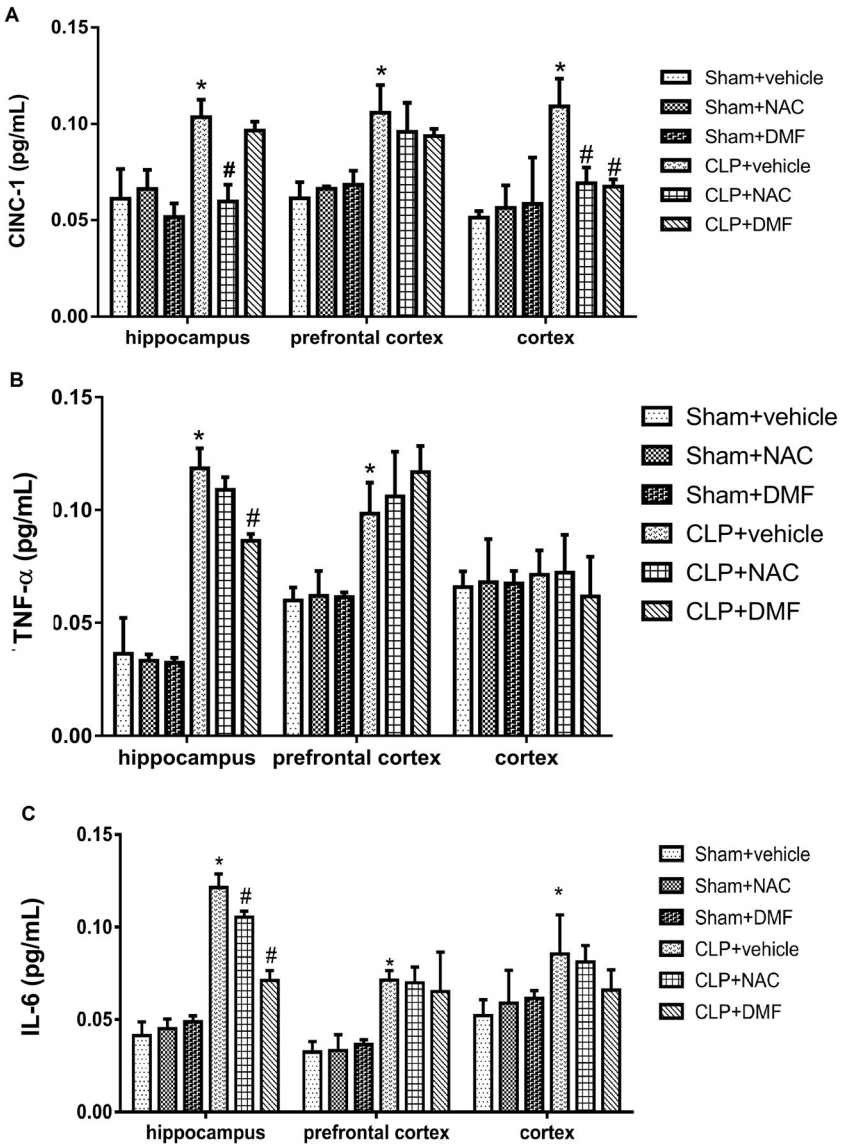

Fig. 2 CINC-1 (**a**), TNF-α (**b**), and IL-6 (**c**) levels in the hippocampus, prefrontal cortex, and total cortex of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is expressed as mean ± standard deviation, analyzed by one-way ANOVA with Tukey *post-hoc* test. $^{*}p < 0.05$ compared to sham + vehicle group and $^{\#}p < 0.05$ compared to CLP + vehicle group

DMF Reduces the Brain Levels of Oxidative Stress After Sepsis

Figure 3 illustrates nitrite/nitrate (N/N) concentration at 24 h after CLP surgery. We observed an increase in N/N concentration in the hippocampus and total cortex of septic animals (CLP + vehicle group), while septic animals treated with DMF (CLP + DMF group) showed a decrease in N/N concentration in the hippocampus and total cortex. With regard to lipid peroxidation (Fig. 4a), there was an increase in MDA equivalent levels in all brain structures of rats subjected to sepsis, while DMF-treated animals exhibited decreased lipid peroxidation in the hippocampus and total cortex. Oxidative damage to protein is represented by protein carbonyls levels (Fig. 4b). All brain structures of CLP + vehicle group were significantly affected, and DMF treatment was effective in diminishing the levels of this oxidative marker in all structures.

SOD activity was decreased in the three brain structures of septic animals (Fig. 5a). DMF treatment was effective in increasing SOD activity only in the hippocampus. When CAT activity was analyzed (Fig. 5b), no changes were observed due to sepsis induction nor the treatments. On the other hand, there was a decrease in GPx activity (Fig. 5c) in all brain structures of CLP + vehicle rats, and the treatment with DMF prevented this alteration in the hippocampus and total cortex.

DMF Prevents Memory Impairment Due to Sepsis

The open field habituation task results are represented in Fig. 6. We observed that all sham animals decreased the number of crossings between the training and test sessions, indicating an adequate memory retention (Fig. 6a). Animals subjected to sepsis (CLP + DMS group) did not present significant difference between both sessions, which demonstrate memory impairment. On the contrary, CLP animals treated with DMF or NAC showed a similar behavior to sham animals, indicating no memory deficit. Regarding the number of rearings (Fig. 6b), there was no difference between treated and untreated CLP groups. Concerning the novel object recognition task (Fig. 7), we noted that animals subjected to sepsis presented memory damage to recognize a new object in short-term (Fig. 7a) and long-term (Fig. 7b) evaluations. Treatment with DMF significantly prevented this alteration only in the short-term evaluation.

Discussion

The development of sepsis cerebral dysfunction contributes to its high mortality, especially because of the exacerbated inflammatory response, as well as the oxidative stress, which causes neurotransmitters and behavioral alterations that negatively impact quality of life (Sonneville et al. 2013). In the present study, we showed for the first time that DMF was effective in reversing neuroinflammation, oxidative brain damage, and cognitive impairment in an animal model of sepsis that is characterized by inducing cognitive impairment associated with deleterious effects in the brain tissue.

The systemic inflammatory response associated with sepsis pathophysiology induces the activation of BBB endothelial cells, which result in the stimulation of proinflammatory mediators in the cerebral parenchyma (Sonneville et al. 2013). Besides, proteins such as matrix metalloproteinases (MMP)-2 and 9 are incite the degradation of tight junctions that guarantee the BBB integrity and its functions (Vafadari et al. 2016). Also, cytokines can activate these MMPs, propitiating the alteration in BBB permeability. Previous research verified that BBB permeability is negatively altered within 24 h after sepsis onset (Dal-Pizzol et al. 2013), being accompanied by increased neutrophil migration to cerebral structures, such as the hippocampus (Comim et al. 2011b). In the present study, we observed an increased neutrophil infiltration, represented by MPO activity, in all brain structures, and the administration of DMF was effective in attenuating this alteration.

DMF, along with its active metabolite monomethyl fumarate (MMF), has been tested to treat inflammatory- and

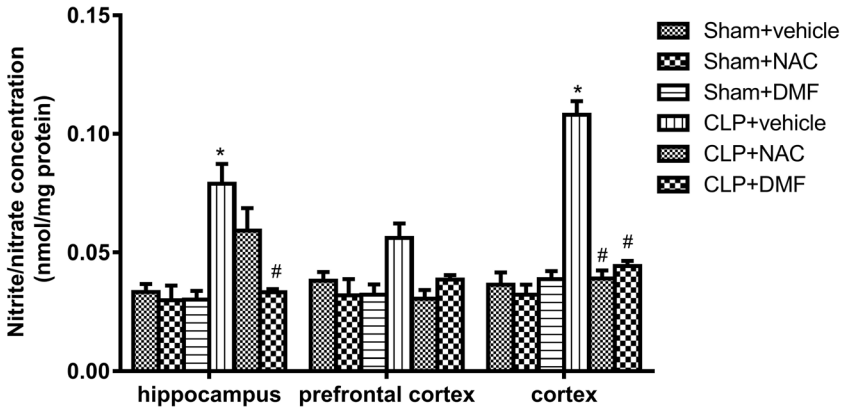

Fig. 3 Nitrite/nitrate concentration in the hippocampus, prefrontal cortex, and total cortex of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is expressed as mean ± standard deviation, analyzed by one-way ANOVA with Tukey *post-hoc* test. $^{*}p < 0.05$ compared to sham + vehicle group and $^{\#}p < 0.05$ compared to CLP + vehicle group

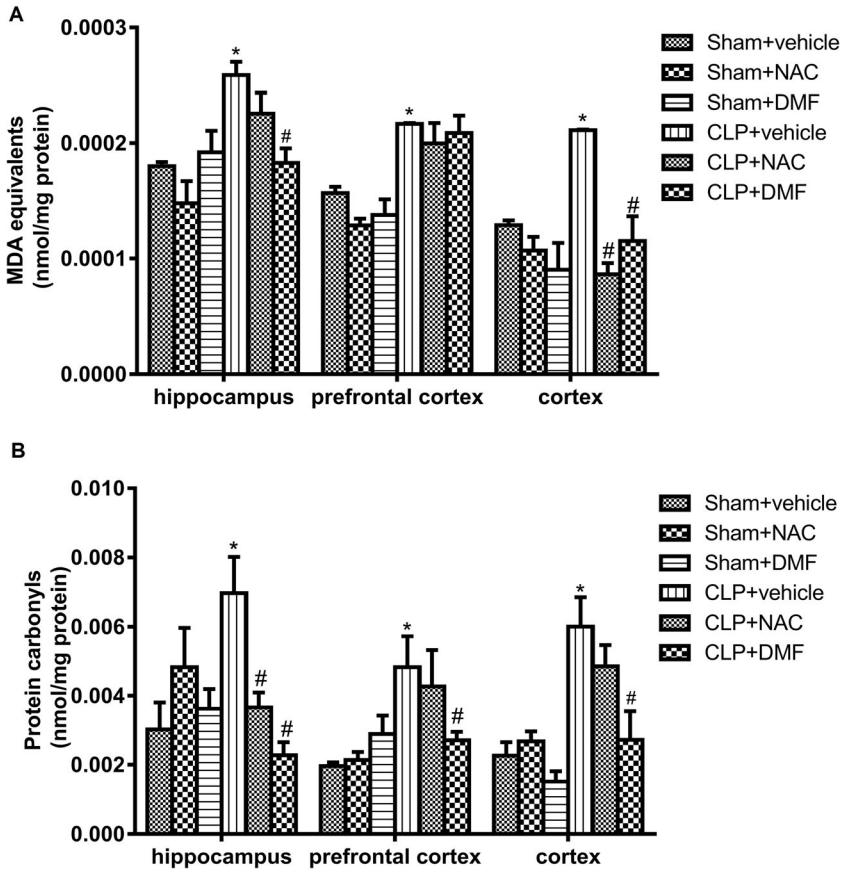

Fig. 4 Lipid peroxidation (**a**) and protein carbonylation (**b**) in the hippocampus, prefrontal cortex, and total cortex of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is expressed as mean ± standard deviation, analyzed by one-way ANOVA with Tukey *post-hoc* test. $^{*}p < 0.05$ compared to sham + vehicle group and $^{\#}p < 0.05$ compared to CLP + vehicle group

oxidative stress-related diseases (Gill and Kolson 2013). After oral administration, DMF is metabolized to MMF, reaching the CNS and having a direct effect on resident cells (Wilms et al. 2010). DMF is considered a potent oral immunomodulatory treatment for patients with relapsing-remitting multiple sclerosis due to its ability to cross the BBB, thus exerting neuroprotection by modulating microglial activation (Wilms et al. 2010). Activated microglia is a critical element of the neuroinflammatory cascade, as this cell starts to release pro-inflammatory cytokines and reactive species that leads to neurodegeneration and altered synaptic transmission, as occurs in neurodegenerative diseases such as multiple sclerosis (Wilms et al. 2010). Consequently, DMF can modulate neuroinflammation by diminishing microglial activation and proinflammatory cytokines levels, with less attraction of immune cells to the region (Lin et al. 2016).

A study of multiple sclerosis (MS) using the experimental model of encephalomyelitis showed that neutrophils infiltrated the CNS, and DMF treatment was able to reverse it through hydrocarboxylic acid activation, a membrane receptor coupled to the G protein. Hydrocarboxylic acid activates the protein extracellular signal-regulated kinase (ERK), which is able to decrease neutrophils migration to brain endothelial cells (Chen et al. 2014). Of note, DMF has been tested in patients with relapsing-remitting MS in clinical trials and the current findings demonstrate significant benefits, including improved quality of life, reductions in relapses, number of new lesions, and lesion volume (Kita et al. 2014; Miller et al. 2015).

Rats subjected to an ischemic stroke model not only presented reduced neutrophil infiltrate with DMF treatment, but also displayed a smaller number of activated microglia in the cerebral ischemic region and decreased levels of proinflammatory cytokines (Lin et al. 2016). Chemokines such as CINC-1, the mouse homolog for interleukin-8 (IL-8) in humans, play a key role in neutrophil chemotaxis and allows the release of other proinflammatory cytokines, e.g. TNF-α and IL-6 (Reddy et al. 2008). When glial cells recognize these molecules, it activates different intracellular pathways, including the transcription factor NF-κB in microglia, with consequent elevation of proinflammatory cytokines (Parodi et al. 2015). In our study, CINC-1, TNF-α, and IL-6 levels were elevated and followed the profile of increased MPO activity in the brain structures. Particularly, studies showed the presence of constitutive cytokine (IL-1β, IL-6, TNF-α) and chemokine receptors around the brain (Cunningham et al. 2002; Otero and Merrill 1994). It appears that in adult mice, a majority of these receptors are located in the hippocampus (Banisadr et al. 2002; Dopp et al. 1997). This may explain the reason for the vulnerability of these regions to the systemic inflammatory

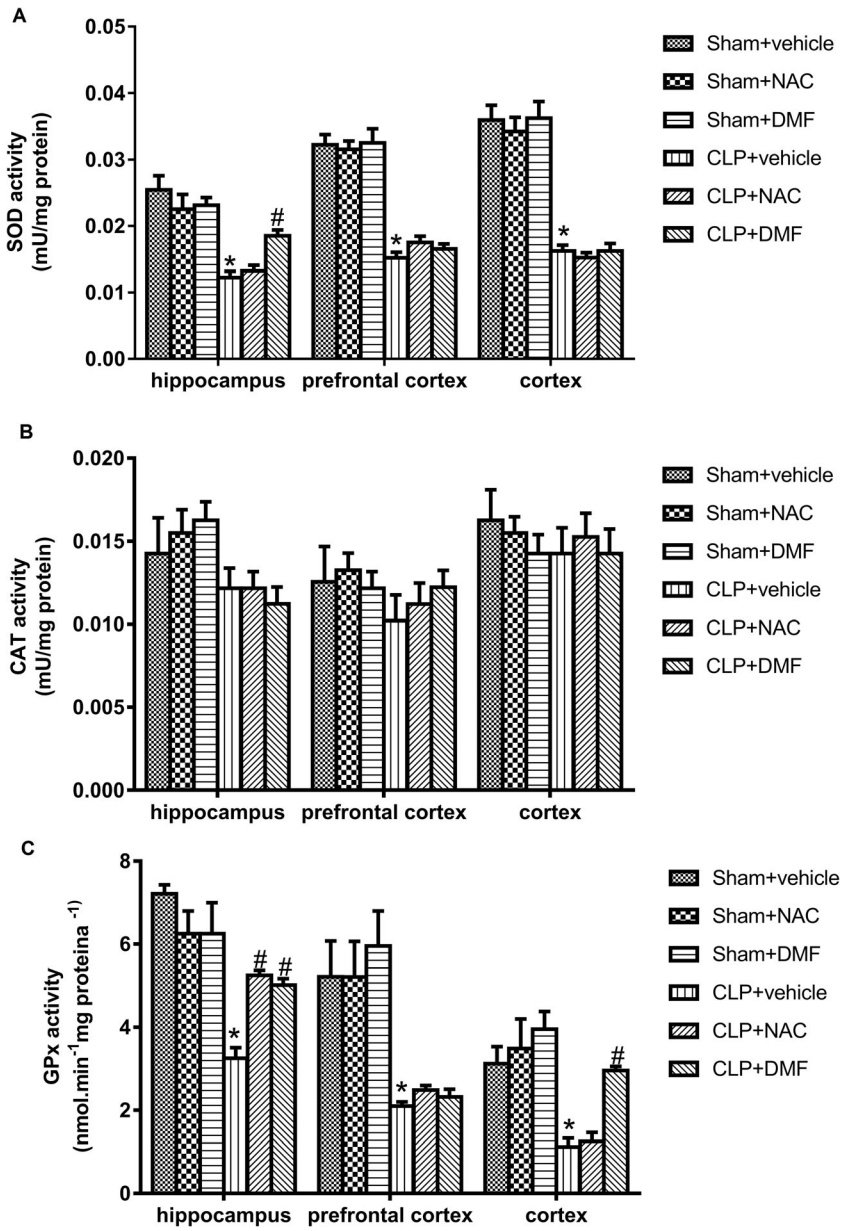

Fig. 5 SOD (**a**), CAT (**b**), and GPx (**c**) activity in the hippocampus, prefrontal cortex, and total cortex of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is expressed as mean ± standard deviation, analyzed by one-way ANOVA with Tukey *post-hoc* test. $^{*}p < 0.05$ compared to sham + vehicle group and $^{\#}p < 0.05$ compared to CLP + vehicle group

response and oxidative stress in sepsis and the effectiveness of DMF treatment.

When activated by bacterial toxins or inflammatory mediators, NF-κB stimulates genes that encode proinflammatory cytokines (Leibowitz and Yan 2016). After microglial activation by lipopolysaccharide (LPS), DMF decreased the release of proinflammatory cytokines through a reduction in NF-κB activation (Michels et al. 2015b), and this antiinflammatory role played by DMF has been demonstrated in several studies (Gerdes et al. 2007; Ghoreschi et al. 2011; Gillard et al. 2015; Kastrati et al. 2016). However, NF-κB inhibition occurs because DMF activates another transcription factor, the Nrf2. Studies using $Nrf2^{-/-}$ knockout mice showed increased instigation of NF-κB and synthesis of cytokines and chemokines after an inflammatory insult by LPS (Wakabayashi et al. 2010) and after a traumatic brain injury (Jin et al. 2008).

Sepsis is associated with increased ROS/RNS production, decreased antioxidant capacity, and the occurrence of oxidative damage (Petronilho et al. 2012; Annane and Sharshar 2014; Vieira et al. 2015). Thus, the inflammatory response in sepsis is characterized by the excessive release of cytokines, associated with increased oxidative stress, leading to mitochondrial dysfunction with consequent cell death (Comim et al. 2011a).

The brain is often exposed to reactive species because of its high oxygen consumption, and during sepsis, this organ is rapidly affected (Comim et al. 2011a), since there is an expressive increase in superoxide (O_2^-) anion (Chuang et al. 2002),

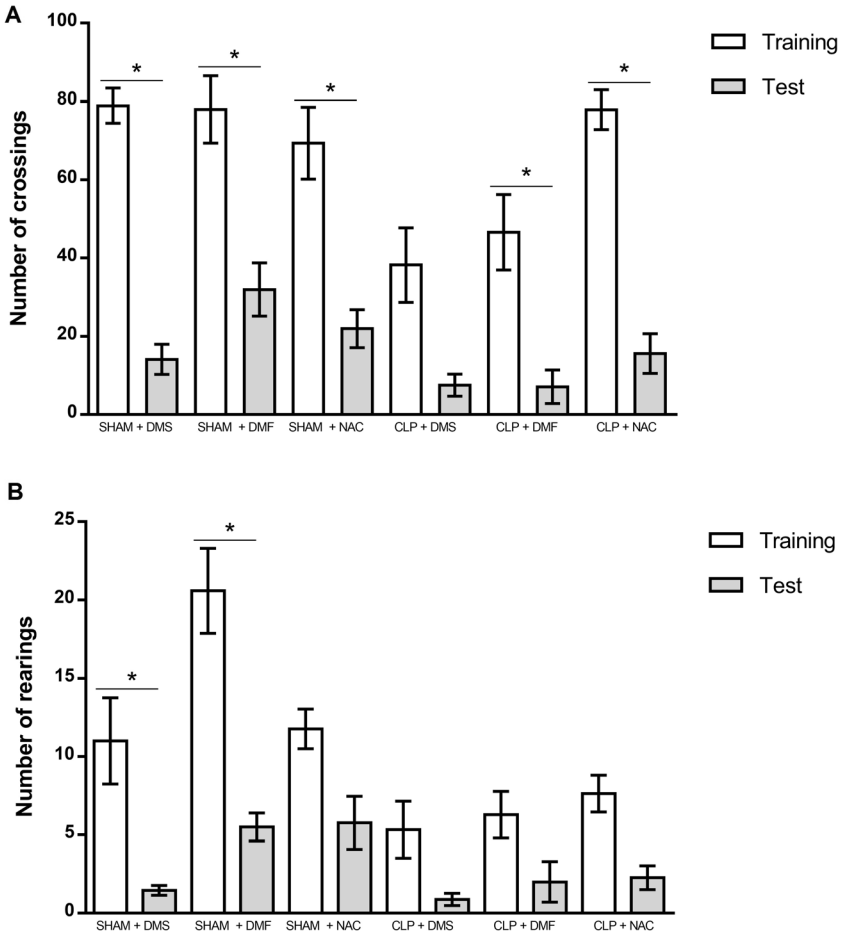

Fig. 6 Number of crossings (**a**) and rearings (**b**) in the habituation to the open field task of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is presented as median ± interquartile rage, analyzed by Mann-Whitney and Wilcoxon test. *$p < 0.05$ compared to training session

nitric oxide (NO), and peroxynitrite (ONOO-) production in the brain tissue after sepsis onset (Berg et al. 2011). As a consequence, mitochondrial degeneration and apoptosis have been observed in different brain regions of septic animals and also in human patients (Bozza et al. 2013; Kasahara and Inoue 2015). In our study, DMF regulated N/N levels, as indicative of NO production, in the hippocampus and prefrontal cortex. Wilms and colleagues have previously shown that DMF decreases NO synthesis in microglia and astrocytes cell culture stimulated with LPS and such response depends on Nrf2 activation (Wilms et al. 2010). Our results demonstrate that the decrease of N/N due to DMF reflected in the reduced oxidative damage to lipids and proteins.

Antioxidant compounds usually act by scavenging reactive species, thus protecting the cells from oxidative damage and reducing neurological conditions related to oxidative stress (Sitar et al. 2016). In sepsis, the production of antioxidant enzymes such as SOD, CAT, and GPx, is disrupted, and our results corroborate the literature, as SOD and GPx activities were decreased in the brain of CLP + vehicle animals. Also, the endogenous machinery is normally satisfactory to restore redox balance and an adequate cellular function; however, sepsis promotes a very intense inflammatory and oxidative response that is not easily diminished by the body itself. This is well observed when we compare sham + vehicle and CLP + vehicle groups, as septic animals display a pronounced reduction in the antioxidant defense system.

When challenged with situations like inflammation and oxidative stress, the primary mechanism for maintenance of cellular redox balance and to reestablish homeostasis is the Nrf2 pathway. Usually, Nrf2 is removed in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) and targeted for degradation. However, reactive species and electrophiles modify cysteine residues on Keap1 that lead to conformational changes and reduce the affinity of KEAP1 for Nrf2, thus allowing Nrf2 to initiate the transcription of many genes involved in the antioxidant and antiinflammatory responses (Itoh et al. 2003, 2004; Kobayashi and Yamamoto 2006; Giustina et al. 2017).

Importantly, DMF acts like an electrophile on Keap1, limiting Nrf2 degradation (Brennan et al. 2017). Thus, DMF contributes to reestablish the levels of antioxidant enzymes. Here we demonstrate that DMF treatment improved the antioxidant activity of SOD and GPx in the hippocampus and total cortex

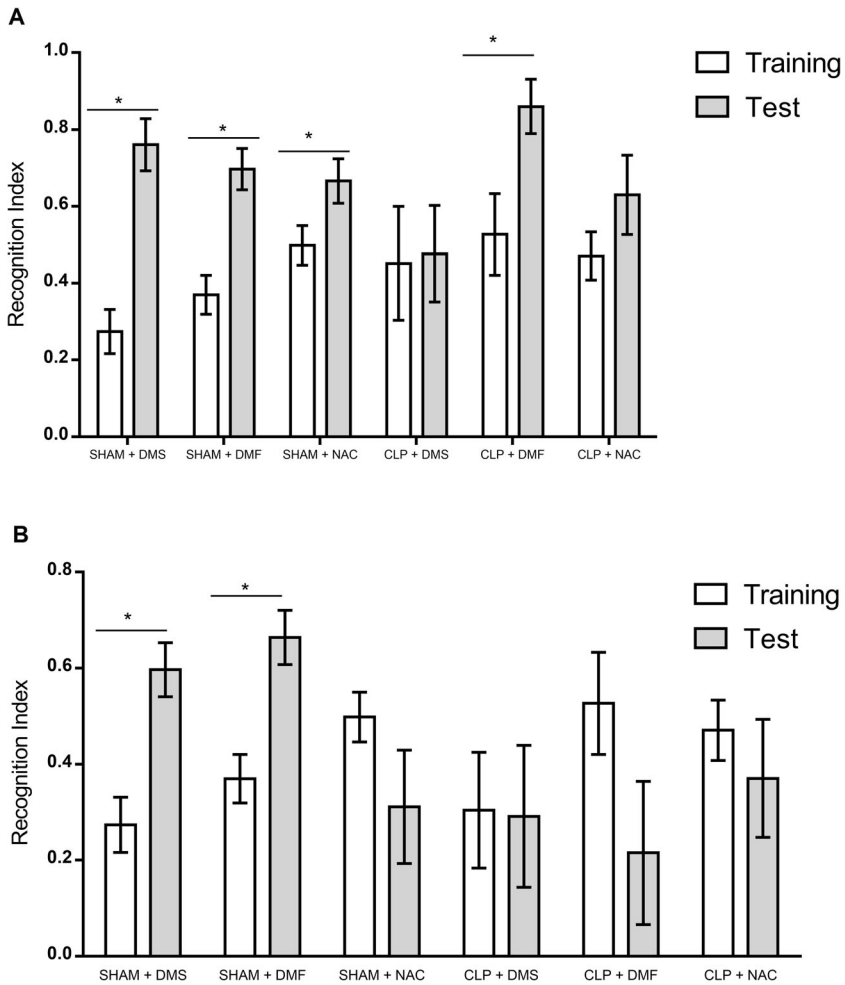

Fig. 7 Short-term (**a**) and long-term (**b**) novel object recognition index of rats subjected to polymicrobial sepsis and treated with DMF or NAC. Data is presented as median ± interquartile rage, analyzed by Mann-Whitney and Wilcoxon test. *$p < 0.05$ compared to training session

of septic rats. Similar findings were also observed by our group in peripheral organs of septic rats subjected to a DMF treatment (Giustina et al. 2017).

In addition, DMF also has cytoprotecting properties in glial cells, oligodendrocytes, and neurons (Bomprezzi 2015). A recent study showed that DMF, by activating Nrf2, attenuated neurological deficits and cerebral edema at 24 and 72 h after an experimental model of intracerebral hemorrhage (Iniaghe et al. 2015). Such positive cytoprotecting impact also influences cognitive function, which is normally disrupted in rodents subjected to the CLP model up to 30 days after surgery, even though they seem fully recovered from infection and motor alterations (Barichello et al. 2005; Tuon et al. 2008; Michels et al. 2015a). In our study, we found that DMF treatment was effective in reversing memory impairment evaluated in the habituation to an open field and novel object recognition tasks, and these findings corroborate with previous studies that evaluated different compounds with directly or indirectly antioxidant effect, e.g. α-lipoic acid (Della Giustina et al. 2017) and vitamin B6 (Danielski et al. 2017).

In the present research, we decided to use NAC as a positive control because it is a classical antioxidant precursor of amino acid cysteine, which is necessary to synthetize glutathione (Minarini et al. 2017). In sepsis, several studies have related the effectiveness of NAC in reducing oxidative damage, both in peripheral and in the CNS (Ritter et al. 2004; Cassol-Jr et al. 2010; Andrades et al. 2011). The use of DMF, on the other hand, seems relevant due to its multiple therapeutic targets beyond the evaluations performed in the present study. Sepsis suppresses the immune function, and one of DMF differentials is its potent immunomodulatory effect (Schulze-Topphoff et al. 2016).

Importantly, our results concerning neutrophil infiltrate have some limitations. Sepsis increases blood/serum MPO activity, and this may have contributed to the MPO activity observed in our results, as we used non-perfused brain tissue to perform the analysis. However, to our knowledge, this research provides novel information about DMF effects in the CNS of septic rats. Therefore, we demonstrated in the present study that DMF reduces sepsis-induced neuroinflammation, oxidative stress, and cognitive impairment in rats subjected with a polymicrobial model of sepsis.

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Compliance with Ethical Standards

All experimental procedures were approved by the Animal Care and Experimentation Committee of UNISUL (protocol number 15.009.4.03.IV), Brazil.

Conflict of Interest The authors declare that they have no conflict of interest.

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