Original Paper

The antidepressant effect of bone marrow mononuclear cell transplantation in chronic stress

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Abstract

Background: Inflammation could be a risk factor for the development of depression and change the outcome of this common chronic-recurrent mental disorder.

Aims: This study aimed to investigate if bone marrow mononuclear cell (BMMC) transplantation is effective in restoring sucrose preference in rats subjected to chronic stress (CS), if it has an anti-inflammatory effect and is able to restore damaged DNA.

Methods: The effect of BMMC transplantation was studied in a controlled protocol (compared with a control group and a selective serotonin reuptake inhibitor escitalopram group) involving sucrose preference in CS in rats. Measurements were taken of the amygdala, hippocampus, frontal cortex, and other brain areas, the spleen and blood pro-inflammatory cytokines, namely interleukin-1β, interleukin-6, tumor necrosis factor-alpha, and interferon-gamma, as well as anti-inflammatory cytokine interleukin-10. Finally, 8-hydroxy-2′-deoxyguanosine (a DNA damage marker) was determined.

Results: BMMC transplantation was as effective as escitalopram in restoring sucrose preference. It also had an anti-inflammatory effect and slightly improved damaged DNA after one week.

Conclusions: These findings suggest administration of BMMC in rats subjected to CS restores sucrose preference, resolves inflammation in both the peripheral and central nervous system, as well as diminishes DNA damage. This effect was similar to that of escitalopram, which is effective in the treatment of depressive patients.

Keywords
Depression, bone marrow mononuclear cells, escitalopram, chronic stress

Introduction

Depression is a common chronic-recurrent mental disorder that causes high morbidity. Recently, the World Health Organization reported that globally >300 million people of all ages suffer from depression, highlighting depression as the leading cause of disability worldwide (WHO, 2017).

Depression, like many other psychiatric conditions, presents complex inflammatory aspects (Miller and Raison, 2016; Slavich and Irwin, 2014; Woelfer et al., 2019). Clinical studies have found that patients with anxiety and depression show increased serum levels of inflammatory markers, such as interleukin-1β (IL-1β), IL-6, tumor necrosis factor-alpha (TNFα) (Köhler et al., 2017; Russo and Nestler, 2013). In recent years, efforts have been made to understand the role of inflammation in depression and develop anti-inflammatory strategies to help in its treatment (Rosenblat et al., 2014; Woelfer et al., 2019), to prevent its progression (Rosenblat et al., 2014), and to avoid the development of other inflammatory diseases associated with depression such as vascular disease and diabetes (Katon et al., 2008; Nicholson et al., 2006). Neuroinflammation due to elevated pro-inflammatory cytokine levels is associated with reduced hippocampal neuroplasticity (e.g. neurogenesis and long-term potentiation and oxidative stress; Eyre and Baune, 2012).

All the anti-inflammatory strategies investigated to date have been of limited impact on patient outcomes (Rosenblat et al., 2014). For example, N-acetylcysteine showed little effect on chronic treatment (Rosenblat et al., 2014). Hence, there is a need to develop anti-inflammatory strategies to normalize this aspect of depression and investigate whether a robust anti-inflammatory strategy might improve depression.

Recent findings from our laboratory suggest bone marrow mononuclear cell (BMMC) transplantation might have the

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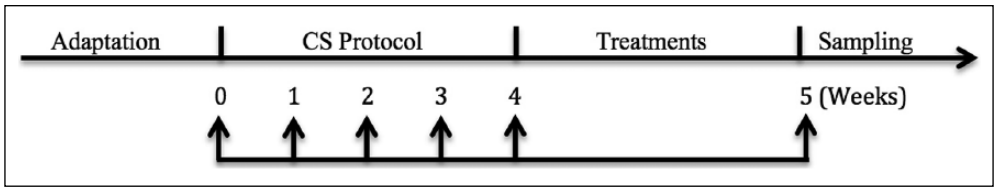

Figure 1. The experiment flowchart showing the adaptation period (until week 0), the CS protocol (from week 1 to 4), the sucrose preference test applications (weeks 0 to 5), the treatment application period (from week 4 to 5), and sampling (after week 5).

potential to repair many types of damaged tissue (Costa-Ferro et al., 2010, 2012; Leal et al., 2014; Venturin et al., 2011; Zanirati et al., 2015). Bone marrow is a semi-fluid component of bone that consists of a diverse population of cell types including those involved in bone development, hematopoiesis, tissue remodeling, and endocrine regulation (Pierce et al., 2019). BMMCs are composed of mesenchymal stem/stromal cells and hematopoietic progenitor cells, which are of potential therapeutic value in promoting tissue repair and regeneration and providing a neuroprotective effect in some disease conditions (Borlongan, 2011; Costa-Ferro et al., 2012). BMMCs have been shown to effectively mediate an anti-inflammatory effect through down-regulation of the inflammatory cytokine IL1, IL-6, and TNF-α, and concomitant upregulation of IL-10 (Costa-Ferro et al., 2012; Leal et al., 2014).

Herein, we hypothesized that BMMCs play a positive role in reducing inflammatory cytokine levels after a sequence of variable, unpredictable chronic stressors (CS). This animal model of depression deviates from the original Chronic Mild Stress proposed by Willner (2005) since we use a combination of mild and moderate stressors. To address this hypothesis, one group of CS rats received a preparation of BMMCs injected into the tail vain, while another group received the selective serotonin reuptake inhibitor (SSRI) escitalopram via peritoneal injection. This design makes it feasible to discriminate between the antidepressant effects of the two strategies (BMMC and escitalopram) in unpredictable CS conditions.

Methods

Animals

For the purposes of this study, 40 two-month-old male Wistar rats were obtained from the Animal House of the Centro de Modelos Biológicos Experimentais of the Pontifícia Universidade Católica do Rio Grande do Sul. The animals were housed in a rat vivarium, four per cage, on a ventilated rack with controlled temperature and humidity. Food and water were available ad libitum, except when food or/and water deprivation was applied as a stress parameter. After a two-week acclimation period, rats were randomly divided into two groups (the CS group and the control group). Rats that received CS ($n = 30$) were housed individually, except when grouping was applied as a stressor. The controls ($n = 10$) were housed in groups in a separate room under standard conditions. The rats were then exposed to the sucrose preference test (Figure 1) once a week. The CS rats were continually exposed to a sequence of variable stressors (CS protocol; see below) for 37 days. On the 30th day of the CS protocol, the rats were divided into three groups: those treated with BMMC (CS-BMMC); saline solution (CS-S), one single injection for seven days; and the selective serotonin reuptake inhibitor antidepressant, escitalopram (CS-SSRI) for seven days. All the CS rats continued to be submitted to the CS protocol until the end of the experiment (Figure 1). The rats in the control group were not exposed to any of the stressors and had free access to food and water. All procedures were in accordance with the guidelines for the care and use of laboratory animals adopted by the National Institute of Health (USA), and with the university's ethical committee, Comissão de Ética de Uso de Animais da Pontificia Universidade Católica do Rio Grande do Sul (CEUA 7943-PUCRS).

CS protocol

To induce unpredictable CS, the animals were subjected to a sequence of events: white noise, social isolation, crowding, 45° box tilting, 24-h food deprivation, 24-h water deprivation, 12-h food and water deprivation, predator odor, tail clamping three sets of three, dark during light cycle, and strobe light. All stressors were of 5–12-h duration and applied individually and continuously, day and night. All the procedures were randomly organized to ensure the unpredictable nature of the experiment. This procedure has been demonstrated to induce depression-like behavioral changes and was based on the protocol used by Baptista et al. (2015).

BMMC and drug administration

Bone marrow cells were harvested from male Wistar rats. The animals were killed using 200 μl of 8% ketamine hydrochloride (Cristália, Brazil) and 2% chlorpromazine (União Química, Brazil), and then dissected. Fresh bone marrow was extracted from the humerus, femurs, and tibiae by flushing with phosphate buffered saline (PBS). After centrifugation, the cell pellet was resuspended in Rosewell Park Memorial Institute medium and fractionated on a density gradient generated by centrifugation over a Ficoll–Hypaque solution (Histopaque 1119 and 1077, 1:1; Sigma, St Louis, MO) at 400 g for 30 min at room temperature. The mononuclear fraction over the Ficoll–Paque layer was collected and washed twice with PBS. Phenotypic characterization of the BMCs was performed using flow cytometry analysis. Briefly, the BMCs were washed in PBS and incubated at 4°C with fluorochrome-conjugated antibodies: CD31, CD45, CD49, and CD90 (1:100, BD Biosciences, Franklin Lakes, NJ). Data acquisition and analysis were performed using a FACSCalibur cytometer and CellQuest software, respectively (BD Biosciences, Franklin Lakes, NJ). At least 50,000 events were collected and analyzed. The cell

markers below were expressed in the BMMCs at the following frequencies: CD31 (5.34%), CD45 (3.12%), CD49 (6.01%), and CD90 (7.14%) (Supplemental Figure 1).

On the 30th day of the CS protocol, one group of animals received a single injection of 10^7 (CS-BMMC, $n = 10$) cells in the tail vein (Costa-Ferro et al., 2010), another group received an equal volume of vehicle (CS-S, $n = 10$, intraperitoneally). A third group was given escitalopram (Lundbeck, Denmark) dissolved in physiological saline at 5 mg/kg, twice a day (Jayatissa et al., 2006) for seven days (07:00–19:00, CS-SSRI, $n = 10$, intraperitoneally).

Behavioral analysis

Sucrose preference test

To test for anhedonic behavior, two water bottles were placed in the housing cage, one with normal tap water and one with a 1% sucrose solution. The bottles were left for 12 h overnight, then the consumption in mL from each bottle was measured and the percentage of sucrose solution from total intake was calculated. Prior to this test, there were no stressors that would directly affect liquid consumption. The first test was used as baseline for the animals. The following tests were performed at 30 days of CS, and one week after the treatments (BMMC, saline, or escitalopram injections) with CS procedures maintained. The percentage of sucrose preference was calculated based on the following formula (Baptista et al., 2015): % sucrose preference = sucrose consumption / (sucrose + water consumption) × 100.

Tissue preparations

Once testing was completed, the rats were anesthetized with isoflurane and decapitated to extract the entire brain, which was dissected on ice. The amygdala, frontal cortex, hippocampus, and other brain structures were removed in that order, followed by the spleen and blood, all of which were frozen and stored at −80°C for analyses of the oxidative stress and cytokines.

Cytokines and 8′2-deoxyguanosine. The isolated brain structures were mechanically macerated with the aid of sterile slides and petri dishes containing Hanks' medium. The homogenate remained in an incubator for 2 h at 37ºC, with 5% CO_2 saturation. Then the material was centrifuged at 2000 rpm for 10 min, and the supernatant was removed and used as a sample for analysis of the cytokines and 8'2-deoxyguanosine. Tissue levels of the IL-1β, IL-6, TNF-alpha, IFN-γ, and IL-10 cytokines were measured using Quantikine Human Kits (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. DNA damage was determined by measuring 8'2-deoxyguanosine using an ELISA immunoassay kit obtained from Abcam (Cambridge, MA, USA) according to the manufacturer's instructions.

Statistical analysis

All data were analyzed using two-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA) software. The significance level was set at $P \leqslant 0.05$.

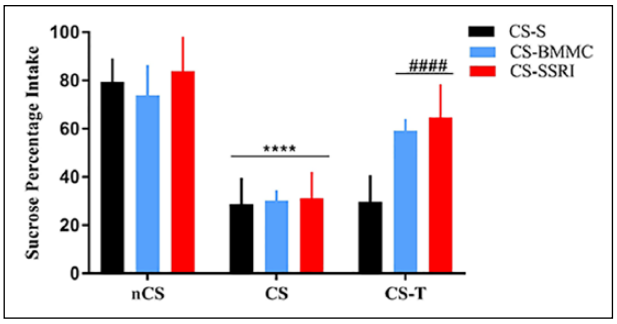

Figure 2. Spontaneous sucrose consumption (% sucrose preference = sucrose consumption / (sucrose + water consumption) × 100) is shown for the three groups (CS, SSRI, and BMMC; 10 rats per group) on day 0 (nCS), after 38 days of the CS protocol (CS), and one week after treatment. Stress diminished sucrose preference, while both BMMC transplantation and escitalopram reversed this stress-induced diminution: nCS (before Chronic Stress), CS (during Chronic Stress and before treatment) and CS-T (after treatment with Escitalopram or BMMC or Saline).
####$p < 0.0001$, CS vs CS-T groups.
****$p < 0.0001$, nCS vs CS (CS-T, treatments: saline, BMMC, or SSRI).

Results

BMMC transplantation restores spontaneous sucrose consumption in CS rats. We investigated the spontaneous preference for sucrose or water consumption in the four groups described in the Methods section (control, CS-S, CS-BMMC, and CS-SSRI) every week (before and during the CS protocol as well as before and after treatment).

Repeated ANOVA measures found significant interaction between groups and treatment (F (4, 93) = 12.29; $p < 0.0001$) regarding sucrose preference. All groups showed a preference for sucrose consumption vis-a-vis water consumption before the CS procedures (no statistical difference between the groups; $p > 0.05$). CS-S exhibited lower sucrose preference compared to the control group ($p < 0.001$). Post hoc analysis found that CS-BMMC and CS-SSRI animals had significantly higher sucrose preference compared to CS-S animals on day 37 (CS-S vs CS-BMMC, $p = 0.0031$; CS-S vs CS-SSRI, $p < 0.0001$) (Figure 2).

The effects of BMMC and escitalopram treatments on CS induced alterations in brain areas, blood, and spleen IL-1β, IL-6, TNFα, and INF-γ levels. As shown in Figure 3, CS induced a significant increase in the levels of IL-1β, IL-6, and TNF-α in the amygdala, hippocampus, frontal cortex, other brain structures, spleen, and blood compared to the control group. A two-way ANOVA (amygdala, hippocampus, frontal cortex, other brain structures, spleen, and blood vs groups) confirmed the CS protocol had an inflammatory effect.

IL-1β levels increased in all of the analyzed brain regions, spleen, and blood in the CS groups. Two-way ANOVA found there was an inflammatory effect × CS interaction (F (15, 48) = 160.7; $p < 0.0001$). No significant differences in IL-1β levels were found between the CS-BMMC and control groups in

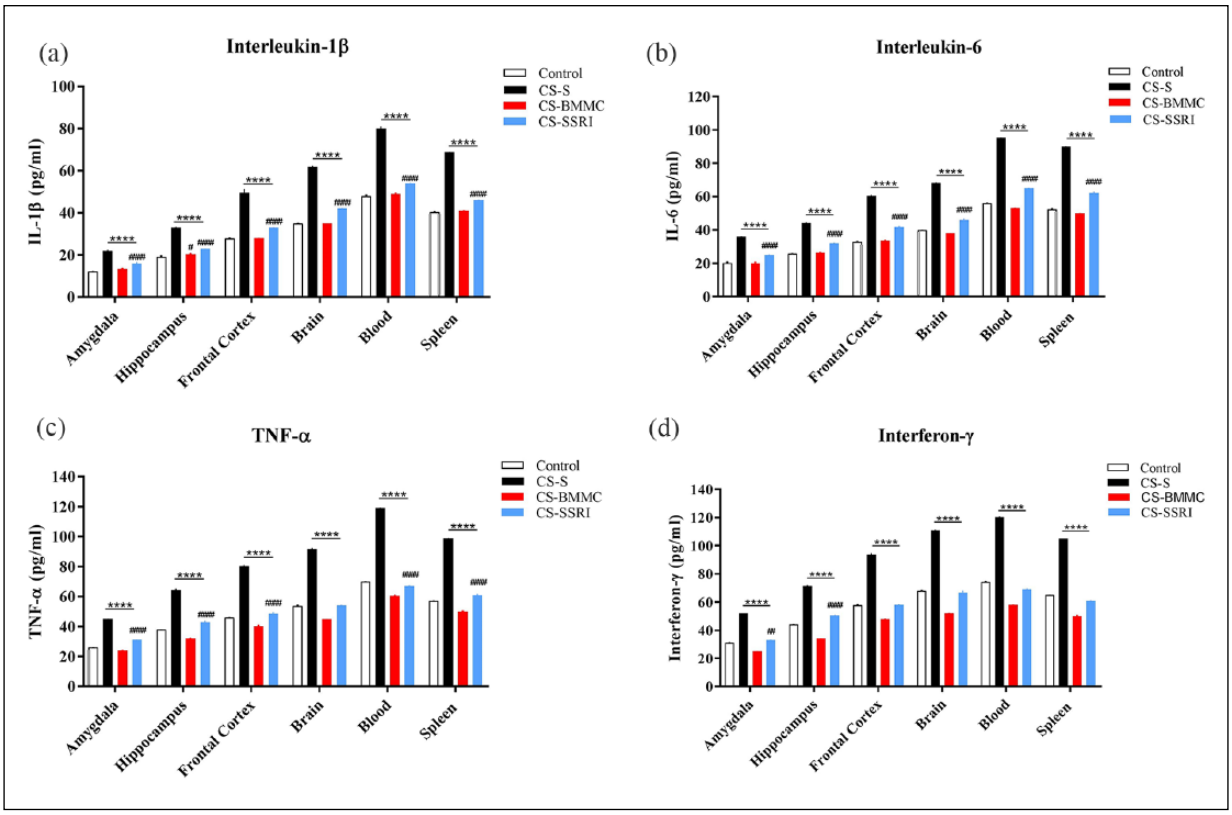

Figure 3. The effects of the BMMC and escitalopram (5 mg/kg) treatments on stress-induced alterations in brain regions, spleen, and blood inflammatory cytokines (a) IL-1β, (b) IL-6, (c) TNFα, and (d) INF-γ levels (10 rats per group). In all analyzed areas, stress increased IL1β, IL6, and TNF-α levels in the CS-S, while both CS-BMMC and CS-SSRI decreased those levels.
$^{\#}p < 0.05$.
$^{\#\#}p < 0.001$.
$^{\#\#\#\#}p < 0.0001$ CS-S, CS-BMMC, or CS-SSRI vs control group.
$^{****}p < 0.0001$ CS-S vs CS-BMMC or CS-SSRI groups.
Brain: other brain structures.

the amygdala, frontal cortex, other brain structures, spleen, and blood ($p > 0.05$) with the exception of the hippocampus ($p < 0.05$) (Supplemental Table 1). However, in all the analyzed areas there was a significant difference between the CS-SSRI group and control group ($p < 0.0001$; Supplemental Table 1)

IL-6 levels increased in all the analyzed brain regions, spleen, and blood in the CS groups. Two-way ANOVA found there was an inflammatory effect × CS interaction (F (15, 48) = 257.8; $p < 0.0001$). Post hoc analysis confirmed there was no statistical difference in IL-6 levels in the amygdala, hippocampus, and frontal cortex between the CS-BMMC group and control group. However, there were slightly lower levels of this cytokine in the other brain areas, blood, and spleen ($p < 0.0001$) of the CS-BMMC rats (Supplemental Table 2). Nonetheless, these differences were not observed in the comparison between the CS-SSRI group and control group (Supplemental Table 2).

TNF-α levels significantly increased in the CS-S group in comparison to the control, CS-BMMC, and CS-SSRI group interaction (F (15, 48) = 347.8; $p < 0.0001$). Post hoc analysis revealed that TNF-α levels were significantly reduced in the CS-BMMC and CS-SSRI groups vs CS-S group ($p = 0.0001$). CS-BMMC TNF-α levels were lower than in the control group in the hippocampus, frontal cortex, other brain regions, spleen, and blood ($p < 0.0001$), as well as in the amygdala ($p = 0.0011$; Supplemental Table 3). When comparing the CS-SSRI vs controls, the only area in which statistical difference was found was the other brain structures ($p = 0.9035$; Supplemental Table 3).

Interferon-γ levels increased in all the analyzed brain regions, spleen, and blood in the CS-S group compared to the control, CS-BMMC and CS-SSRI group interaction (F (15, 48) = 303.9; $p < 0.0001$). However, in the CS-BMMC, interferon-y levels were decreased in all the analyzed areas (brain regions, spleen, and blood; $p < 0.0001$) when compared to the control group. Interferon-y levels increased in the amygdala and hippocampus in the CS-SSRI in comparison with the control group (Supplemental Table 4).

The effects of the BMMC and escitalopram treatments on CS induced alterations in brain areas, spleen, and blood IL10 levels. Figure 4 illustrates the levels of the anti-inflammatory cytokine IL-10 in different brain regions, spleen, and blood of the four previously described group interactions (F (15, 48) = 75.65; $p < 0.0001$). Post hoc analysis showed the CS-S rats had lower hippocampal IL-10 levels than the control rats ($p < 0.0001$). No significant differences in IL-10 levels were found between the CS-BMMC rats and controls in the hippocampus ($p = 0.9703$), frontal cortex ($p = 0.999$), or blood ($p = 0.5423$). However, there was significant difference with higher levels in in the amygdala ($p = 0.0001$) and spleen ($p = 0.0148$) in the

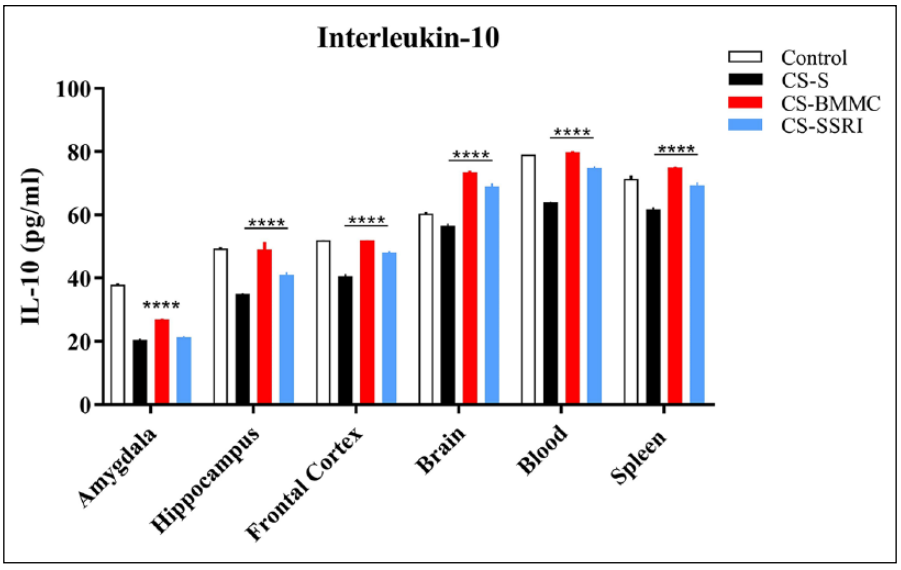

Figure 4. The effect of the BMMC and escitalopram (5 mg/kg) treatments on stress-induced alterations in IL-10 levels. BMMC significantly increased IL-10 levels when compared to the CS-S group. Escitalopram increased IL-10 levels in the areas analyzed with the exception of the amygdala ($p > 0.05$). IL-10 levels were significantly increased only in the other brain structures and spleen in the BMMC group compared to the control group, and in the CS-SSRI group those levels only increased in other brain structures when compared to the control group.
****$p < 0.0001$ CS-S vs CS-BMMC or CS-SSRI groups.
Brain: other brain structures.

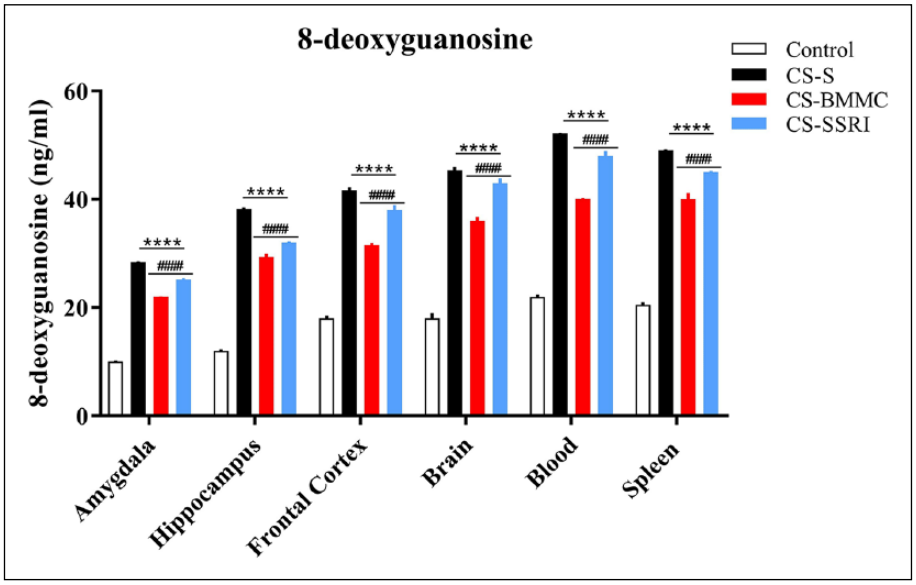

Figure 5. The effect of the BMMC and escitalopram (5 mg/kg) treatments on stress-induced alterations regarding 8′2-deoxyguanosine levels. All the groups showed significantly increased 8′2-deoxyguanosine levels compared to the control group. BMMC transplantation and escitalopram reduced 8′2-deoxyguanosine levels in stressed rats (CS-S).
****$p < 0.0001$ CS-S vs CS-BMMC or CS-SSRI groups.
####$p < 0.0001$ CS-S, CS-BMMC, or CS-SSRI vs control groups.
Brain: other brain structures.

CS-SSRI group when compared with controls (Supplemental Table 4).

CS-BMMC rats exhibited higher levels of IL-10 in the amygdala, hippocampus, frontal cortex, other brain structures, spleen, and blood ($p < 0.0001$) compared to the CS-S group. In the comparison between the CS-SSRI and CS-S groups, the results were similar in the hippocampus, frontal cortex, other brain structures, spleen, and blood ($p < 0.0001$), with the exception of the amygdala, where the levels were higher in the CS-SSRI group ($p = 0.5554$).

The effects of the BMMC and escitalopram treatments on CS induced alterations in the brain areas, spleen, and blood 8'2-deoxyguanosine levels. Figure 5 shows data from the two-way ANOVA for 8'2-deoxyguanosine levels in the previously described brain regions, spleen, and blood in the group interactions ($F(15, 48) = 40.17; p < 0.0001$). CS significantly increased 8'2-deoxyguanosine levels in all brains regions, spleen, and blood as compared to the control group ($p < 0.0001$). By contrast, administration of BMMC or SSRI to the CS-exposed rats significantly decreased 8'2-deoxyguanosine levels as compared to the CS-S group ($p < 0.0001$). Levels were higher in the CS-BMMC group compared with the CS-SSRI ($p < 0.0001$) group (Supplemental Table 5).

Discussion

Our data suggest there was an antidepressant-like effect after one week following BMMC transplantation in the CS-induced depression-like behaviors, as indicated by the sucrose preference test. In addition, in the same rats, we found production of the pro-inflammatory mediators IL-1β, IL-6, TNFα, and INF-γ, and the anti-inflammatory IL-10 was normalized.

When tested at 30 days of the CS protocol, animals presented a robust decrease in preference for sucrose in comparison with the baseline test (about 50% reduction). Although the range of decrease usually observed in such tests is between 20 and 30%, a decrease of 50% has been previously reported (Jayatissa et al., 2006; Zhou et al., 2018). We can even suppose that the animals developed an aversion to sucrose, since they preferred water to sucrose solution. This unusual response could be a result of the combination of mild and moderated stress, although even in mild stress this could happen (Janakiraman et al., 2016). It should be noted that the sucrose preference in the CS-S was the same on the 30th and 37th days, suggesting those animals displayed the maximum effect this protocol could provoke. We think that a decrease of this magnitude could be considered a measurement of anhedonia.

The results of the present study suggest BMMC transplantation had a robust anti-inflammatory effect, with an increase in anti-inflammatory cytokine IL-10 in the amygdala, hippocampus, frontal cortex, other brain structures, and outside the brain in the spleen and blood, as well as a decrease in pro-inflammatory cytokine levels (IL-1 β, IL-6, TNFα, and INF-γ) in the same structures. The findings are similar for treatment with escitalopram, with the exception of the IL-10 in the amygdala, where the medication was found to show no effect.

The role of inflammation in the pathophysiology of depression has been recognized over the last decade (Rosenblat et al., 2014; Slavich and Irwin, 2014). However, no primarily anti-inflammatory treatment alone has been shown to be effective in the treatment of depression, despite recognition of the role played by inflammation and inflammatory cytokines in the neuroprogression of major depressive disorders (Moylan et al., 2012; Setiawan et al., 2018). CS can also increase pro-inflammatory cytokine levels, such as TNFα, interleukin 1β (Il-1β), and interleukin 6 (IL-6). The present research has sought to address the question as to whether an anti-inflammatory intervention has an antidepressant effect in the CS paradigm given that BMMC cell migration was not expected to reach the brain (Costa-Ferro et al., 2012).

In our previous studies, using BMMC transplantation in epileptic rats, we found that in epilepsy, where there is a permeability of the blood–brain barrier (as opposed to depression and stress, where no such permeability is expected), a small number of transplanted cells reached the brain tissue but did not differentiate into neurons or glia (Costa-Ferro et al., 2012). However, the same study demonstrated that BMMC transplantation stimulated robust endogenous neurogenesis in the hippocampus, thus reducing neuronal loss and gliosis. Those results suggest BMMC has a paracrine effect on the brain in an animal model of epilepsy (Costa-Ferro et al., 2012). As previously demonstrated, those cells migrate preferably to bone marrow and spleen (de Freitas-Souza et al., 2012). Thus, we can speculate that it is not the migration of BMMCs to the brain that produces the antidepressant effect, but rather it is a peripheral effect. As previously demonstrated, stress-induced inflammatory signals could be transmitted from the periphery to the brain by humoral, cellular, and neural routes, suggesting the existence of intense cross-talk between brain and peripheral inflammation (Miller and Raison, 2016). Our data support the possibility that a change in cytokine-level profiles may influence such an effect. Peripherally produced pro-inflammatory cytokines are known to trigger microglia and brain macrophages, which, in turn, activate the kynurenine pathway by activating indoleamine dioxygenase and the production of reactive oxygen and nitrogen species. This mechanism is known to be related to the development of depression (Miller and Raison, 2016).

However, as BMMC transplantation is a nonspecific procedure, transplanting diverse cell populations, including lymphocytes, monocytes, mesenchymal stem cells, and hematopoietic progenitor cells and various cell-signaling peptides, our data do not allow us to state with certainty that anti-inflammation is the cause of the antidepressant effect found with BMMC transplantation.

Finally, we demonstrated that BMMC transplantation has a small effect in diminishing 8'2-deoxyguanosine, one of the major products of DNA oxidation. Inflammation and oxidative stress linked to depression could damage DNA. A meta-analysis including four studies with patients suffering from major depressive disorders, four studies with patients suffering from bipolar disorder, and five studies including patients showing depressive symptoms demonstrated 8'2-deoxyguanosine augmentation (Black et al., 2015). Biomarkers of oxidative stress have been reported in many neurological and psychiatric conditions, including Alzheimer's disease, Parkinson's disease, schizophrenia, and bipolar disorder (Butterfield et al., 2001; Martin and Teismann, 2009; Yao et al., 2006). Oxidative stress is an important mechanism in the neuroprogression of mood disorders (Rosenblat et al., 2014) and may be a common pathophysiological mechanism by which depressed individuals become vulnerable to comorbid medical conditions (Forlenza and Miller, 2006). Inflammation and oxidative stress provoked by CS and depression could also damage DNA. Our results demonstrate that CS provoked DNA oxidative damage, as evidenced by the increased 8'2 deoxyguanosine levels in the frontal cortex, amygdala, hippocampus, whole brain, spleen, and blood, which is in accordance with the literature. The present data confirm that rats exposed to the CS procedure show significant increases in levels of protein oxidation and oxidative nuclear DNA damage (Liu et al., 1996). Furthermore, they are partially concordant with another study

using mice and the CS paradigm that demonstrated augmented 8'2 deoxyguanosine levels in the frontal cortex but not in the amygdala (Maluach et al., 2017).

In the present study, although far from returning to normal levels, we also found decreased 8'2 deoxyguanosine levels in the BMMC- or SSRI-treated stressed rats compared to the saline-stressed rats, suggesting these treatments lowered oxidative stress. This result is more impressive, given that the CS protocol continued during the period of seven days after BMMC transplantation until the sample collection. The maintenance of the stress protocol after the BMMC transplantation together with the short time for DNA recovery (seven days) could have led to the persistence of DNA damage, albeit at a lower level.

Our study has several limitations. It would be interesting to determine whether, as in the rat model of epilepsy, any BMMCs migrated to the brain, and, if so, whether they differentiated into neurons or glia and, if not so, as expected, where those transplanted cells went. Moreover, in our protocol we observed the effect at one week after transplantation; thus, it would be useful to conduct tests after a longer time period. Finally, it would be helpful to identify which of the BMMCs produce the antidepressant effect.

Our data point to the need for more research into the potential effect of BMMC transplantation as a treatment for depression, mainly resistant-depression, where options are limited. In summary, this study reveals the ability of BMMCs to modulate inflammation and that the effect was similar to that found with the use of an SSRI in rats subjected to CS, but certainly with a different and original "action mechanism". Finally, it would be interesting to have a group of animals not submitted to CS protocol treated with BMMC, in order to explore the effect of those cells alone in normal rats.

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Declaration of conflicting interests

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Supplementary material

Supplemental material for this article is available online.

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