Pharmacological Reports 71 (2019) 24–31

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Imipramine treatment reverses depressive- and anxiety-like behaviors, normalize adrenocorticotropic hormone, and reduces interleukin-1β in the brain of rats subjected to experimental periapical lesion

Lutiana R. Simões[a], Soraia Netto[a], Jaqueline S. Generoso[a,*], Renan A. Ceretta[a], Rodrigo F. Valim[a], Diogo Dominguini[a], Monique Michels[b], Gislaine Z. Réus[c], Samira S. Valvassori[c], Felipe Dal-Pizzol[b], Tatiana Barichello[a,d,e]

[a] Laboratory of Experimental Microbiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[b] Laboratory of Experimental Pathophysiology, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[c] Laboratory of Neurosciences, Graduate Program in Health Sciences, Health Sciences Unit, University of Southern Santa Catarina (UNESC), Criciúma, SC, Brazil
[d] Translational Psychiatry Program, Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA
[e] Neuroscience Graduate Program, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences, Houston, TX, USA

ARTICLE INFO

Article history:
Received 13 December 2017
Received in revised form 20 June 2018
Accepted 7 August 2018
Available online 8 August 2018

Keywords:
Imipramine
Major depressive disorder
Anxiety
Inflammation
Periodontal lesion

ABSTRACT

Background: A periodontal lesion is a consequence of chronic inflammatory processes, itself triggered by a bacterial infection of the pulpal and endodontic microenvironment. Evidence suggests that periodontal lesion induction could alter inflammatory cytokines leading to behavior changes. These effects in the context of anxiety and depressive behavior have been not full investigated. We aimed to observe anxiety- and depressive-like behavioral in rodent subjected to periapical dental lesions.
Methods: Pro-inflammatory cytokines levels also were investigated in the frontal cortex and hippocampus. Parameters related to hypothalamic–pituitary–adrenal (HPA) axis activation also were evaluated. Wistar rats were divided in groups: control/saline; control/imipramine; periapical lesion/saline; and periapical lesion/imipramine. Three weeks after induction of the periapical dental lesion, they were subjected to behavioral tests.
Results: In the periapical lesion group was demonstrated anhedonic behavior and depressive-like behavior. In the elevated plus-maze test the periapical lesion group had an increase in the number of entries and spent more time in the closed arms. Imipramine treatment was able to reverse depressive- and anxiety-like behaviors. In the hippocampus and frontal cortex tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, and serum adrenocorticotropic hormone (ACTH) levels were higher in the periapical lesion group. However, rats treated with imipramine had lower IL-1β and ACTH levels.
Conclusions: Our results revealed depressive- and anxiety-like behaviors following induction of a specific dental lesion. These effects could be associated to higher levels of brain pro-inflammatory cytokines and HPA axis changes. Antidepressants treatments could be an alternative to treat comorbidities associated to periodontal lesions.

Introduction

Periodontal diseases are the most prevalent oral diseases worldwide especially in developing countries [1]. Periodontal lesions are chronic inflammatory processes triggered by bacterial infections of the pulpal and endodontic microenvironment. Periodontal lesions are described as a decrease of mineralized tissue surrounding the root apex the result of the host immune

Abbreviations: ACTH, adrenocorticotropic hormone; ASC, apoptosis-associated speck-like protein; HPA, hypothalamic–pituitary–adrenal; IL-1R, interleukin-1 receptor; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide; MDD, major depressive disorder; NaCl, sodium chloride; NLRP3, NOD-like receptor protein 3; PAMPs, pathogen-associated molecular patterns; PRRs, pattern-recognition receptors; SPSS, Statistical Package for the Social Science; TNF-α, tumor necrosis factor-α.

* Corresponding author.
E-mail address: jsg@unesc.net (J.S. Generoso).

https://doi.org/10.1016/j.pharep.2018.08.003
1734-1140/

response [2–4]. In this context, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 β (IL-1β) play an important role in the modulation of inflammatory immune responses within the periapical microenvironment. These cytokines are critical determinants in the outcome of the lesion [5–7].

The interaction between pathogen-associated molecular patterns (PAMPs) and pattern-recognition receptors (PRRs) promotes the release of mediators, such as cytokines and chemokines which propagate and regulate the response necessary to remove invasive microorganisms [8]. These peripherally produced cytokines are capable of impacting neurotransmitter processes in the brain, contributing to induction of behavioral changes. A substantial body of evidence suggests that products of peripheral inflammatory processes contribute to the pathogenesis of major depressive disorder (MDD) as well as anxiety [9]. Even in the absence of identifiable somatic illness, subjects with depressive symptoms have been shown to exhibit evidence of immune activation (i.e. higher concentration of specific inflammatory cytokines) [10]. These patients also experienced higher levels of pro-inflammatory cytokines than in normal healthy controls [11,12]. Corroborating with these results, animal models of meningitis or sepsis presented a host immune response with high levels of cytokines in the brain and depressive-like behavior [13,14]. In addition, the administration of TNF-α or IL-1β induced depressive-like behavior in rodents [15,16]. In this fashion, the immune system has been shown to be a protagonist in behavioral changes as this has been demonstrated by the aforementioned studies in both laboratory animals and human patients [11,17].

Alterations in the hypothalamic-pituitary-adrenal (HPA) axis have been found in patients with MDD [18]. Administration of pro-inflammatory cytokines or lipopolysaccharide (LPS), a constituent of the cell wall of Gram-negative bacteria, demonstrated similar results. Neonatal LPS exposure elevated the circulating levels of corticosterone activating the animal's HPA axis [19]. Previously we reported that in adult experimental meningitis, the cytokines remained elevated even when the animals were free of infection. At the same time Wistar rats showed changes in HPA axis and anhedonia which were reversed with imipramine treatment [20]. Although there is limited data on this issue, imipramine treatment effectively inhibited the production of TNF-α, IL-1β, and interleukin-6 (IL-6) in neural stem cells incubated with LPS [21,22].

Based on these observations, we hypothesized that experimental periapical lesions, a peripheral inflammation by oral microflora, could induce behavior changes. First, we investigated if periapical lesions could trigger depressive- and anxiety-like behaviours and changes in the HPA axis in Wistar rats. Second, we evaluated the levels of TNF-α, IL-1β, and IL-6 in the hippocampus and frontal cortex after a periapical lesion. Finally, we verified the effects of imipramine treatment on depressive- and anxiety-like behaviours, cytokines, and the HPA axis in adult rats subjected to periapical lesions.

Experimental procedures

Animal model of periapical lesion

Adult male Wistar rats (age of two months, 250–350 g body weight) from our breeding colony were used for the experiments. The rats were housed under standard light conditions (12 h light/12 h dark with lights on between 6 a.m. and 6 p.m.). The male rats as adult were housed with food and water available ad libitum. All procedures were approved by the Animal Care and Experimentation Committee of UNESC/046/2013-2, Brazil, and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised in 1996). The animals were anesthetized by an intraperitoneal administration of ketamine (6.6 mg/kg), xylazine (0.3 mg/kg), and acepromazine (0.16 mg/kg) [23]. The surgical exposure of molars and the infection of the dental pulp were from the oral environment, inducing reproducible results in the development of periapical lesions and the destruction of bone.

Pulp necrosis was induced on the left mandibular first molars during adulthood. Dental pulps were exposed by drilling cavities on the central portion of the occlusal surface with a 1011 H L round bur in high speed (KG Sorensen, Cotia, SP, Brazil) to a depth nearly equal to the bur diameter (1 mm) [24]. The periapical lesion was confirmed by radiography. The x-ray unit operated at 7 mA at 70 kVp, with a size 2 phosphor plate14 (Dabi Atlante, São Paulo) and exposure time of 0.2 s. Digital x-ray system was used to capture images scanned (Vista Scan Durr, São Paulo) at the resolution of 1000 dpi.

Imipramine treatment

The animals were divided into four groups: control (n = 10), control/imipramine (n = 10), periapical lesion/saline (n = 10), and periapical lesion/imipramine (n = 10) (40 animals for each of the 3 behavioral tests, 120 animals in total). The same animals were used for biochemical measurements. Imipramine (10 mg/kg) or sodium chloride (NaCl) sterile solution (0,9%) were administered intraperitoneally once daily for 14 days. The last dose was administered 60 min before the tests. Imipramine was obtained from Novartis Pharmaceutical Industry (Brazil) and dissolved in NaCl sterile solution immediately before the injections. The treatments were administered in a volume of 1 mL/kg [25].

Behavioral tests

On day 21 after periapical lesion induction [26], the animals were subjected to sweet food consumption to evaluate anhedonia, to the forced swimming test to evaluate immobility, and to elevated plus maze to evaluate anxiety. Immediately following the behavioral tests, the animals were euthanized and decapitated (Fig. 1).

Sweet food consumption (anhedonia)

The preference for consumption of sweet foods was measured in all the groups to evaluate anhedonia for 7 days (14th to 21st day after periapical lesion induction). For this purpose, the animals were placed in a lighted rectangular box (40 × 15 × 20 cm) with a glass ceiling, floor, and wooden side walls. The box was divided into nine equal rectangles by black lines. Ten Froot Loops® cereal pieces (Kellogg's pellets of wheat, corn starch, and sucrose) were placed at one end of the box. The animals were subjected to five 3-min trials once daily for 5 days to become familiarized with the food (14th to 19th day). After being habituated, the animals were exposed to two test sessions of 3 min each, when the number of ingested pellets was measured (20th–21st day). This test was conducted in a light cycle and the evaluation was conducted by a researcher blinded to the groups. The test was considered complete when the animal ate all, $^{1}/_{2}$, or $^{1}/_{4}$ part of the Froot Loops®, in accordance with previous studies [27,28]. The two test sessions were held with the animals fasting for a period of 22 h prior to the behavioural test. Food deprivation is a motivating stimulus and might be an acute stressor for the animals.

Forced swimming test

This test was conducted according to previous reports [29–31] and was used as a model of depressive-like behaviour. The test involves two exposures to a cylindrical water tank in which rats could not touch the bottom or escape. The tank is constructed of transparent plexiglass and is 80 cm tall and 30 cm in diameter. The

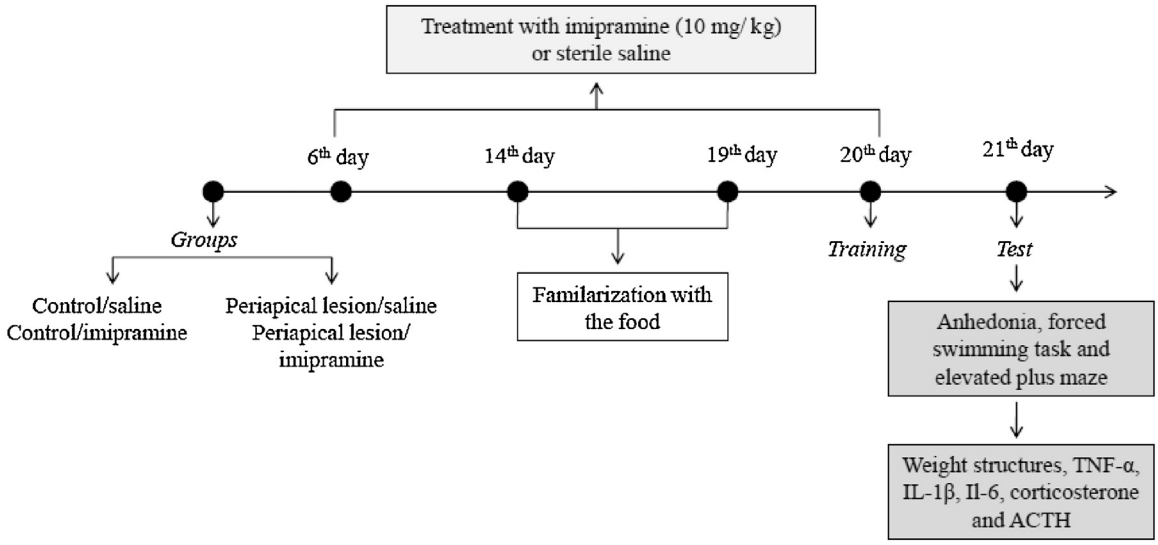

Fig. 1. Timeline of treatment and behavior tasks during experimental periapical lesion.

tank was filled with water (22–23 °C) to a depth of 40 cm. The water in the tank was changed for each rat. For the first exposure, the rats were placed in the water for 15 min (training session) [32]. After 24 h, the rats were placed in the water again for a 5 min session (test session). The time of immobility was analysed in the test session. The rats were judged to be immobile whenever they stopped swimming and remained floating in the water with their heads above the surface.

Elevated plus maze

Rats were subjected to the elevated plus-maze test as previously described [33]. The elevated plus-maze apparatus, entirely made of wood, consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) separated by a central platform (5 × 5 cm). The height of the maze was 50 cm and the tests were conducted under dim red light (level of luminosity measured in the center of the apparatus, 100 lx) in a quiet room. Animals were allowed a 5-minute exposure to red light in their own home cages before the testing procedure. Next, they were placed individually on the central platform of the plus-maze facing an open arm. The number of entries into and the time spent in open and enclosed arms of the apparatus were recorded over a 5-minute session. The elevated plus-maze test was performed 24 h after the last injection.

Adrenal gland and hippocampus weight

After death, the hippocampus and adrenal gland were removed through craniotomy and laparotomy, respectively, and weighted in an analytical scale [27].

Assessment of TNF-α, IL-1β, and IL-6 levels

At the end of 1 h, following completion of behavioural tests, the animals were sacrificed and decapitated and the hippocampus and frontal cortex was removed for the evaluation of TNF-α, IL-1β, and IL-6 levels. Briefly, the hippocampus and frontal cortex were homogenized in extraction solution containing aprotinin (100 mg of tissue per 1 mL) containing: 0.4 mol/L NaCl, 0.05% Tween 20, 0.5% 7 BSA, 0.1 mmol/L Phenyl methyl sulfonyl fluoride, 0.1 mmol/L benzethonium chloride, 10 mmol/L EDTA and 20 KI aprotinin, using Ultra-Turrax (Fisher Scientific, Pittsburgh, PA, USA). The concentrations of cytokines/chemokines in the hippocampus and frontal cortex were determined using commercially available ELISA assays, following the instructions supplied by the manufacturer (DuoSet kits, R&D Systems, Minneapolis, MN, USA). The results are reported in pg/100 mg of hippocampal and frontal cortex tissue. Protein was measured using the method of Lowry et al. [34] using bovine serum albumin as a standard.

Corticosterone and ACTH levels

The blood was collected to evaluate ACTH and corticosterone levels [35,36]. After 1 h of sweet food consumption, forced swimming and elevated plus maze tasks exposure, the rats were sacrificed by decapitation, between 1 and 3 p.m. Thus, we obtained 2 blood trunk samples from each animal. The first sample was collected in tubes containing EDTA (As recommended by the manufacturer (Monovette, Sarstedt), collection tubes contained 1.2–2 g of potassium EDTA/L, with a maximum 1% dilution effect of liquid EDTA). The sample was centrifuged for 10 min at 4000×*g* at room temperature (22 °C), and the plasma supernatant was collected to evaluate ACTH levels [37], (using Enzyme-Linked Immunosorbent Assay Kit from mark Life Science Inc, PRC). The second sample was centrifuged immediately after collection for 10 min at 4000×*g* at room temperature (22 °C). Then the serum was utilized to evaluate corticosterone levels (the primary glucocorticoid in rats) [27,28]. The corticosterone levels were determined using enzyme immunoassay kits (from corticosterone EIA Kit manufactured by Cayman Chemical Company, USA) for animals.

Statistics

The data was analysed for normal distribution using the Shapiro-Wilk test. The results for sweet food consumption, forced swimming test, elevated plus maze, TNF-α, IL-1β, IL-6, ACTH levels, adrenal gland weight and hippocampal weight analysis are reported as a mean ± SEM of 10 rats per group. Differences among experimental groups were determined by two-way ANOVA, followed by Tukey's *post-hoc* testing. Corticosterone concentrations were not normally distributed. These concentrations are reported as medians with interquartile bars. Corticosterone differences across experimental groups were tested for significance using Kruskal-Wallis testing. All analyses were performed using the Statistical Package for the Social Science (SPSS) software version 20.0 (IBM, Chicago, IL, USA).

Results

Fig. 2 illustrates the sweet food consumption in rats subjected to periapical lesions. In the periapical lesion group that received saline, the sucrose consumption decreased as compared to the control group/saline. In the periapical lesion group that received imipramine treatment, the sucrose consumption increased when compared to the periapical lesion group/saline. Imipramine reversed depressive-like behaviour in this group. Results from two-way ANOVA are as follows: periapical lesion group [F(lesion) = 21.69, $p < 0.001$], for imipramine administration [F(imipramine) = 4.3006, $p < 0.0448$], for periapical lesions and imipramine interaction [F(interaction) = 4.30, $p < 0.0448$].

In the forced swimming test, in the periapical lesion group that received saline was observed to have more time in immobility when compared with the control group/saline. The periapical lesion group presented with depressive-like behaviour in this test. In the periapical lesion group that received imipramine treatment, the time in immobility decreased when compared with the periapical lesion group/saline. Imipramine treatment reversed depressive-like behaviour in this group. Data from two-way ANOVA for the group [F(lesion) = 13.3031, $p < 0.001$], for imipramine administration [F(imipramine) = 17.44, $p < 0.001$], for periapical lesions and imipramine administration interaction [F(interaction) = 14.46, $p < 0.001$] (Fig. 3).

Fig. 4 illustrates the elevated plus maze in rats submitted to periapical lesions. The periapical lesion group had an increased number of entries in the close arms when compared with control/saline group. The imipramine treatment decreased the number of entries in the close arms when compared with periapical lesion/saline group (Fig. 4A). The periapical lesion group decreased the number of entries in the open arms when compared with control/saline group. The imipramine treatment increased the number of entries in the open arms when compared with periapical lesion/saline group (Fig. 4B). The periapical lesion group increased the time spent in the close arms when compared with control/saline group. The imipramine treatment decreased the time spent in the close arms when compared with periapical lesion/saline group (Fig. 4C). The periapical lesion group decreased the time spent in the open arms when compared with control/saline group. The imipramine treatment increased the time spent in the open arms when compared with periapical lesion/saline group (Fig. 4D). Data from two-away ANOVA for the group number of entries closed arms [F(lesion) = 0.9679, $p = 0.331597$], for imipramine administration [F(imipramine) = 15.4332, $p < 0.001$], for periapical lesions and imipramine administration interaction [F(interaction) = 9.2681, $p = 0.004278$]. For number of entries open arms [F(lesion) = 11.2008, $p = 0.0018$], for imipramine administration [F(imipramine) = 8.1446, $p = 0.006880$], for periapical lesions and imipramine administration interaction [F(interaction) = 7.5886, $p = 0.008879$]. For time closed arms [F(lesion) = 5.086, $p = 0.029809$], for imipramine administration [F(imipramine) = 6.028, $p = 0.018646$], for periapical lesions and imipramine administration interaction [F(interaction) = 0.018646, $p < 0.001$]. For time open arms [F(lesion) = 4.8630, $p = 0.033402$], for imipramine administration [F(imipramine) = 6.1801, $p = 0.017311$], for periapical lesions and imipramine administration interaction [F(interaction) = 20.7990, $p < 0.001$].

Fig. 5 illustrates the TNF-α, IL-1β, and IL-6 levels in the hippocampus and frontal cortex of rats submitted to periapical

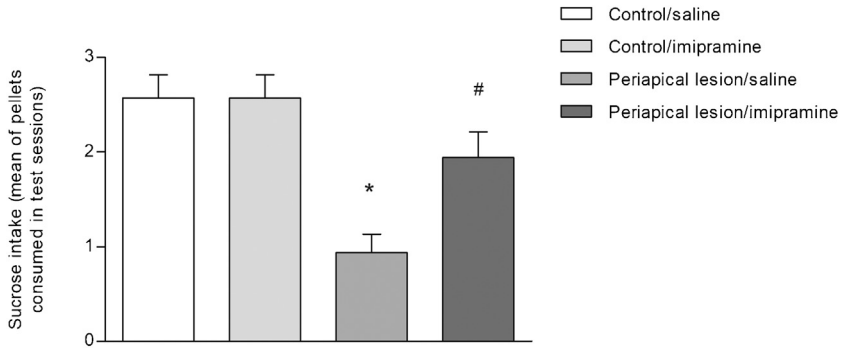

Fig. 2. The sweet food consumption in rats submitted to experimental periapical lesion. The data are reported as the mean ± SEM 10 rats per group. The differences among the experimental groups were determined by two-way ANOVA, followed by Tukey's *post-hoc* test. The symbols $^{*}p < 0.05$ indicate statistical significance in the comparison with the control/saline group; $^{\#}p < 0.05$ indicates statistical significance compared with the periapical lesion/saline group.

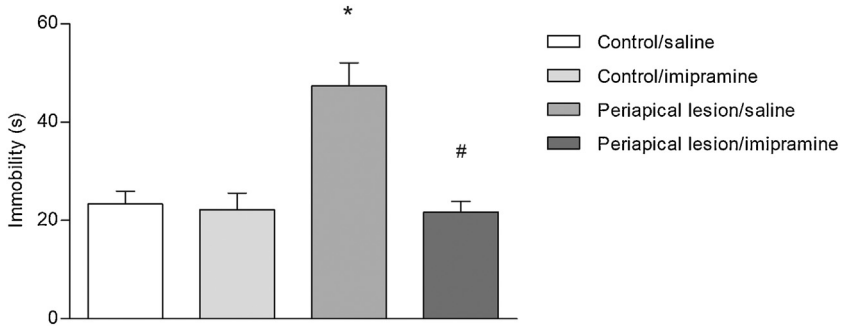

Fig. 3. The forced swimming test in rats submitted to experimental periapical lesion. The data are reported as the mean ± SEM of 10 animals per group. The differences among the experimental groups were determined by two-way ANOVA, followed by Tukey's *post-hoc* test. The symbols $^{*}p < 0.05$ indicate statistical significance in comparison with the control/saline group; $^{\#}p < 0.05$ indicates statistical significance compared with the periapical lesion/saline group.

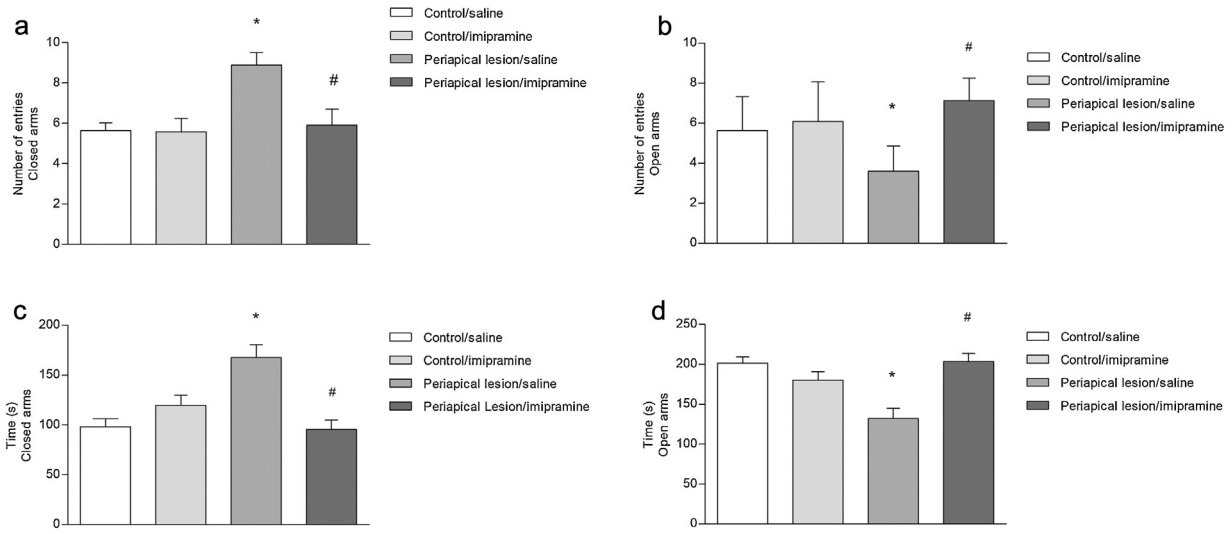

Fig. 4. The Elevated plus maze test in rats submitted to experimental periapical lesion. (A and B) number of entries closed or open arms, (C and D) time closed or open arms. The data are reported as the mean ± SEM of 10 animals per group. The differences among the experimental groups were determined by two-way ANOVA, followed by Tukey's *post-hoc* test. $^{*}p < 0.05$ indicates statistical significance compared with the control/saline group. $^{\#}p < 0.05$ indicates statistical significance compared with the periapical lesion/saline group.

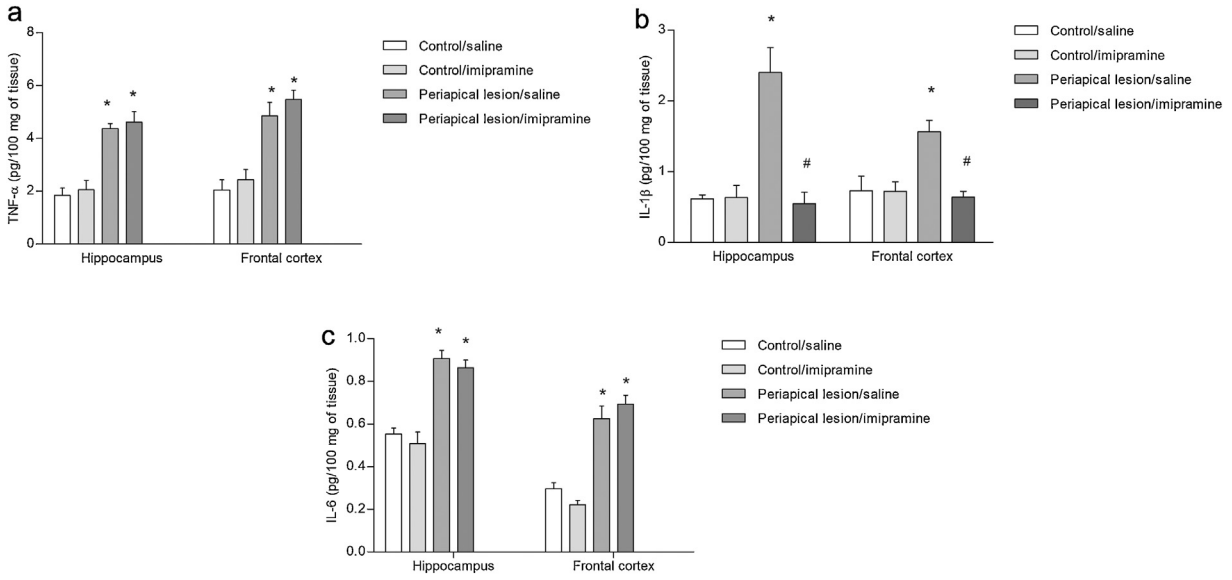

Fig. 5. Kinetics of TNF-α, IL-1β, and IL-6 levels assessed by ELISA in the hippocampus and frontal cortex after submitted to experimental periapical lesion. Results are expressed as the mean ± SEM of 10 animals in each group and determined by two-way analysis of ANOVA and Tukey's test; $^{*}p < 0.05$ indicates statistical significance compared with the control/saline group, $^{\#}p < 0.05$ indicates statistical significance compared with the periapical lesion/saline group.

lesion. The TNF-α levels increased in the hippocampus and frontal cortex in both periapical lesion/saline and periapical lesion/imipramine groups (Fig. 5A). IL-1β levels increased in the hippocampus and frontal cortex in the periapical lesion/saline group. However, the imipramine treatment decreased IL-1β levels in the hippocampus and frontal cortex (Fig. 5B). IL-6 levels increased in the hippocampus and frontal cortex in both periapical lesion/saline and periapical lesion/imipramine groups (Fig. 5C). Data from the two-way ANOVA for periapical lesions TNF-α [F(hippocampus) = 63.78, $p < 0.001$; F(frontal cortex) = 49.35, $p < 0.001$], IL-1β [F(hippocampus) = 72.682, $p < 0.001$; F(frontal cortex) = 24.934, $p < 0.001$], IL-6 [F(hippocampus) = 76, $p < 0.001$; F(frontal cortex) = 97, $p < 0.001$], for imipramine administration TNF-α [F(hippocampus) = 0.49, $p = 0.49$; F(frontal cortex) = 1.47, $p = 0.24$], IL-1β [F(hippocampus) = 84.319, $p < 0.001$; F(frontal cortex) = 38.374, $p < 0.001$], IL-6 [F(hippocampus) = 1.19, $p = 0.29$; F(frontal cortex) = 0.0096, $p = 0.9233$], for periapical lesions versus imipramine administration TNF-α [F(hippocampus) = 0.014, $p = 0.97$; F(frontal cortex) = 0.08, $p = 0.77$], IL-1β [F(hippocampus) = 87.861, $p < 0.001$; F(frontal cortex) = 37.326, $p < 0.001$], IL-6 [F(hippocampus) = 0, $p = 0.98$; F(frontal cortex) = 3.14, $p = 0.95$].

Fig. 6 illustrates the ACTH levels from the blood of rats subjected to periapical lesions. The ACTH levels increased in the periapical lesion/saline group when compared with the control/saline and periapical lesion/imipramine groups. The ACTH presented a main effect on periapical lesion [F(lesion) = 7.3456, $p = 0.011539$], and on imipramine administration [F(imipramine) = 7.5748, $p = 0.010448$]. However, it did not present any effect on periapical lesions *versus* imipramine administration [F(interaction) = 3.6927, $p = 0.065264$].

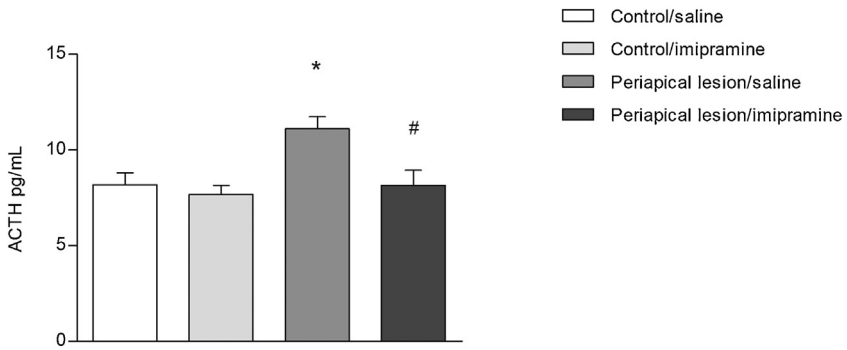

Fig. 6. The ACTH levels in rats submitted to experimental periapical lesion. The boxes represent the mean ± SEM of 10 animals per group. The differences among the experimental groups were determined by two-way ANOVA followed by the Tukey *post-hoc* test. The symbols $^{*}p < 0.05$ indicate statistical significance compared with the control/saline group; $^{\#}p < 0.05$ indicates statistical significance compared with the periapical lesion/saline group.

In the Fig. 7, the corticosterone levels did not present a statistically significant difference in Kruskal-Wallis test [$p = 0.065817$].

Fig. 8 illustrates the weight of adrenal gland (A) and hippocampus (B) from rats subjected to periapical lesion. There was no statistically significant difference of the adrenal glands weight in the group periapical lesion/imipramine when compared with the group periapical lesion/saline [$p = 0.193$] (Fig. 8A). The hippocampus weight did not change in any of the groups. For hippocampal right weight [$p = 0.286$], for imipramine administration [$p = 0.265$], for periapical lesions *versus* imipramine administration [$p = 0.244$] (Fig. 8B). For hippocampal left weight [$p = 0.662$], for imipramine administration [$p = 0.794$], for periapical lesions *versus* imipramine administration [$p = 0.19021$].

Discussion

This current study showed that 21 days after the periapical lesion, cerebral inflammatory mediators, such as IL1β, and alteration of the HPA axis can lead to behavior changes (Fig. 9). Adulthood rats subjected to periapical lesions presented depressive-like behaviour (anhedonia and immobility to swim) and anxiety-like behaviour (the rodent entered and spent more time in the closed arms). Moreover, these animals showed an increase of TNF-α, IL-1β, and IL-6 levels in the hippocampus and frontal cortex and increased levels of ACTH in the blood. However, imipramine, a tricyclic antidepressant of the dibenzazepine group, reversed depressive-and anxiety-like behaviours, normalized ACTH levels in the blood, and decreased IL-1β in the brain.

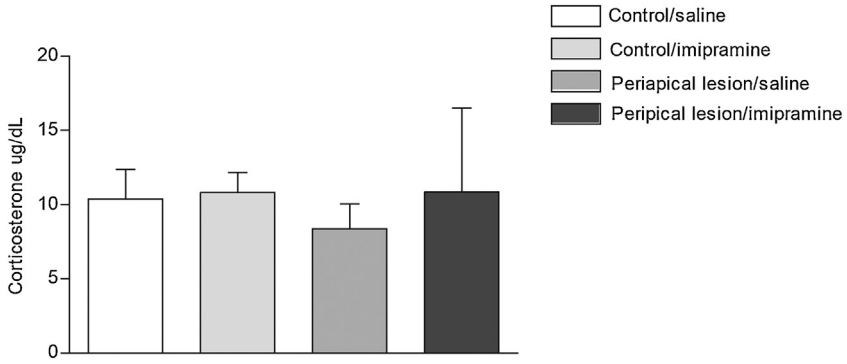

Fig. 7. The corticosterone levels in rats submitted to experimental periapical lesion. Levels are reported median and bars represent interquartile range. The differences among the experimental groups were determined by Kruskal-Wallis.

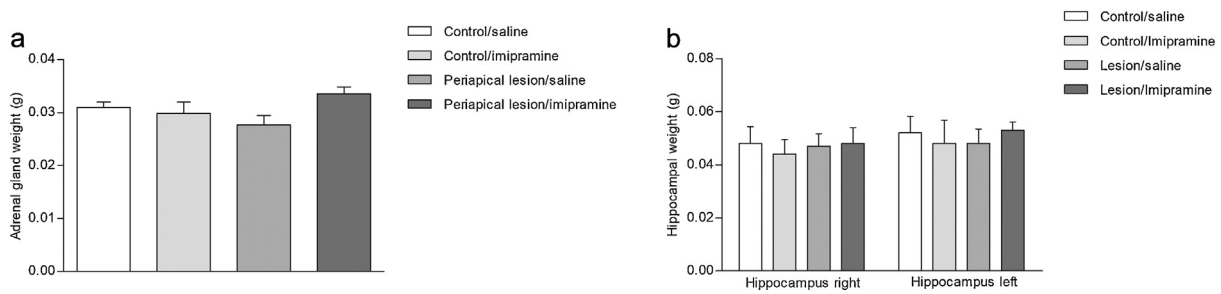

Fig. 8. Weight of the adrenal gland and hippocampus in rats submitted to experimental periapical lesion. (A) adrenal gland weight, (B) hippocampal weight. The differences among the experimental groups were determined by two-way ANOVA followed by Tukey's *post-hoc* test.

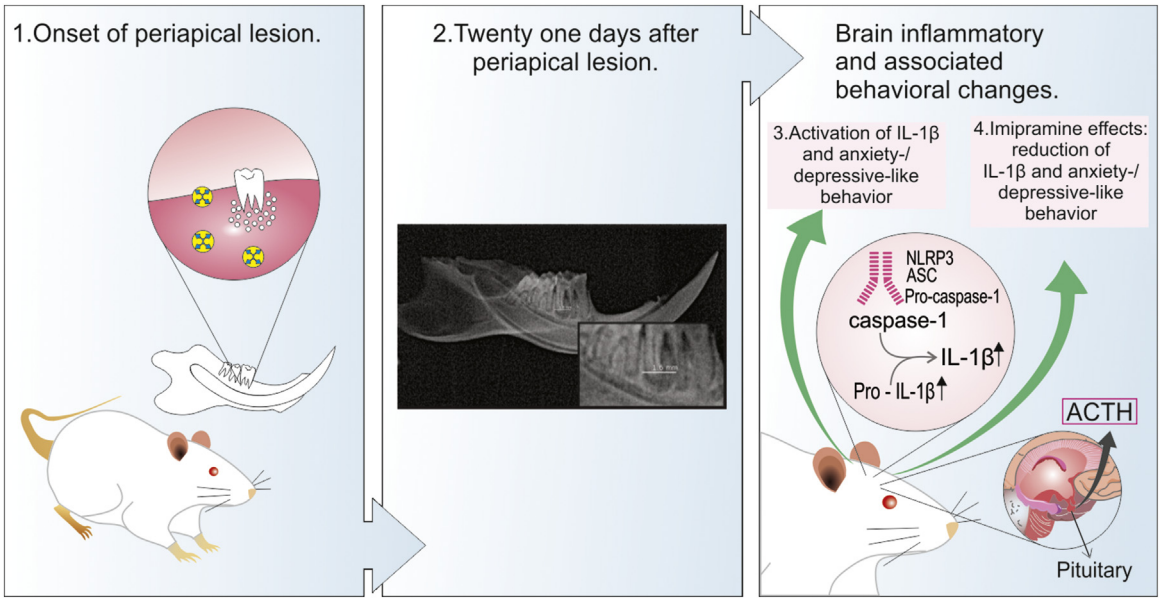

Fig. 9. Interaction between modulation of inflammatory immune responses and alterations in the hypothalamic-pituitary-adrenal. ASC, apoptosis-associated speck-like protein; IL-1R, interleukin-1 receptor; IL-1β, interleukin-1 beta; ACTH, adrenocorticotropin hormone; NLRP3, NOD-like receptor protein 3.

The site of infection, the periapical lesion, may contribute to systemic conditions by enhancing pro-inflammatory cytokines production [2]. In response to systemic inflammation, the soluble mediators can increase the permeability of blood-brain barrier leading to an imbalance of brain homeostasis [38]. Physiological effects of host immune activation can be responsible for several behavioural, neuroendocrinologic, and neurochemical alterations [39]. Subsequently, this process can result in cognitive and behavioural manifestations such as anorexia, discomfort, and MDD. These symptoms conjointly are known as sickness behaviour [17,38]. In this study the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 levels maintained elevated in the rat brain 21 days after periapical lesion induction. However, imipramine treatment decreased exclusively the IL-1β levels. The opposite result was showed i*n vitro* studies that evaluated the effects of imipramine on cells. Imipramine inhibited TNF-α, IL-1β, and IL-6 production by monocytes stimulated by LPS [40]. Similar studies, utilizing neural stem cells or microglial cells, imipramine treatment inhibited the production of the same cytokines [22,41]. In addition, imipramine treatment reversed cytokines changes in the cerebrospinal fluid and serum induced by animal model of depression induced by maternal deprivation [42]. Although IL-1β and TNF-α are both macrophage-derived pro-inflammatory cytokines, this study demonstrated a distinctive sensitivity of these cytokines to the suppressive effects of the imipramine. In the meantime, imipramine treatment reverted depressive- and anxiety-like behaviors. Another study with rodent chronic mild stress of paradigm depression, imipramine inhibited the production of IL-1β in association with neurobehavioral improvement [43]. Moreover, some studies have shown that depression is accompanied by increasing levels of IL-1β and Interleukin-1 receptor (IL-1R) [44,45].

Corroborating with our findings, in an animal model of depression that evaluated the chronic stress of rats after 7 weeks of exposure the mRNA levels of the pro-inflammatory cytokines IL-1β and IL-6 were increased in dorsal hippocampus. The expression of IL-1β was still significantly upregulated after 7 weeks, and these changes were normalized by chronic treatment with imipramine, differently from what observed for IL-6, whose changes were ameliorated only in part by imipramine and agomelatine. Given the apparent 'resistance' of IL-6 to the pharmacological treatment, it may be inferred that the elevation of its levels contributes to residual symptoms that may impair or limit clinical remission of depression [46].

Cytokines, such as TNF-α, IL-1β, and IL-6 are known to activate the HPA axis. Particularly, IL-1β effectively increases the HPA axis by triggering corticotropin-releasing and ACTH [44,45]. In patients with MDD, it were observed hyperactivity of the HPA axis [47,48]. It is well known that inflammation is a reflexive response to infection and the HPA axis in particular is essential in limiting and resolving the inflammatory process [18]. Several rodent models of infection such as sepsis and meningitis have demonstrated a simultaneous increase of HPA axis hormones and depressive-like behavior. In this study, experimental periapical lesions also triggered an increase of ACTH level in the blood, which were stabilized with imipramine treatment. Therefore, it seems that the rodent depressive and anxiety-like behavior could be attributed to either increased ACTH levels or increased secretion of the IL-1β.

Conclusion

In conclusion, we revealed that experimental periapical lesion induced depressive- and anxiety-like behaviours in rats. Moreover, this is the first study that demonstrates positive effects of imipramine antidepressant under these behavioural parameters induced by periapical lesion. Periapical lesion also induced an increase in the pro-inflammatory cytokines in the brain areas involved with the pathophysiology of MDD and anxiety, as well changes in the HPA axis, suggesting that changes in cytokines and HPA axis induced by periapical lesions could be, at least in part, involved to depressive- and anxiety-like behaviours. Lastly, these pathophysiological changes reversed following antidepressant treatment with imipramine further underscores the clinical translational potential of our findings and is suggest that immune modulators or other antidepressant could be important to patients with inflammatory periapical lesions. however, further studies are needed to elucidate the role of inflammation in behavioral changes after periapical injury and its treatment with antidepressants.

Author contributions

All authors participated in the design and interpretation of the studies, analysis of the data and review of the manuscript; Netto, Generoso, Ceretta and Valim, conducted the experiments; Michels, Valvassori and Dal-Pizzol were responsible the biochemical analyzes; Dominguini were responsible for the behavioral tests; and Simões, Réus and Barichello wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The Translational Psychiatry Program (USA) is funded by the Department of Psychiatry and Behavioral Sciences, McGovern Medical School, The University of Texas Health Science Center at Houston (UTHealth). Research from Laboratory of Experimental Microbiology, Neuroscience, and Experimental Pathophysiology (Brazil) is supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC), and Universidade do Extremo Sul Catarinense (UNESC).

References

[1] Bansal M., Mittal N, Singh TB. Assessment of the prevalence of periodontal diseases and treatment needs: a hospital-based study. J Indian Soc Periodontol 2015;19(2):211–5.
[2] Graves DT, Oates T, Garlet GP. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J Oral Microbiol 2011;3:.
[3] Siqueira Jr. JF, Rocas IN. Diversity of endodontic microbiota revisited. J Dent Res 2009;88(11):969–81.
[4] Narayanan LL, Vaishnavi C. Endodontic microbiology. J Conserv Dent 2010;13 (4):233–9.
[5] Colic M, Gazivoda D, Vucevic D, Vasilijic S, Rudolf R, Lukic A. Proinflammatory and immunoregulatory mechanisms in periapical lesions. Mol Immunol 2009;47(1):101–13.
[6] Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, et al. Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth. J Med Microbiol 2005;54(Pt 8):777–83.
[7] Fan R, Sun B, Zhang CF, Lu YL, Xuan W, Wang QQ, et al. Receptor activator of nuclear factor kappa B ligand and osteoprotegerin expression in chronic apical periodontitis: possible association with inflammatory cells. Chin Med J (Engl) 2011;124(14):2162–6.
[8] Hanke ML, Kielian T. Toll-like receptors in health and disease in the brain: mechanisms and therapeutic potential. Clin Sci 2011;121(9):367–87.
[9] Depino AM. Perinatal inflammation and adult psychopathology: from preclinical models to humans. Semin Cell Dev Biol 2017;7(17) 30309-9.
[10] Reus GZ, Fries GR, Stertz L, Badawy M, Passos IC, Barichello T, et al. The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. Neuroscience 2015;300:141–54.
[11] Yang K, Xie G, Zhang Z, Wang C, Li W, Zhou W, et al. Levels of serum interleukin (IL)-6, IL-1beta, tumour necrosis factor-alpha and leptin and their correlation in depression. Aust N Z J Psychiatry 2007;41(3):266–73.
[12] Kim YK, Na KS, Shin KH, Jung HY, Choi SH, Kim JB. Cytokine imbalance in the pathophysiology of major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2007;31(5):1044–53.
[13] Barichello T, Dos Santos I, Savi GD, Simoes LR, Generoso JS, Comim CM, et al. Depressive-like-behavior and proinflamatory interleukine levels in the brain of rats submitted to pneumococcal meningitis. Brain Res Bull 2010;82(5-6):243–6.
[14] Comim CM, Cassol-Jr OJ, Constantino LC, Petronilho F, Constantino LS, et al. Depressive-like parameters in sepsis survivor rats. Neurotox Res 2010;17(3):279–86.
[15] Neis VB, Manosso LM, Moretti M, Freitas AE, Daufenbach J, Rodrigues AL. Depressive-like behavior induced by tumor necrosis factor-alpha is abolished by agmatine administration. Behav Brain Res 2014;261:336–44.
[16] Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. Proc Natl Acad Sci U S A 2008;105(2):751–6.
[17] Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9(1):46–56.
[18] Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 2012;37(1):137–62.
[19] Walker FR, March J, Hodgson DM. Endotoxin exposure in early life alters the development of anxiety-like behaviour in the Fischer 344 rat. Behav Brain Res 2004;154(1):63–9.
[20] Barichello T, Milioli G, Generoso JS, Cipriano AL, Costa CS, Moreira AP, et al. Imipramine reverses depressive-like parameters in pneumococcal meningitis survivor rats. J Neural Transm 2012;119(6):653–60.
[21] Calabrese F, Rossetti AC, Racagni G, Gass P, Riva MA, Molteni R. Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. Front Cell Neurosci 2014;8:430.
[22] Peng CH, Chiou SH, Chen SJ, Chou YC, Ku HH, Cheng CK, et al. Neuroprotection by Imipramine against lipopolysaccharide-induced apoptosis in hippocampus-derived neural stem cells mediated by activation of BDNF and the MAPK pathway. Eur Neuropsychopharmacol 2008;18(2):128–40.
[23] Barichello T, Generoso JS, Silvestre C, Costa CS, Carrodore MM, Cipriano AL, et al. Circulating concentrations, cerebral output of the CINC-1 and blood-brain barrier disruption in Wistar rats after pneumococcal meningitis induction. Eur J Clin Microbiol Infect Dis 2012;31(8):2005–9.
[24] Scarparo RK, Dondoni L, Bottcher DE, Grecca FS, Rockenbach MI, Batista Jr EL. Response to intracanal medication in immature teeth with pulp necrosis: an experimental model in rat molars. J Endod 2011;37(8):1069–73.
[25] Barichello T, Generoso JS, Collodel A, Moreira AP, Almeida SM. Pathophysiology of acute meningitis caused by Streptococcus pneumoniae and adjunctive therapy approaches. Arq Neuropsiquiatr 2012;70(5):366–72.
[26] Barichello T, Netto S, Ceretta RA, Generoso JS, Simoes LR, Ribeiro PA, et al. Association between experimental bacterial meningitis and periapical lesion. J Clin Diagn Res 2015;9(5):1.
[27] Katz RJ. Animal models and human depressive disorders. Neurosci Biobehav Rev 1981;5(2):231–46.
[28] Gamaro GD, Manoli LP, Torres IL, Silveira R, Dalmaz C. Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochem Int 2003;42(2):107–14.
[29] Tuon L, Comim CM, Petronilho F, Barichello T, Izquierdo I, Quevedo J, et al. Time-dependent behavioral recovery after sepsis in rats. Intensive Care Med 2008;34(9):1724–31.
[30] Einat H, Clenet F, Shaldubina A, Belmaker RH, Bourin M. The antidepressant activity of inositol in the forced swim test involves 5-HT(2) receptors. Behav Brain Res 2001;118(1):77–83.
[31] Porsolt RD. Animal model of depression. Biomedicine 1979;30(3):139–40.
[32] Reus GZ, Stringari RB, Ribeiro KF, Luft T, Abelaira HM, Fries GR, et al. Administration of cannabidiol and imipramine induces antidepressant-like effects in the forced swimming test and increases brain-derived neurotrophic factor levels in the rat amygdala. Acta Neuropsychiatr 2011;23(5):241–8.
[33] Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14(3):149–67.
[34] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265–75.
[35] Gadek-Michalska A, Tadeusz J, Rachwalska P, Spyrka J, Bugajski J. Effect of prior stress on interleukin-1beta and HPA axis responses to acute stress. Pharmacol Rep 2011;63(6):1393–403.
[36] Gadek-Michalska A, Tadeusz J, Rachwalska P, Spyrka J, Bugajski J. Brain nitric oxide synthases in the interleukin-1beta-induced activation of hypothalamic-pituitary-adrenal axis. Pharmacol Rep 2012;64(6):1455–65.
[37] Reisch N, Reincke M, Bidlingmaier M. Preanalytical stability of adrenocorticotropic hormone depends on time to centrifugation rather than temperature. Clin Chem 2007;53(February (2)):358–9.
[38] Sankowski R, Mader S, Valdes-Ferrer SI. Systemic inflammation and the brain: novel roles of genetic, molecular, and environmental cues as drivers of neurodegeneration. Front Cell Neurosci 2015;9:28.
[39] Vidrih B, Karlovic D, Pasic MB, Uremovic M, Mufic AK, Matosic A. A review of the psychoneuroimmunologic concepts on the etiology of depressive disorders. Acta Clin Croat 2012;51(3):403–9.
[40] Xia Z, DePierre JW, Nassberger L. Tricyclic antidepressants inhibit IL-6, IL-1 beta and TNF-alpha release in human blood monocytes and IL-2 and interferon-gamma in T cells. Immunopharmacology 1996;34(1):27–37.
[41] Obuchowicz E, Bielecka AM, Paul-Samojedny M, Pudelko A, Kowalski J. Imipramine and fluoxetine inhibit LPS-induced activation and affect morphology of microglial cells in the rat glial culture. Pharmacol Rep 2014;66(1):34–43.
[42] Reus GZ, Dos Santos MA, Abelaira HM, Ribeiro KF, Petronilho F, Vuolo F, et al. Imipramine reverses alterations in cytokines and BDNF levels induced by maternal deprivation in adult rats. Behav Brain Res 2013;242:40–6.
[43] Kubera M, Symbirtsev A, Basta-Kaim A, Borycz J, Roman A, Papp M, et al. Effect of chronic treatment with imipramine on interleukin 1 and interleukin 2 production by splenocytes obtained from rats subjected to a chronic mild stress model of depression. Pol J Pharmacol 1996;48(5):503–6.
[44] Maes M, Song C, Yirmiya R. Targeting IL-1 in depression. Expert Opin Ther Targets 2012;16(11):1097–112.
[45] Maes M, Bosmans E, Suy E, Vandervorst C, DeJonckheere C, Raus J. Depression-related disturbances in mitogen-induced lymphocyte responses and interleukin-1 beta and soluble interleukin-2 receptor production. Acta Psychiatr Scand 1991;84(4):379–86.
[46] Rossetti AC, Papp M, Gruca P, Paladini MS, Racagni G, Riva MA, et al. Stress-induced anhedonia is associated with the activation of the inflammatory system in the rat brain: restorative effect of pharmacological intervention. Pharmacol Res 2016;103:1–12.
[47] Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry 2001;49(5):391–404.
[48] Pariante CM, Lightman SL. The HPA axis in major depression: classical theories and new developments. Trends Neurosci 2008;31(9):464–8.