Brain Research Bulletin 155 (2020) 1–10

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Minocycline treatment prevents depression and anxiety-like behaviors and promotes neuroprotection after experimental ischemic stroke

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ARTICLE INFO

Keywords:
Anxiety
Depression
Inflammation
Ischemia
Minocycline
Neuropathology
Neurotrophic factors
Mice

ABSTRACT

Depression and anxiety have been reported as the major neuropsychiatric consequences following stroke. Minocycline, a neuroprotective drug has minimized depressive symptoms in patients with major depressive disorders and anxiety-like symptoms. In addition, minocycline demonstrated efficacy and seemed a promising neuroprotective agent in acute stroke patients. The present studied evaluated the effects of minocycline treatment on the depression and anxiety-like behaviors, brain damage and expression of inflammatory and neuroprotective mediators after transient global cerebral ischemia in C57BL/6 mice. Brain ischemia was induced by bilateral occlusion of the common carotids (BCCAo) for 25 min and subsequent reperfusion. Sham and BCCAo animals received minocycline at a dose of 30 mg/kg by intraperitoneal injection during 14 days. The locomotor activity, depression and anxiety-like behaviors were assessed by open field, forced swim and elevated plus maze tests, respectively. Then, the brains were removed and processed to evaluate brain damage by histological and morphometric analysis, hippocampal neurodegeneration using Fluoro-Jade C histochemistry, microglial activity using iba-1 immunohistochemistry, brain levels of TNF, IFN-γ, IL-6, IL-10, IL-12p70 and CCL2 by CBA, CX3CL1 and BDNF by ELISA assays. The animals developed depression and anxiety-like behaviors post-stroke and minocycline treatment prevented those neurobehavioral changes. Moreover, minocycline-treated BCCAo animals showed less intense brain damage in the cerebral cortex, brainstem and cerebellum as well as significantly reduced hippocampal neurodegeneration. BCCAo groups exhibited up-regulation of some cytokines at day 14 after ischemia and brain levels of CX3CL1 and BDNF remained unaltered. Our data indicate that the depression and anxiety-like behavioral improvements promoted by minocycline treatment might be related to its neuroprotective effect after brain ischemia in mice.

1. Introduction

Ischemic stroke has been associated with high incidence of sensorimotor dysfunctions as well as cognitive deficits (Chemerinski and Robinson, 2000; Kapoor et al., 2019). Additionally, depression and anxiety have been reported as the major neuropsychiatric consequences following stroke, leading to a negative impact on functional rehabilitation and increase in mortality (Buijck et al., 2012; De Mello et al., 2016; Pedroso et al., 2018). The predictive value of stroke topography in post-stroke neuropsychiatric disorders has been considered

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https://doi.org/10.1016/j.brainresbull.2019.11.009
Received 4 September 2019; Received in revised form 28 October 2019; Accepted 17 November 2019
Available online 19 November 2019
0361-9230/

controversial. Some studies have suggested an association between intensity of post-stroke depression and lesion location (Castellanos-Pinedo et al., 2011; Shi et al., 2017). On the other hand, some authors demonstrated no relationship between ischemic stroke location and post-stroke depressive symptoms (Sagnier et al., 2019). Brain injury after stroke promotes microglial activation and secretion of pro-inflammatory mediators, cytotoxic substances as well as anti-inflammatory cytokines and neurotrophic factors (Eldahshan et al., 2019; Jayaraj et al., 2019; Tejeda et al., 2019). Of importance, brain-derived neurotrophic factor (BDNF) plays an important role in neuroprotection during acute stroke (Luo et al., 2019; Tejeda et al., 2019; Victoria et al., 2017)et al., 2019; Victoria et al., 2017) and change in those levels have been related to mechanisms underlying the pathophysiology of depression and anxiety (Aldoghachi et al., 2019; Kojima et al., 2019; Mizui et al., 2019; Verduijn et al., 2015; Zemdegs et al., 2018). Moreover, some researchers also described higher serum levels of the chemokine CX3CL1/fractalkine, which mediated neuron–microglia cross-talk in patients with major depressive disorder (Merendino et al., 2004).

Minocycline, a second generation tetracycline showed anti-inflammatory and neuroprotective effects by inhibition of microglial activation. Minocycline demonstrated efficacy and seemed a promising neuroprotective agent in acute stroke patients (Malhotra et al., 2018; Sheng et al., 2018). Furthermore, minocycline minimized depressive symptoms in patients with major depressive disorder (Dean et al., 2014), bipolar disorder (Murrough et al., 2018; Savitz et al., 2018), and anxiety-like symptoms after stress (Liu et al., 2018; Wang et al., 2018; Zhang et al., 2019). In rats submitted to transient bilateral occlusion of common carotid arteries and treated for seven days, minocycline pre-treatment prevented memory impairment, reduced brain damage and the levels of pro-inflammatory cytokines (Naderi et al., 2017). Other study showed that minocycline treatment attenuated neurological deficits, infarct volume and up-regulation of pro-inflammatory cytokines in the brain two days after focal brain ischemia in mice (Jin et al., 2015). Minocycline administration also improved memory impairment and increased hippocampal levels of CREB, pCREB, and BDNF after permanent bilateral occlusion of common carotid arteries in rats (Zhao et al., 2015). However, to best of our knowledge there are no data regarding the effects of minocycline treatment on depression and anxiety-like behaviors associated with brain damage, brain levels of inflammatory cytokines, CX3CL1 and BDNF after transient global cerebral ischemia in mice.

2. Material and methods

2.1. Animals

Nine to ten week old, male, C57BL/6 were obtained from Animal Care Facilities of the Biological Sciences Institute of the Federal University of Minas Gerais (CEBIO-ICB-UFMG). The animals were maintained on a 12-h light/dark cycle with water and food ad libitum. This project was approved by the Ethics Committee on the Use of Animals (CEUA/UFMG) under protocol number 209/2016.

2.2. Induction of ischemia and reperfusion (BCCAo) and minocycline treatment

Mice were pre-anesthetized by intraperitoneal injection of a mixture of 80 mg / kg ketamine and 10 mg/kg xylazine. Transient global cerebral ischemia was induced by occlusion of common carotid arteries via non-traumatic surgical clips as previously described (Toscano et al., 2016). After 25 min, the circulation was reestablished with clip removal, characterizing transient global cerebral ischemia. All animals were kept in a warm environment for approximately 1 h and then were kept alive and undergoing experimentation for 14 consecutive days in appropriated home cages with free access to water and soft food. Minocycline hydrochloride treatment was performed following a modification of the method reported by (Jin et al., 2015). Mice were injected intraperitoneally with 30 mg/kg of minocycline, with the initial dose being 1 h after the induction of brain ischemia and maintained daily for fourteen days. The animals from untreated sham and BCCAo groups received the same volume of saline phosphate buffer (PBS). The mice were randomly assigned to the following groups: sham, minocycline-treated sham, BCCAo and minocycline-treated BCCAo.

2.3. Behavioral tests

Fourteen days after cerebral ischemia and reperfusion, the open field test (OFT), the elevated plus maze (EPM), and the forced swim test (FST) were conducted.

2.4. Open Field Test (OFT)

The Open Field test was used to investigate locomotor activity (Hall, 1941). The Open Field (Insight®, SP, Brazil) was performed in a white square arena (50 cm × 50 cm × 30 cm). Each mouse was placed in the center of the field and allowed to freely explore the arena for 5 min. The total distance traveled during the exploration was registered using the Ethovision software (Noldus Information Technology).

2.5. Forced swim test (FST)

Forced swim test is the most common test to evaluate depressive-like behavior in rodents and climbing behavior is a type of active behavior consisting of upward directed movements of the forepaws along the side of the swim chamber (Bagdas et al., 2019). In the pre-test session, mice were placed in the cylinder with water in a temperature of 25 °C for 6 min and recorded. The last 4 min were used to address the depressive-like behavior. The first 2 min is considered a water adaptation period. For the behavioral record, the animals were filmed and the immobility and swimming time were measured. At the end of the test each animal was removed from the container and dry towels. Climbing was recorded when vigorous movements with forepaws in and out of the water, usually directed against the wall of the cylinder.

2.6. Elevated plus-maze (EPM) test

The elevated plus maze was performed to evaluate anxiety-like behavior (Soares et al., 2013). The EPM apparatus (Insight®, SP, Brazil) is a plus-shaped maze formed by two open arms and two closed arms. In the EPM test, each animal was placed in the center of the platform and allowed to freely explore the labyrinth for 5 min. Behavior sections were recorded to measure the percentage of open arms entries and the percentage of time spent in open arms.

2.7. Histopathology, immunohistochemistry and morphometry

Animals were anesthetized with an intraperitoneal injection of a mixture of ketamine (50 mg/kg) plus xylazine (20 mg/kg) and were euthanized at day 14 post-induction. The brains were removed and post fixed in formalin for 24 h. Then, the brains were cut into coronal slices of 2 mm in thickness with a mouse brain slicer matrix (Insight LTDA, Ribeirão Preto, SP, Brazil). After fixation, 5 μm thickness sections were cut and processed for hematoxylin and eosin (H&E) staining and for immunohistochemistry. For morphometric analysis, 45 fields at a final magnification of ×200 for each animal obtained from three sections from whole brain were captured. The capture system was used with a Qcolor 3-RTV-CLR-10 camera coupled to an Olympus BX40 microscope. The total tissue area and cavitations were measured using ImageJ software to calculate the ratio of necrotic cavity area to total tissue area, which was expressed as percentage of necrotic area. In addition, it was quantified the frequency of ischemic neurons around necrotic

areas, with a graticule of 25 points, according to (Toscano et al., 2016). Intersection points corresponding to ischemic neurons were counted until a total of 1000 points for each animal. The values were shown as percentage of the ratio of intersection points coinciding ischemic neurons regions versus total intersection points for animal. To evaluate the microglia activation in the areas adjacent to the infarct areas, the immunohistochemistry technique was performed using the ionized calcium binding adapter molecule 1 antibody (Iba-1), as described by Faleiros et al. (2014). Serial sections of 5 μm were submitted to antigen recover with sodium-citrate buffer (pH 6) for 12 min in microwave and blocked for endogenous peroxidase activity with H_2O_2 and PBS solution (10%) for 30 min. After, the protein block was made with non-fat dry milk in PBS 6% (w/v) and 0.1% Tween-20. Brain sections were then incubated with rabbit monoclonal antibody against iba-1 (Abcam), which were diluted 1:1000 and incubated overnight at 4 ˚C. Biotinylated polyclonal link and streptavidin-HRP (Leica) were applied and the sections were incubated with diaminobenzidine 5% (v/v) (Leica). Then, the sections were counterstained with Harris hematoxylin and examined microscopically.

2.8. Fluoro-Jade C analysis

FJC was used to detect neurons in death process. At day 14 post induction, mice were anesthetized with an intraperitoneal injection of a mixture of ketamine (150 mg/kg) plus xylazine (10 mg/kg). Then, they were perfused with saline solution followed by cold 4% paraformaldehyde. Brains were removed and fixed in cold 4% paraformaldehyde overnight. Subsequently, the brains were moved to a 30% sucrose solution, until complete saturation, then were frozen in isopentane 99% and dry ice for 20 s and stored at −80 ˚C. Brains were sliced into 30-μm-thick sections at −20 ˚C with the aid of a cryostat. The evaluation and quantification of the number of degenerating neurons in hippocampal regions was done by Fluoro Jade C (FJC) staining (Jain et al., 2019). The sections were mounted on gelatinized slides (1% gelatin in dH_2O) and dried on a hot plate at 50–55 ˚C for 1 h. After that, the slides were immersed in a solution containing 1% NaOH in 80% ethanol for 5 min. After, the slides were placed in 70% ethanol for 2 min and in distilled water for 2 min and then incubated in 0.06% potassium permanganate solution for 20 min. The slides were then washed with dH_2O for 2 min and incubated in 0.00.1% FJC solution for 20 min. The FJC solution was prepared by first stocking 0.01% FJC dye solution in distilled water and then adding 1 mL of the stock solution to 99 mL of 0.1% acetic acid. The slides were washed twice for 1 min and then dried in an oven at 37 ˚C and then mounted. Sections were then photographed on a Zeiss fluorescent microscope (Zeiss, Oberkochen, Germany) using a 488 nm excitation. Fluorescence intensity quantification was performed using the ImageJ software. The number of fluorescent spots in each image was evaluated by a blind investigator for all experimental groups.

2.9. Cytometric Bead Array (CBA) of pro-inflammatory and anti-inflammatory cytokines and of the chemokine CCL2

Brain tissues were carefully removed and homogenized in a PBS-buffer extraction solution containing a protease-inhibitor cocktail. Lysates were centrifuged at 13,000 *g* for 10 min at 4 ˚C and stocked at -70 ˚C until use. Analyses of brain cytokine and chemokine levels were determined using BD™ CBA Mouse Inflammation Kit (CBA; BD Biosciences, San Diego, CA) in accordance to the manufacturer's instructions and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The following cytokines and chemokine were quantified: TNF-α, IFN-γ, IL-6, IL-10, IL-12p70 and CCL2. The detection limit of the CBA assay was around 0.2 pg/mL.

2.10. Enzyme-linked immunosorbent assay of neurotrophic factor BDNF and chemokine CX3CL1

Brain samples from all animals were collected and stored at −80 ˚C. The samples were homogenized in an extraction solution (100 mg of tissue per milliliter), containing 0.4 M NaCl, 0.05% Tween 20, 0.5% BSA, 0.1 mM phenyl methyl sulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA, and 20 IU aprotinin, with Ultra-Turrax (Fisher Scientific, Pittsburgh, PA, USA). Lysates were centrifuged at 13,000 *g* for 10 min at 4 ˚C, and supernatants were collected. Concentrations of neurotrophin brain derived neurotrophic factor (BDNF) and chemokine (CX3CL1) were determined by ELISA (R&D Systems, Minneapolis, MN, USA), in accordance to the manufacturer's instructions. The detection limit of the ELISA assay was around 5 pg/mL.

2.11. Statistical analysis

All analyses were performed using GraphPad Prism 5 software (GraphPad softaware, San Diego, CA, USA). The obtained data were checked by Kolmogorov-Smirnov normality test. Student's *t*-test or analysis of variance (ANOVA) was used for normally distributed data. Mann-Whitney test or Kruskal-Wallis non-parametric test was performed for nonparametric variables. The data are presented as mean and standard error of the mean (SEM). A value of $p < 0.05$ was considered significant.

3. Results

3.1. Minocycline prevented BCCAo-associated depressive and anxiety-like behaviors

All groups had similar values in distance travelled in the OFT, showing no changes in locomotor activity at day 14 (Fig.1A). BCCAo animals displayed depressive-like behavior as shown by a significant decrease in duration of climbing ($p < 0.05$) in the FST. The administration of minocycline improved the depressive-like behavior by increasing the climbing time ($p < 0.05$) at 14 days after transient global cerebral ischemia (Fig.1B). Moreover, BCCAo animals presented an anxiety-like behavior as revealed by a significant decrease in the percentage of open arm time ($p < 0.05$) and entries ($p < 0.05$) of the EPM test compared with sham animals. Importantly, the minocycline treatment significantly prevented the BCCAo-associated anxiety-like behavior.

3.2. Minocycline treatment reduced brain pathology induced by BCCAo

Next, we investigated the protective effect of minocycline on brain lesions induced by cerebral ischemia and reperfusion. Non-treated sham and treated sham animals showed normal histological appearance (Fig. 2A-B). In contrast, both BCCAo groups exhibited necrotic cavitations surrounded by a penumbra zone scattered throughout the cerebral cortex, brainstem and cerebellum (Fig. 2C). Neuronal loss was also found along hippocampal areas, predominantly in CA1 and CA3 regions. Additionally, BCCAo animals exhibited focal areas of gliosis. All histopathological lesions were less intense in minocycline-treated BCCAo animals (Fig. 2D). The morphometric analysis confirmed those findings. The minocycline treatment promoted reduction in the percentage of necrotic area ($p < 0.05$) and ischemic neurons ($p < 0.05$) in BCCAo group (Fig. E–F). To investigate the effects of chronic minocycline treatment in the activation of microglia, we evaluated the expression of the marker, Iba-1 by immunohistochemistry. We confirmed the inhibition of microglial activation by minocycline by using Iba-1immunohistochemistry. Occasional immunopositive cells were observed in the brains from both sham and minocycline-treated sham groups. Numerous Iba-1 positive cells were detected throughout the brains of BCCAo animals and some Iba-1 immunopositive cells were

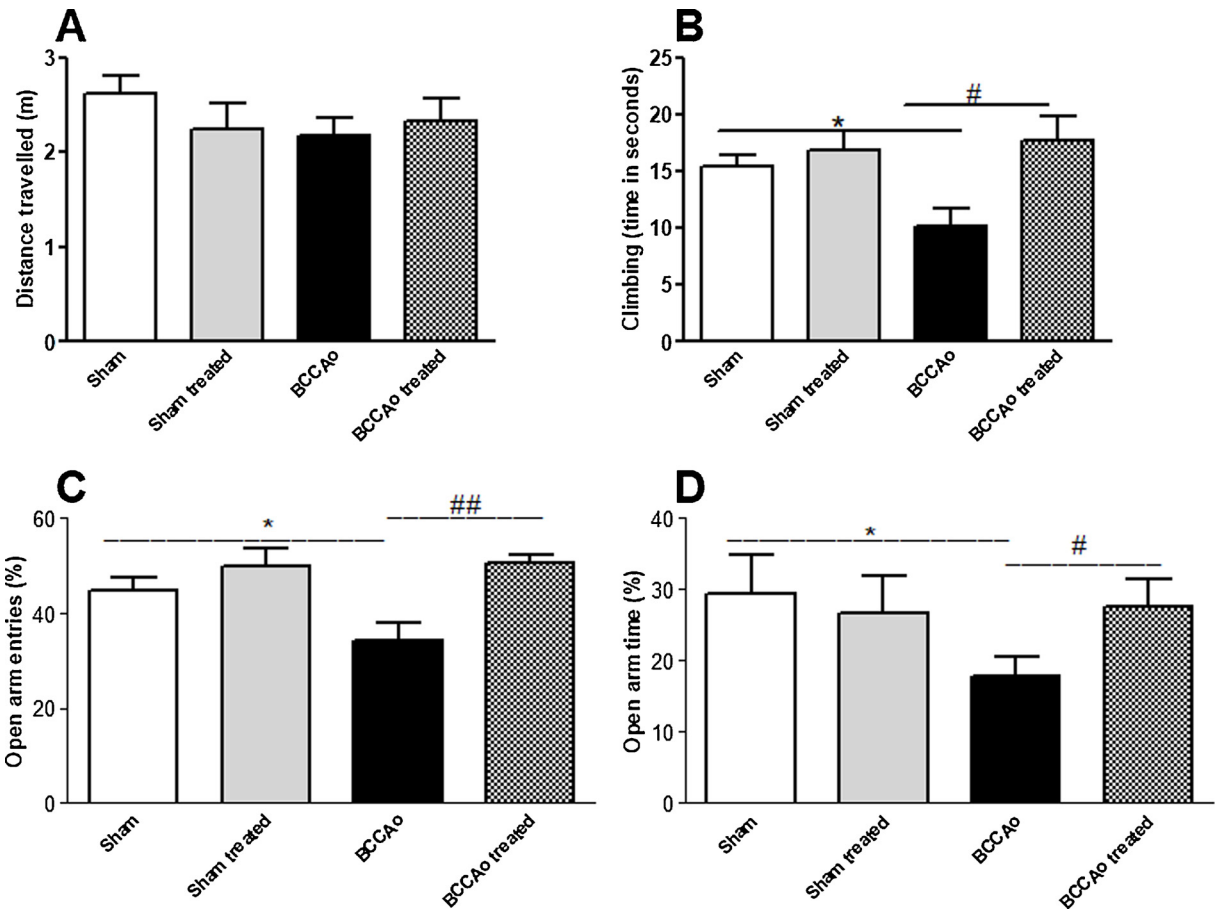

Fig. 1. Effects of minocycline treatment on antidepressant-like and anxiety-like behaviors after global cerebral ischemia in mice. All mice were submitted to open field for locomotor activity evaluation (A), the forced swimming test for depressive-like behavior (B) analyses and the elevated plus maze to anxiety assessment (C–D). Sham (n = 5), minocycline-treated Sham (n = 5), BCCAo (n = 7) and minocycline-treated BCCAo (n = 10). Asterisks indicate statistical differences where *$p < 0.05$, BCCAo versus sham; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, non-treated versus minocycline-treated BCCAo mice.

visualized in the minocycline-treated BCCAo mice (data not shown).

3.3. The effects of minocycline on neuronal cell damage in the hippocampus induced by BCCAo

In order to further evaluate the effects of minocycline on neuronal damage, we used a well-established method to stain the brain sections with Fluoro-Jade C, which specifically stains degenerating neurons. A significant augment of neuronal cell damage ($p < 0.05$) was observed in CA3 region and in the dentate gyrus in BCCAo animals, compared with sham mice. On the other hand, minocycline reduced neuronal death in CA2 ($p < 0.05$), CA3 regions ($p < 0.005$) and in the dentate gyrus ($p < 0.05$), showing the neuroprotective effect induced by minocycline treatment after brain ischemia (Fig.3).

3.4. Brain levels of pro-inflammatory and anti-inflammatory cytokines after treatment with minocycline in BCCAo animals

Many cytokines and chemokines are produced during brain ischemia and reperfusion, contributing to the pathogenesis of the disease. In this study, we measured the brain levels of TNF, IFN-γ, IL-6, IL-10, IL-12 p70 and CCL2. BCCAo induced up-regulation of IFN-γ, IL-12 p70, IL-10 and CCL2 ($p < 0.05$) fourteen days after the induction. Increased levels of IFN-γ and IL-12 p70 ($p < 0.05$) was also observed in the brains of minocycline-treated BCCAo mice. All animals had similar levels of TNF and IL-6 in the evaluated period (Fig.4).

3.5. Minocycline treatment did not alter the brain expression of CX3CL1 (fractalkine) and the neurotrophic factor BDNF

Similar brain levels of CX3CL1 (fractalkine) (Fig.5A) and BDNF (Fig.5B) were observed in all groups at day 14 post-stroke.

4. Discussion

This is the first study focusing the antidepressant and anxiolytic effects of minocycline administration in the transient global cerebral ischemia in mice. Depression and anxiety are very common psychiatric complications in patients with ischemic stroke and have been associated with poor outcome ((Buijck et al., 2012; De Mello et al., 2016; Pedroso et al., 2018). BCCAo mice exhibited depressive and anxiety-like behaviors, demonstrating that our mouse model resemble clinical aspects of the disease. Importantly, minocycline was able to prevent those psychiatric disorders. Minocycline also reduced depressive symptoms in patients with major depressive disorder (Dean et al., 2014), bipolar disorder (Murrough et al., 2018; Savitz et al., 2018), and anxiety-like symptoms after stress (Liu et al., 2018; Wang et al., 2018; Zhang et al., 2019). Furthermore, minocycline improved anxiety and cognitive deficits and attenuated the hypoxia–ischemia induced brain injury in juvenile rats (Fan et al., 2006). The pathophysiological mechanisms underlying the development of post-stroke anxiety and depression are still unknown. These disorders have been associated with immune-inflammatory dysfunction, neurotransmitter imbalance, increased production of neurotoxic substances and neuronal cell death (Becker, 2016; Pascoe et al., 2011). In the present study, we observed hippocampal

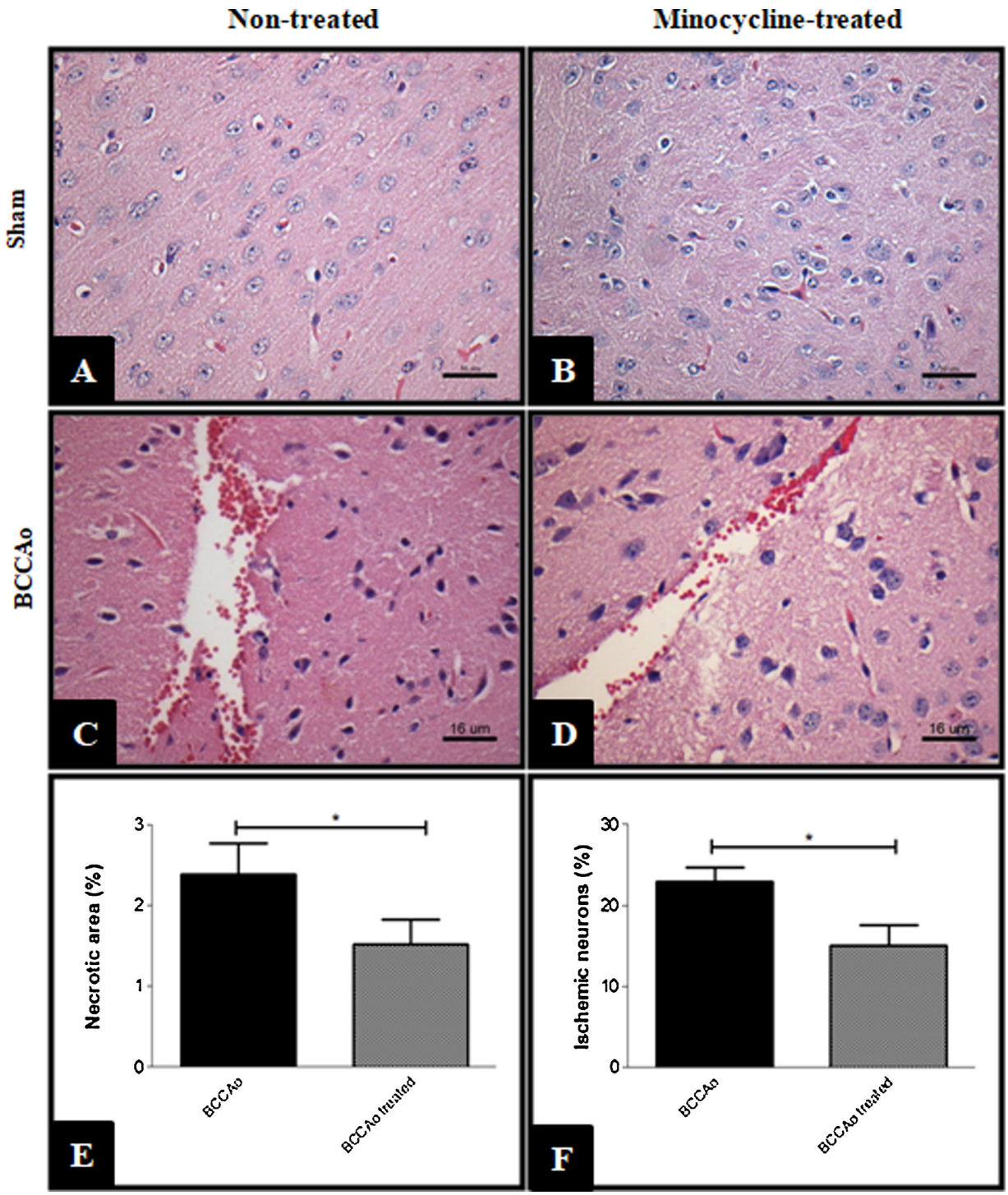

Fig. 2. Minocycline treatment promoted less intense histopathological lesions. Photomicrographs of frontal cortex from sham group (A), minocycline-treated sham group (B), BCCAo group (C) and minocycline-treated BCCAo group (D). Normal histological aspect (A–B); Necrotic cavity surrounded by ischemic neurons (C–D). H&E stain. Minocycline-treated BCCAo mice had decreased percentage of necrotic cavities (E) and frequency of ischemic neurons in the penumbra zone (F), compared with BCCAo group. Results are expressed as mean ± SEM and are representative of at least two independent experiments (n = 10 per group). Asterisks indicate statistical differences where *p < 0.05.

neurodegeneration in the CA subfields, which are usually selective areas for neuronal cell death after transient cerebral global ischemia (Silva et al., 2015; Soares et al., 2013). In addition, ischemic stroke promoted damage in the cerebrum, brainstem and cerebellum. Our data demonstrated that the treatment with minocycline for fourteen days attenuated those lesions, resulting in reduction of infarct area and ischemic neurons in mice. Indeed, improvement in neurological outcomes has been observed in patients with ischemic stroke who received oral minocycline daily for five days (Amiri-Nikpour et al., 2015). Minocycline has the ability to protect neurons against brain ischemia (Cho et al., 2007; Naderi et al., 2017). Some authors suggested that the neuroprotective effect from minocycline is mediated by inhibition of cell death pathways, such as cytochrome c release from mitochondria and caspase-1 and caspase-3 expressions (Matsukawa et al., 2009). It

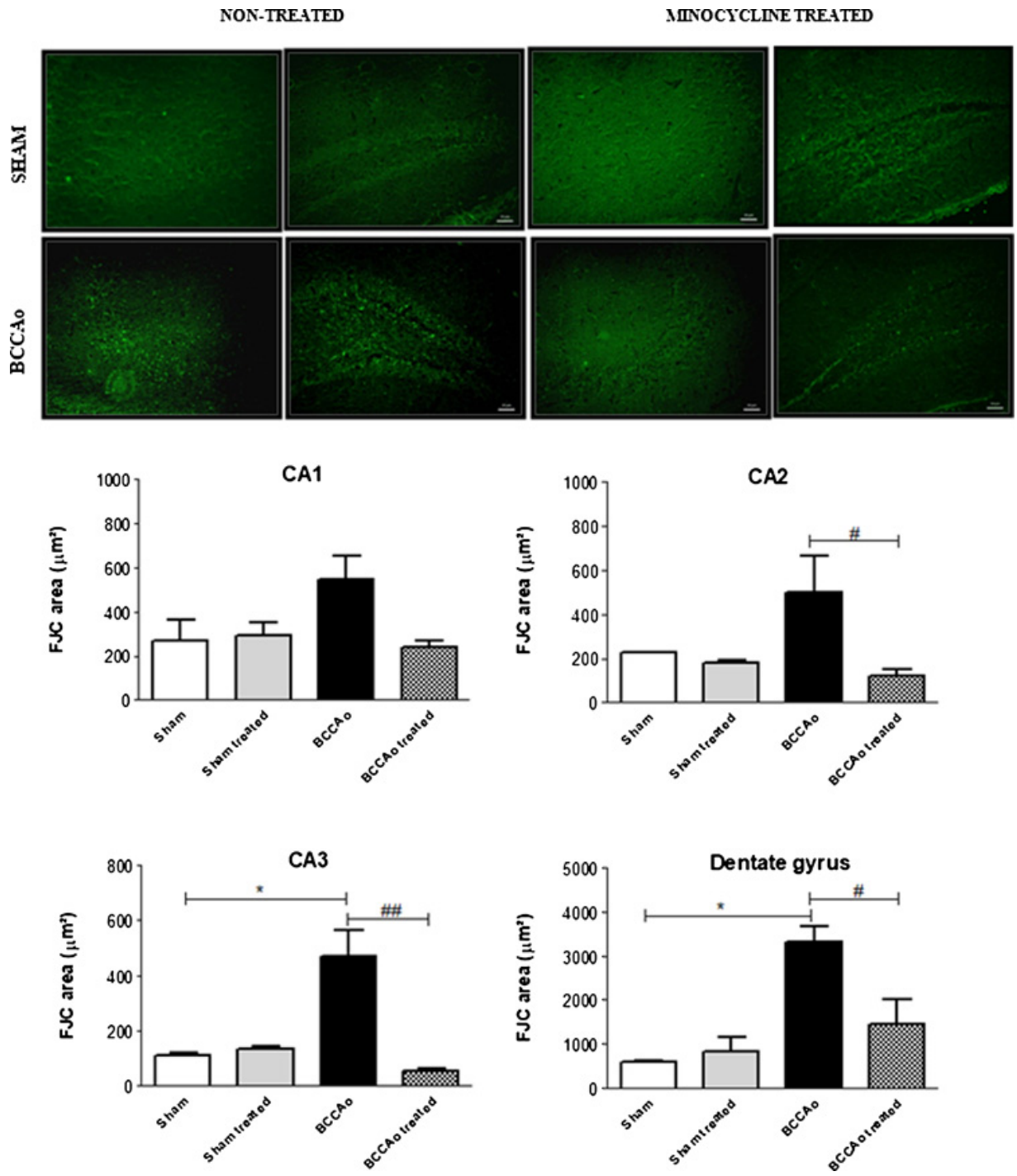

Fig. 3. Effects of minocycline on the number of FJC-positive cells in the CA1, CA2, CA3 and dentate gyrus of hippocampus from BCCAo animals. Minocycline treated ischemic mice showed less intense neurodegeneration as established by FJC staining (n = 5 per group). Results are expressed as mean ± SEM. Asterisks indicate statistical differences where *$p < 0.05$, BCCAo versus sham; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, non-treated versus minocycline-treated BCCAo mice.

has already been described reduction in infarct size and prevention of tissue loss in the ischemic hemispheres of adult spontaneously hypertensive rats submitted to transient middle cerebral artery occlusion and treated with a single dose of minocycline (Yang et al., 2015). Some studies have reported that minocycline treatment for two days after focal brain ischemia attenuated neurological deficits and infarct volume in mice (Jin et al., 2015). Of note, minocycline administration for seven days also prevented brain damage and reduced pyramidal cell death and microglial activation in the CA1 region of hippocampus in rats submitted to brain ischemia and reperfusion (Naderi et al., 2017). These studies are in accordance to our findings and reinforce the minocycline protective effect on neurodegeneration.

In the current study, we also found a significant increase of brain IFN-γ, IL-12p70, IL-10 and CCL2 concentrations of BCCAo mice, indicating that those mediators may also contribute to development of depression and anxiety-like behaviors induced by ischemia injury. However, the brain levels of IFN-γ and IL-12p70 remained elevated and no difference in CCL2 amounts was observed after minocycline treatment in BCCAo animals. Increased serum levels of TNF, IL-6, IFN-γ has been observed up to 12th months after stroke in patients with post-

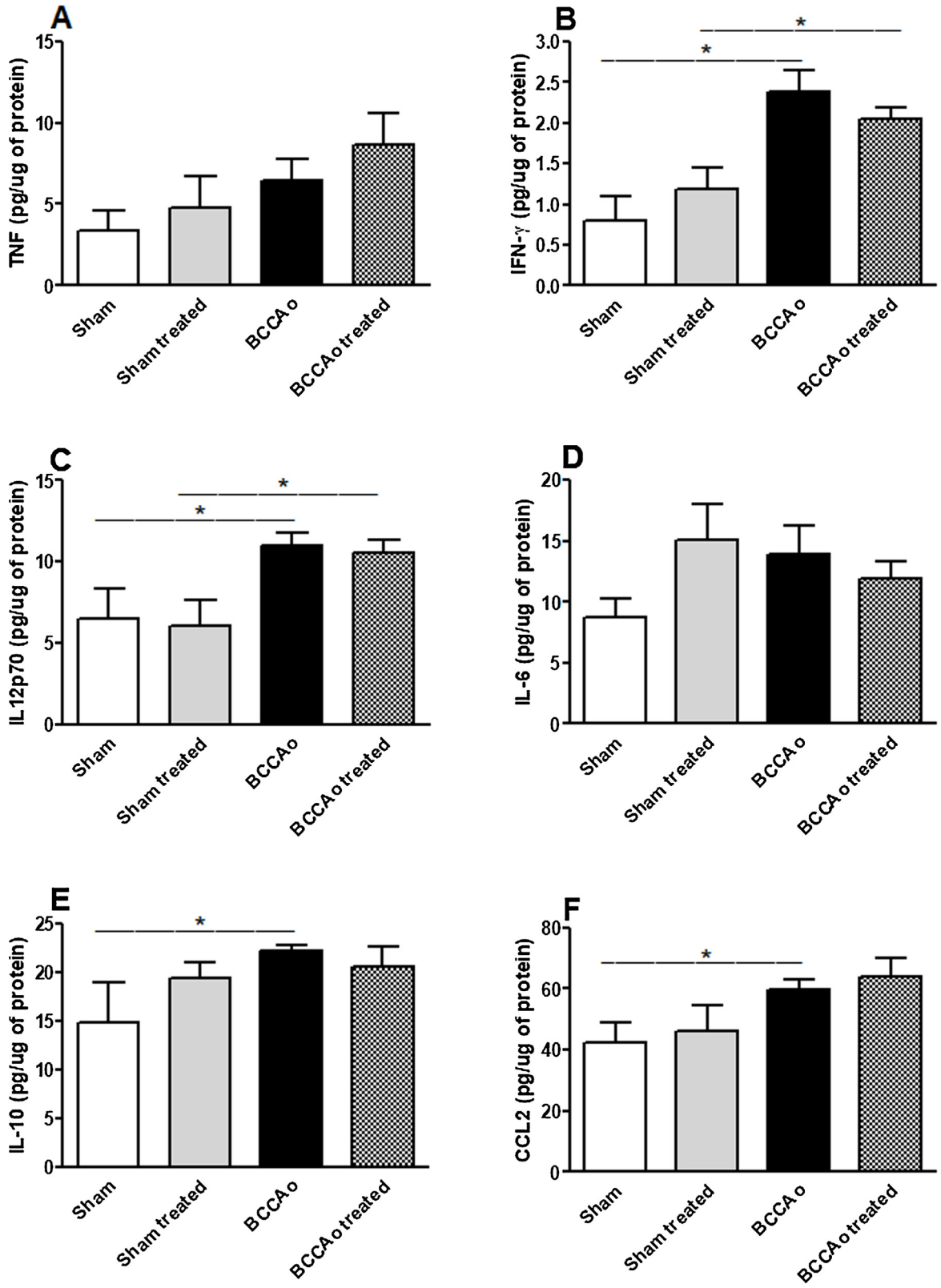

Fig. 4. Effects of minocycline treatment in the inflammatory response from brain ischemic animals. Brain levels of TNF, IFN-γ, IL12p70, IL-6, IL-10 and CCL2 levels were assessed by cytometric bead array (CBA). Results are expressed as mean ± SEM (n = 5 per group). Asterisks indicate statistical differences where *$p < 0.05$, non-treated versus minocycline-treated BCCAo mice.

stroke depression (Su et al., 2012). Furthermore, higher peripheral levels of IL-12, CCL2 and IL-10 were detected in patients with major depressive disorder (Köhler et al., 2017). Some authors hypothesized that the pro-inflammatory processes, predominantly in limbic regions, activated indoleamine 2,3-dioxygenase 1 (IDO1), promoting reduction of serotonin production in paralimbic areas and consequently post-stroke depression (Spalletta et al., 2006). Interesting, IFN-γ can induce depressive-like behavior by alteration in IDO1 activity and in the levels

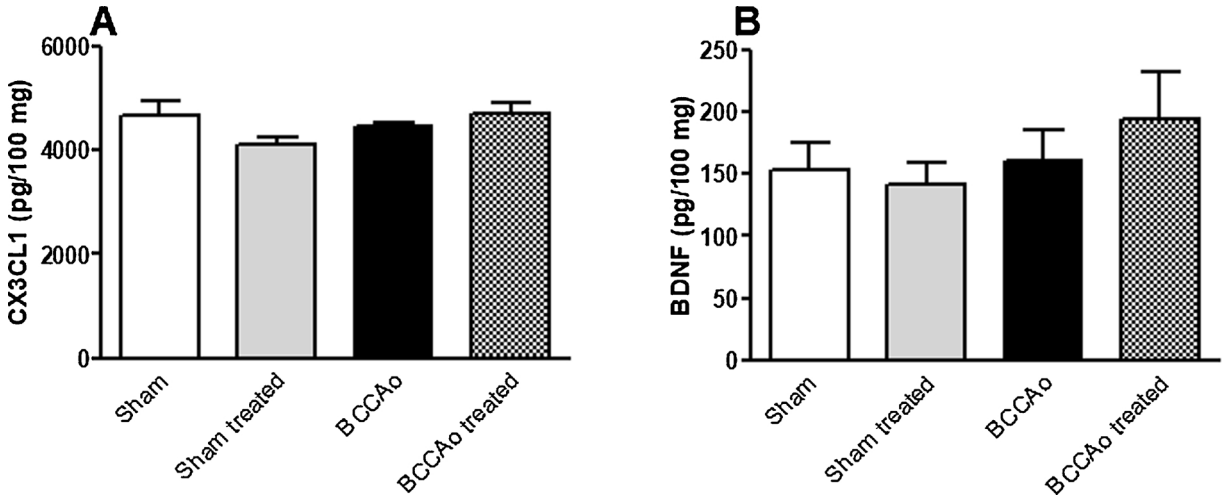

Fig. 5. Levels of CX3CL1 (A) and BDNF (B) was measured by ELISA in the brains from sham and BCCAo mice after treatment with minocycline. Results are expressed as mean ± SEM (n = 5 per group).

of L-tryptophan in the serum and frontal cortex of mice (Murakami et al., 2016). Additionally, IL-12/IFN-γ can interfere with inflammatory cytokine signaling, and the blockage of IL-12p70 and IL-23 efficiently suppressed injury and improved recovery of neurological deficits in an ischemia-reperfusion model (Konoeda et al., 2010). Moreover, levels of IL-12p70 were found to be higher in the serum of patients with stroke in comparison with controls, and correlated significantly with the volume of early brain CT hypodense areas (Zaremba and Losy, 2006).

We evaluated the brain expression of the chemokine fractalkine (CX3CL1) and similar levels were detected fourteen days after BCCAo. Microglial activation is usually regulated by CX3CL1 and CX3CR1 receptor and their signaling has been demonstrated to dampen neuroinflammation (Ahn et al., 2019). Less severe neurological score associated with reduction of brain damage and inflammation were observed three days after transient occlusion of the middle cerebral artery in CX3CR1 deficient mice (Tang et al., 2014). The expression of CX3CL1 in the hippocampal CA1 field from gerbils following transient global cerebral ischemia was verified by western blot and immunohistochemistry (Ahn et al., 2019). Interesting, these authors demonstrated a decrease of CX3CL1 protein levels at six hours and a peak at one day after ischemia-reperfusion induction. Then, CX3CL1 values returned to lower levels until 10 days after brain ischemia. This transient elevation of neuronal CX3CR1 has been associated with ischemia-induced apoptotic neuronal death (Ahn et al., 2019; Wang et al., 2018). But none of these works evaluated the brain levels of CX3CL1 after minocycline treatment. Our work showed similar levels of CX3CL1 in the brain and the minocycline treatment did not alter those values fourteen days after ischemia in mice. These data suggested that the peak of production of this chemokine after brain ischemia could occur before fourteen days and that probably had an adjustment of CX3CL1 release by survived neurons. Several works have been shown elevation of BDNF in the brain after stroke in rodents, suggesting that BDNF is involved in post-lesional plasticity and survival of neurons via the promotion of anti-apoptotic pathways (Cao et al., 2011; Victoria et al., 2017; Zhao et al., 2015). A possible neuroprotective mechanism attributed to minocycline after ischemic brain injury is via enhancement of BDNF expression by modulation of miR-155-mediated BDNF repression along with increased expression of Bcl-2/Bax ratio and reduced expression of caspase-3 (Lu et al., 2017). Béjot et al. (2011) demonstrated an increase in brain BDNF levels at four and twenty-four hours post-embolization, and brain BDNF levels were similar to control animals eight days after stroke. These data corroborated with our results that BDNF levels were not changed at day 14 after brain ischemia. On the other hand, the ratio of mBDNF/proBDNF and BDNF levels were significantly decreased four weeks after middle cerebral artery occlusion in post-stroke depression rats (Luo et al., 2019).

In conclusion, these results show that minocycline treatment prevents neuropsychiatric-like behaviors and diminished brain damage in post-stroke mice. Minocycline has a therapeutic potential for treating depression and anxiety as well as to promote neuroprotection in post-stroke patients. Future studies are clearly warranted to better understand the underlying molecular mechanisms.

Declaration of Competing Interest

None.

Acknowledgments

We are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig) for their financial support.

References

Ahn, J., Kim, D., Park, J., Lee, T., Lee, H., Won, M., Lee, C., 2019. Expression changes of CX3CL1 and CX3CR1 proteins in the hippocampal CA1 field of the gerbil following transient global cerebral ischemia. Int. J. Mol. Med. 939–948. https://doi.org/10.3892/ijmm.2019.4273.

Aldoghachi, A.F., Tor, Y.S., Redzun, S.Z., Bin Lokman, K.A., Abdul Razaq, N.A., Shahbudin, A.F., Badamasi, I.M., Cheah, P.S., Stanslas, J., Veerakumarasivam, A., Rosli, R., Ibrahim, N., Lye, M.S., Ling, K.H., 2019. Screening of brain-derived neurotrophic factor (BDNF) single nucleotide polymorphisms and plasma BDNF levels among Malaysian major depressive disorder patients. PLoS One 14, 1–19. https://doi.org/10.1371/journal.pone.0211241.

Amiri-Nikpour, M.R., Nazarbaghi, S., Hamdi-Holasou, M., Rezaei, Y., 2015. An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect. Acta Neurol. Scand. 131, 45–50. https://doi.org/10.1111/ane.12296.

Bagdas, D., AlSharari, S., Roni, M.A., Campbell, V.C., Muldoon, P.P., Carroll, F.I., Damaj, M.I., 2019. Blockade of nicotinic acetylcholine receptor enhances the responsiveness to bupropion in the mouse forced swim test. Behav. Brain Res. 360, 262–269. https://doi.org/10.1016/j.bbr.2018.12.027.

Becker, K.J., 2016. Inflammation and the silent sequelae of stroke. Neurotherapeutics 13, 801–810. https://doi.org/10.1007/s13311-016-0451-5.

Béjot, Y., Mossiat, C., Giroud, M., Prigent-Tessier, A., Marie, C., 2011. Circulating and brain BDNF levels in stroke rats. Relevance to clinical studies. PLoS One 6, 6–11. https://doi.org/10.1371/journal.pone.0029405.

Buijck, B.I., Zuidema, S.U., Spruit-Van Eijk, M., Geurts, A.C.H., Koopmans, R.T.C.M., 2012. Neuropsychiatric symptoms in geriatric patients admitted to skilled nursing facilities in nursing homes for rehabilitation after stroke: a longitudinal multicenter study. Int. J. Geriatr. Psychiatry 27, 734–741. https://doi.org/10.1002/gps.2781.

Cao, Y., Mao, X., Sun, C., Zheng, P., Gao, J., Wang, X., Min, D., Sun, H., Xie, N., Cai, J., 2011. Baicalin attenuates global cerebral ischemia/reperfusion injury in gerbils via anti-oxidative and anti-apoptotic pathways. Brain Res. Bull. 85, 396–402. https://doi.org/10.1016/j.brainresbull.2011.05.002.

Castellanos-Pinedo, F., Hernández-Pérez, J.M., Zurdo, M., Rodríguez-Fúnez, B.,

Hernández-Bayo, J.M., García-Fernández, C., Cueli-Rincón, B., Castro-Posada, J.A., 2011. Influence of premorbid psychopathology and lesion location on affective and behavioral disorders after ischemic stroke. Journal of Neuropsychiatry Clinical Neuroscience 23, 340–347. https://doi.org/10.1176/appi.neuropsych.23.3.340.

Chemerinski, E., Robinson, R.G., 2000. The neuropsychiatry of stroke. Psychosomatics 41, 5–14. https://doi.org/10.1016/S0033-3182(00)71168-6.

Cho, K.O., Kim, S.K., Cho, Y.J., Sung, K.W., Kim, S.Y., 2007. Regional differences in the neuroprotective effect of minocycline in a mouse model of global forebrain ischemia. Life Sci. 80, 2030–2035. https://doi.org/10.1016/j.lfs.2007.03.005.

De Mello, R.F., De Souza Santos, I., Alencar, A.P., Benseñor, I.M., Lotufo, P.A., Goulart, A.C., 2016. Major depression as a predictor of poor long-term survival in a brazilian stroke cohort (Study of stroke mortality and morbidity in adults) EMMA study. J. Stroke Cerebrovasc. Dis. 25, 618–625. https://doi.org/10.1016/j.jstrokecerebrovasdis.2015.11.021.

Dean, O.M., Maes, M., Ashton, M., Berk, L., Kanchanatawan, B., Sughondhabirom, A., Tangwongchai, S., Ng, C., Dowling, N., Malhi, G.S., Berk, M., 2014. Protocol and rationale-the efficacy of minocycline as an adjunctive treatment for major depressive disorder: a double blind, randomised, placebo controlled trial. Clin. Psychopharmacol. Neurosci. 12, 180–188. https://doi.org/10.9758/cpn.2014.12.3.180.

Eldahshan, W., Fagan, S.C., Ergul, A., 2019. Inflammation within the neurovascular unit: focus on microglia for stroke injury and recovery. Pharmacol. Res. 147, 104349. https://doi.org/10.1016/j.phrs.2019.104349.

Faleiros, B.E., Miranda, A.S., Campos, A.C., Gomides, L.F., Kangussu, L.M., Guatimosim, C., Camargos, E.R.S., Menezes, G.B., Rachid, M., Teixeira, A.L., 2014. Up-regulation of brain cytokines and chemokines mediates neurotoxicity in early acute liver failure by a mechanism independent of microglial activation. Brain Res. 1578, 49–59. https://doi.org/10.1016/j.brainres.2014.07.001.

Fan, L.W., Lin, S., Pang, Y., Rhodes, P.G., Cai, Z., 2006. Minocycline attenuates hypoxia-ischemia-induced neurological dysfunction and brain injury in the juvenile rat. Eur. J. Neurosci. 24, 341–350. https://doi.org/10.1111/j.1460-9568.2006.04918.x.

Hall, C.S., 1941. Emotional behavior in the rat. J. Comp. Psychol. 18, 385–403. https://doi.org/10.1037/h0071444.

Jayaraj, R.L., Azimullah, S., Beiram, R., Jalal, F.Y., Rosenberg, G.A., 2019. Neuroinflammation: friend and foe for ischemic stroke. J. Neuroinflammation 16, 1–24. https://doi.org/10.1186/s12974-019-1516-2.

Jin, Z., Liang, J., Wang, J., Kolattukudy, P.E., 2015. MCP-induced protein 1 mediates the minocycline-induced neuroprotection against cerebral ischemia/reperfusion injury in vitro and in vivo. J. Neuroinflammation 12, 1–12. https://doi.org/10.1186/s12974-015-0264-1.

Kapoor, A., Scott, C., Lanctot, K.L., Herrmann, N., Murray, B.J., Thorpe, K.E., Lien, K., Sicard, M., Swartz, R.H., 2019. Symptoms of depression and cognitive impairment in young adults after stroke/transient ischemic attack. Psychiatry Res. 279, 361–363. https://doi.org/10.1016/j.psychres.2019.06.022.

Köhler, C.A., Freitas, T.H., Maes, M., de Andrade, N.Q., Liu, C.S., Fernandes, B.S., Stubbs, B., Solmi, M., Veronese, N., Herrmann, N., Raison, C.L., Miller, B.J., Lanctôt, K.L., Carvalho, A.F., 2017. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. Acta Psychiatr. Scand. 135, 373–387. https://doi.org/10.1111/acps.12698.

Kojima, M., Matsui, K., Mizui, T., 2019. BDNF pro-peptide: physiological mechanisms and implications for depression. Cell Tissue Res. 377, 73–79. https://doi.org/10.1007/s00441-019-03034-6.

Konoeda, F., Shichita, T., Yoshida, H., Sugiyama, Y., Muto, G., Hasegawa, E., Morita, R., Suzuki, N., Yoshimura, A., 2010. Therapeutic effect of IL-12/23 and their signaling pathway blockade on brain ischemia model. Biochem. Biophys. Res. Commun. 402, 500–506. https://doi.org/10.1016/j.bbrc.2010.10.058.

Liu, H.Y., Yue, J., Hu, L.N., Cheng, L.F., Wang, X.S., Wang, X.J., Feng, B., 2018. Chronic minocycline treatment reduces the anxiety-like behaviors induced by repeated restraint stress through modulating neuroinflammation. Brain Res. Bull. 143, 19–26. https://doi.org/10.1016/j.brainresbull.2018.08.015.

Lu, Y., Huang, Z., Hua, Y., Xiao, G., 2017. Minocycline promotes BDNF expression of N2aCells via inhibition of miR-155-Mediated repression after oxygen-glucose deprivation and reoxygenation. Cell Molecular Neurobiology 38, 1305–1313. https://doi.org/10.1007/s10571-018-0599-0.

Luo, L., Li, C., Deng, Y., Wang, Y., Meng, P., Wang, Q., 2019. High-intensity interval training on neuroplasticity, balance between brain-derived neurotrophic factor and precursor brain-derived neurotrophic factor in poststroke depression rats. J. Stroke Cerebrovasc. Dis. 28, 672–682. https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.11.009.

Malhotra, K., Chang, J.J., Khunger, A., Blacker, D., Switzer, J.A., Goyal, N., Hernandez, A.V., Pasupuleti, V., Alexandrov, A.V., Tsivgoulis, G., 2018. Minocycline for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials. J. Neurol. 265, 1871–1879. https://doi.org/10.1007/s00415-018-8935-3.

Matsukawa, N., Yasuhara, T., Hara, K., Xu, L., Maki, M., Yu, G., Kaneko, Y., Ojika, K., Hess, D.C., Borlongan, C.V., 2009. Therapeutic targets and limits of minocycline neuroprotection in experimental ischemic stroke. BMC Neurosci. 10, 126. https://doi.org/10.1186/1471-2202-10-126.

Merendino, R.A., Di Pasquale, G., De Luca, F., Di Pasquale, L., Ferlazzo, E., Martino, G., Palumbo, M.C., Morabito, F., Gangemi, S., 2004. Involvement of fractalkine and macrophage inflammatory protein-1 alpha in moderate-severe depression. Mediators Inflamm. 13, 205–207. https://doi.org/10.1080/09511920410001713484.

Mizui, T., Hattori, K., Ishiwata, S., Hidese, S., Yoshida, S., Kunugi, H., Kojima, M., 2019. Cerebrospinal fluid BDNF pro-peptide levels in major depressive disorder and schizophrenia. J. Psychiatr. Res. 113, 190–198. https://doi.org/10.1016/j.jpsychires.2019.03.024.

Murakami, Y., Ishibashi, T., Tomita, E., Imamura, Y., Tashiro, T., Watcharanurak, K., Nishikawa, M., Takahashi, Y., Takakura, Y., Mitani, S., Fujigaki, H., Ohta, Y., Kubo, H., Mamiya, T., Nabeshima, T., Kim, H.C., Yamamoto, Y., Saito, K., 2016. Depressive symptoms as a side effect of Interferon-α therapy induced by induction of indoleamine 2,3-dioxygenase 1. Sci. Rep. 6, 1–12. https://doi.org/10.1038/srep29920.

Murrough, J.W., Huryk, K.M., Mao, X., Iacoviello, B., Collins, K., Nierenberg, A.A., Kang, G., Shungu, D.C., Iosifescu, D.V., 2018. A pilot study of minocycline for the treatment of bipolar depression: effects on cortical glutathione and oxidative stress in vivo. J. Affect. Disord. 230, 56–64. https://doi.org/10.1016/j.jad.2017.12.067.

Naderi, Y., Sabetkasaei, M., Parvardeh, S., Moini Zanjani, T., 2017. Neuroprotective effects of pretreatment with minocycline on memory impairment following cerebral ischemia in rats. Behav. Pharmacol. 28, 214–222. https://doi.org/10.1097/FBP.0000000000000297.

Pascoe, M.C., Crewther, S.G., Carey, L.M., Crewther, D.P., 2011. Inflammation and depression: why poststroke depression may be the norm and not the exception. Int. J. Stroke 6, 128–135. https://doi.org/10.1111/j.1747-4949.2010.00565.x.

Pedroso, V.S.P., Brunoni, A.R., Vieira, É.L.M., Jorge, R.E., Lauterbach, E.C., Teixeira, A.L., 2018. Early psychiatric morbidity in a Brazilian sample of acute ischemic stroke patients. Clinics 73, 1–5. https://doi.org/10.6061/clinics/2018/e055.

Sagnier, S., Munsch, F., Bigourdan, A., Debruxelles, S., Poli, M., Renou, P., Olindo, S., Rouanet, F., Dousset, V., Tourdias, T., Sibon, I., 2019. Journal of Stroke Cerebrovasccular Disease 28, 1236–1242. https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.01.010.

Savitz, J.B., Teague, T.K., Misaki, M., Macaluso, M., Wurfel, B.E., Meyer, M., Drevets, D., Yates, W., Gleason, O., Drevets, W.C., Preskorn, S.H., 2018. Treatment of bipolar depression with minocycline and/or aspirin: an adaptive, 2×2 double-blind, randomized, placebo-controlled, phase IIA clinical trial. Transl. Psychiatry 8. https://doi.org/10.1038/s41398-017-0073-7.

Sheng, Z., Liu, Y., Li, H., Zheng, W., Xia, B., Zhang, X., Yong, V.W., Xue, M., 2018. Efficacy of minocycline in acute ischemic stroke: a systematic review and meta-analysis of rodent and clinical studies. Front. Neurol. 9. https://doi.org/10.3389/fneur.2018.01103.

Shi, Y., Zeng, Y., Wu, L., Liu, Z., Zhang, S., Yang, J., Wu, W., 2017. A study of the brain functional network of post-stroke depression in three different lesion locations. Sci. Rep. 7, 14795. https://doi.org/10.1038/s41598-017-14675-4.

Silva, B., Sousa, L., Miranda, A., Vasconcelos, A., Reis, H., Barcelos, L., Arantes, R., Teixeira, A., Rachid, M.A., 2015. Déficit de memória associado ao aumento dos níveis cerebrais de citocinas pró-inflamatórias e neurodegeneração na isquemia cerebral aguda. Arq. Neuropsiquiatr. 73, 655–659. https://doi.org/10.1590/0004-282X20150083.

Soares, L.M., Schiavon, A.P., Milani, H., De Oliveira, R.M.W., 2013. Cognitive impairment and persistent anxiety-related responses following bilateral common carotid artery occlusion in mice. Behav. Brain Res. 249, 28–37. https://doi.org/10.1016/j.bbr.2013.04.010.

Spalletta, G., Bossù, P., Ciaramella, A., Bria, P., Caltagirone, C., Robinson, R.G., 2006. The etiology of poststroke depression: a review of the literature and a new hypothesis involving inflammatory cytokines. Mol. Psychiatry 11, 984–991. https://doi.org/10.1038/sj.mp.4001879.

Su, J.A., Chou, S.Y., Tsai, C.S., Hung, T.H., 2012. Cytokine changes in the pathophysiology of poststroke depression. Gen. Hosp. Psychiatry 34, 35–39. https://doi.org/10.1016/j.genhosppsych.2011.09.020.

Tang, Z., Gan, Y., Liu, Q., Yin, J.X., Liu, Q., Shi, J., Shi, F.D., 2014. CX3CR1 deficiency suppresses activation and neurotoxicity of microglia/macrophage in experimental ischemic stroke. J. Neuroinflammation 11, 1–13. https://doi.org/10.1186/1742-2094-11-26.

Tejeda, G.S., Esteban-Ortega, G.M., San Antonio, E., Vidaurre, Ó., Díaz-Guerra, M., 2019. Prevention of excitotoxicity-induced processing of BDNF receptor TrkB-FL leads to stroke neuroprotection. EMBO Mol. Med. https://doi.org/10.15252/emmm.201809950. e9950.

Toscano, E.C.D.B., Silva, B.C., Victoria, E.C.G., Cardoso, A.C.D.S., Miranda, A.S.D., Sugimoto, M.A., Sousa, L.P., Carvalho, B.A.D., Kangussu, L.M., Silva, D.G.D., Rodrigues, F.G., Barcelos, L.D.S., Vasconcelos, A.C., Amaral, F.A., Teixeira, M.M., Teixeira, A.L., Rachid, M.A., 2016. Platelet-activating factor receptor (PAFR) plays a crucial role in experimental global cerebral ischemia and reperfusion. Brain Res. Bull. 124. https://doi.org/10.1016/j.brainresbull.2016.03.022.

Verduijn, J., Milaneschi, Y., Schoevers, R.A., Van Hemert, A.M., Beekman, A.T.F., Penninx, B.W.J.H., 2015. Pathophysiology of major depressive disorder: mechanisms involved in etiology are not associated with clinical progression. Transl. Psychiatry 5https://doi.org/10.1038/tp.2015.137. e649-9.

Victoria, E.C.G., De Brito Toscano, E.C., De Sousa Cardosoa, A.C., Da Silva, D.G., De Miranda, A.S., Da Silva Barcelos, L., Sugimoto, M.A., Sousa, L.P., De Assis Limae, I.V., De Oliveira, A.C.P., Brant, F., Machado, F.S., Teixeira, M.M., Teixeira, A.L., Rachid, M.A., 2017. Knockdown of C-C chemokine receptor 5 (CCR5) is protective against cerebral ischemia and reperfusion injury. Curr. Neurovasc. Res. 14. https://doi.org/10.2174/1567202614666170313113056.

Wang, W., Wang, R., Xu, J., Qin, X., Jiang, H., Khalid, A., Liu, D., Pan, F., Ho, C.S.H., Ho, R.C.M., 2018. Minocycline attenuates stress-induced behavioral changes via its anti-inflammatory effects in an animal model of post-traumatic stress disorder. Front. Psychiatry 9, 1–10. https://doi.org/10.3389/fpsyt.2018.00558.

Yang, Y., Salayandia, V.M., Thompson, J.F., Yang, L.Y., Estrada, E.Y., Yang, Y., 2015. Attenuation of acute stroke injury in rat brain by minocycline promotes blood-brain barrier remodeling and alternative microglia/macrophage activation during recovery. J. Neuroinflammation 12, 1–15. https://doi.org/10.1186/s12974-015-0245-4.

Zaremba, J., Losy, J., 2006. Interleukin-12 in acute ischemic stroke patients. Folia Neuropathol. 44, 59–66.

Zemdegs, J., Rainer, Q., Grossmann, C.P., Rousseau-Ralliard, D., Grynberg, A., Ribeiro, E.,

Guiard, B.P., 2018. Anxiolytic- and antidepressant-like effects of fish oil-enriched diet in brain-derived neurotrophic factor deficient mice. Front. Neurosci. 12, 1–16. https://doi.org/10.3389/fnins.2018.00974.

Zhang, C., Zhang, Y.P., Li, Y.Y., Liu, B.P., Wang, H.Y., Li, K.W., Zhao, S., Song, C., 2019. Minocycline ameliorates depressive behaviors and neuro-immune dysfunction induced by chronic unpredictable mild stress in the rat. Behav. Brain Res. 356, 348–357. https://doi.org/10.1016/j.bbr.2018.07.001.

Zhao, Y., Xiao, M., He, W., Cai, Z., 2015. Minocycline upregulates cyclic AMP response element binding protein and brain-derived neurotrophic factor in the hippocampus of cerebral ischemia rats and improves behavioral deficits. Neuropsychiatr. Dis. Treat. 11, 507–516. https://doi.org/10.2147/NDT.S73836.