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Title: Minocycline Improves Memory in a Passive Avoidance Task Following Cerebral Ischemia-Reperfusion By Enhancing Hippocampal Synaptic Plasticity and Restoring Antioxidant Enzyme Activity in Rats

Short title: Minocycline Restores Synaptic Plasticity

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Abstract

Introduction: Oxidative stress plays a crucial role in the impairment of synaptic plasticity following cerebral ischemia which ultimately results in memory dysfunction. Hence, application of antioxidant agents could be beneficial in the management of memory deficit after brain ischemia. Minocycline is a tetracycline antibiotic with antioxidant effect. The main objective of this work was to assess minocycline effect on the impairment of synaptic plasticity and memory after cerebral ischemia-reperfusion in rats.

Methods: Transient occlusion of common carotid arteries was used to induce ischemia-reperfusion injury in rats. Single or multiple (once daily for 7 days) doses of minocycline were administered before (pretreatment) or after (treatment) brain ischemia. Seven days after ischemia-reperfusion, passive avoidance performance, hippocampal long-term potentiation, and the activity of antioxidant enzymes were assessed.

Results: The results of passive avoidance test showed that minocycline (20 and 40 mg/kg) significantly increased step-through latency while reduced the duration of staying in dark chamber in the treatment (but not pretreatment) group. In electrophysiological experiments, the rats which were treated (but not pretreated) with minocycline (40 mg/kg) showed a significant increase in the amplitude of the field excitatory postsynaptic potentials in the dentate gyrus area of hippocampus. The treatment (but not pretreatment) with minocycline (20 and 40 mg/kg) resulted in a significant increase in the activity of catalase, glutathione peroxidase, and superoxide dismutase in the hippocampus.

Conclusion: It was ultimately determined that minocycline attenuates memory dysfunction after cerebral ischemia-reperfusion in rats through the improvement of hippocampal synaptic plasticity and restoration of antioxidant enzymes activity.

Keywords: Brain ischemia; Memory; Minocycline; Synaptic plasticity; Antioxidant enzymes
Plain Language Summary

Stroke is a common neurological disease with a relatively high rate of mortality and disabilities in all around the world. More than half of the patients who have had an episode of stroke suffer from the impairment of sensorimotor function and language problems as well as learning and memory disorders. Oxidative stress plays an important role in the impairment of memory following brain ischemia. Hence, application of antioxidant agents could be beneficial in the management of memory deficit after stroke. Minocycline is a tetracycline antibiotic which is basically used for the treatment of infectious diseases; nevertheless, it can function as a potent antioxidant medication. Hence, we hypothesized that minocycline could attenuate memory impairment after brain ischemia. We examined this hypothesis in a rat model of brain ischemia. In this model, the main arteries that supply the brain with oxygenated blood were occluded and as a result, brain ischemia was induced in the animals. Then, minocycline was administered in the rats which were subjected to brain ischemia. Seven days later, memory function in the rats was evaluated. The results showed that minocycline could enhance the activity of antioxidant enzymes in the brain which physiologically fight off oxidative stress. This function of minocycline enabled it to protect brain cells against ischemic injury and thereby to increase the transmission of neuronal signals from one cell to another cell in the memory centers in the brain. These minocycline effects ultimately led to an increase in the memory function of rats which was evident in the behavioral memory test. Overall, the results obtained from this study suggest that minocycline can be considered as a memory enhancer drug in the patients who suffer from learning and memory disorders following stroke.
1. Introduction

Learning and memory disorder is one of the most common outcomes of cerebral ischemia in patients who suffer from stroke, vascular dementia, and cardiac arrest. Currently, the administration of fibrinolytic agents is the only approved medications to manage acute phase of ischemic stroke. However, there is no effective therapeutic approach to attenuate neuronal damage in long-term period which underlie sensorimotor and cognitive disorders, particularly memory impairment (Zhao & Willing, 2018).

The hippocampus is one of the most important areas of the brain which implicates the formation of memory. It is well established that the formation of long-term potentiation (LTP) is a fundamental process for memory acquisition in the hippocampal formations (Lynch, 2004). Nonetheless, the physiological induction of LTP could remarkably get impaired under pathological conditions particularly oxidative stress. In this regard, it has been demonstrated that the increase in free radical generation which is often concomitant with a reduction in antioxidant enzyme capacity, adversely affect the induction of LTP (Knapp & Klann, 2002). Accordingly, in cerebral ischemia in which excessive amounts of free radicals are produced (Warner, Sheng, & Batinić-Haberle, 2004), LTP formation in the hippocampus is disrupted and ultimately leads to memory impairment (Xu et al., 2010).

Studies have shown that hippocampal neurons are highly susceptible to oxidative stress and thereby damaged during ischemia-reperfusion (Baron, Yamauchi, Fujioka, & Endres, 2014). The occurrence of oxidative stress during cerebral ischemia causes a noticeable generation of reactive oxygen species (ROS), such as peroxide, superoxide and radical hydroxyl. In fact, oxidative stress occurs when ROS generation predominates over the antioxidant defense system. Contrarily, intracellular antioxidant enzymes including glutathione peroxidase, catalase, and superoxide dismutase are of the most important antioxidant mechanisms in protecting cells against destructive effects of ROS (Manzanero, Santro, & Arumugam, 2013). Considering massive production of ROS during cerebral ischemia (Warner, Sheng, & Batinić-Haberle, 2004) and the impairment of synaptic plasticity due to oxidative stress (Serrano & Klann, 2004; Kishida & Klann, 2007), it could be anticipated that the utilization of antioxidant agents results in the attenuation of neuronal damage and memory disorders following cerebral ischemia (Karimi et al., 2013; Chen, Yin, Hwang, Tang, & Yang, 2012).

Minocycline is a tetracycline molecule which possesses anti-apoptotic, anti-inflammatory, and antioxidant effects in addition to its antibiotic effect. Besides, minocycline can act as a free-radical
scavenger and efficiently remove several reactive radical molecules (Chen, Yin, Hwang, Tang, & Yang, 2012). Moreover, it has been recently reported that pretreatment or treatment with minocycline suppresses the process of lipid peroxidation and neuroinflammation in the brain and attenuate neuronal injury during global ischemia in rat brain (Naderi, Sabetkasaei, Parvardeh, & Moini Zanjani, 2017). Although, several mechanisms of action have been reported to describe the protective effect of minocycline against ischemic conditions in the brain (Naderi, Sabetkasaei, Parvardeh, & Zanjani, 2017; Sheng et al., 2018), the neuroprotective effect of this antibiotic on hippocampal synaptic plasticity and enzymatic antioxidant activity following cerebral ischemia has not yet been evaluated. Considering the fundamental role of synaptic plasticity in memory formation (Lynch, 2004) and enzymatic antioxidant defense system in the restoration of neuronal function (Lalkovičová, & Danielisová, 2016), it was hypothesized that the aforementioned mechanisms may underlie the attenuating effect of minocycline on memory impairment during cerebral ischemia. Thus, the aim of this study was to clarify whether minocycline could act to enhance memory function through improving synaptic plasticity and restoration of antioxidant enzyme activity in the hippocampus after neuronal injury induced by cerebral ischemia-reperfusion in rats.

2. Methods

2.1. Animals

In this study, male Wistar rats weighing 200 to 250 g were used. Animals were kept in a standard temperature, light and humidity conditions and always had free and easy access to tap water and food. All experiments and procedures were carried out under the supervision and approval of the Ethics Committee settled in the School of Medicine, Shahid Beheshti University of Medical Sciences (Code of Ethics: IR.SBMU.SM.REC.1394.6).

2.2. Induction of cerebral ischemia

Bilateral common carotid artery occlusion was used to induce cerebral ischemic injury in rats as described previously (Naderi, Parvardeh, Zanjani, & Sabetkasaei, 2018). For the induction of cerebral ischemia, common carotid arteries on both sides of vertebral column in the area of the neck were exposed in rats under anesthesia induced by chloral hydrate (300 mg/kg, intraperitoneally (i.p.); Sigma-Aldrich). Then, the blood flow through the common carotid arteries was blocked by using bulldog microclamps. After 20 min, the microclamps were removed and the blood flow to the brain was restored.
2.3. Study design

The rats were divided into four main groups (n=6). In the treatment group, minocycline (Sigma-Aldrich) was injected i.p. to the rats immediately after reperfusion. In this group, minocycline was administered with the doses of 10, 20, and 40 mg/kg, once daily for 7 days. In pretreatment group, single doses of minocycline (10, 20, and 40 mg/kg, i.p.) was administered to the rats, 60 min before the obstruction of common carotid arteries. The rats in control group received normal saline. In sham-operated group, the animals underwent surgery and their common carotid arteries were exposed, but not occluded. Minocycline dosage was selected based on previous studies (Naderi, Sabetkasaei, Parvardeh, & Zanjani, 2017).

2.4. Passive avoidance test

Seven days after cerebral ischemic injury, a passive avoidance test was conducted to assess memory performance in rats. The procedure was executed by using shuttle box according to the method described by Arabian et al. (2017). Primarily, the animals were placed in the shuttle box to habituate with the surrounding. Thirty min after habituation trial, the rats were placed in the lighted chamber of the shuttle box. After 5 seconds, the guillotine door between the lighted and dark compartments was opened and allowed the animal to enter the dark chamber. Immediately after the entrance of rats to the dark compartment, the valve between the two chambers was closed and an electric shock (1 mA for 2 seconds) was applied. Twenty seconds later, the rats were transferred to their cages. After 24 hours, a retention trial was performed in which the rats were placed once more in the lighted compartment and after 5 seconds, the door between two chambers was opened. The latency to step-through the dark chamber and the time duration which animals remained in dark compartment were recorded. At this stage, no shock was given to the animal when entered to the dark chamber. The cut-off limit to enter the dark chamber was considered 300 seconds in retention trial.

2.5. Recording of LTP in the hippocampus

A day after the passive avoidance test, LTP recording was performed to evaluate the synaptic plasticity in the hippocampus. The animals were first anesthetized by urethane (Merck) with the dosage of 1.5 g/kg, i.p. and then placed in a stereotactic device. Two small holes of 1 mm in diameter were created in the animal’s skull to enter electrodes into the brain. The stimulating electrode was placed in the hippocampal perforant pathway (PP) zone in accordance with the atlas of rat’s brain (AP: -8.1 mm, ML: +4.3 mm, and DV: 3.2 mm. The recording electrode was put in the granular cell layer of dentate gyrus (DG) area in the hippocampus (AP: -3.8 mm, ML: +2.3 mm, and DV: 2.7-3.2 mm) (Paxinos &
The electrodes were made of Teflon-coated stainless steel with 125 μm in diameter (A-M Systems, USA). A two-channel Electromodule amplifier (R12, ScienceBeam, Iran) was used to record field excitatory postsynaptic potentials (fEPSPs). The recorded signals were amplified (×1000) and digitized at 1 kHz. A bandwidth filter was set at 1-3000 Hz prior to digitization. The acquisition of biosignal and data analysis was carried out using eTrace software (ScienceBeam). The input-output curve was generated by applying a series of stimulating currents in the range of 100 to 900 mA to obtain maximum amplitude of field excitatory postsynaptic potentials (fEPSP). Then, the test stimulus with 0.033 Hz frequency and a stimulation intensity that aroused a fEPSP with amplitude of 40% of maximum response was applied in the PP area. The recording of fEPSP in the DG area was performed for 30 minutes as baseline. To induce LTP, a high frequency (100 Hz) stimulation consisting of 10 burst, each containing 4 shocks at intervals of 200 ms was applied in the PP area. Immediately after tetanic stimulation, electrical stimulation similar to pre-tetanic stimulation was applied and fEPSPs were recorded for two hours. The changes in the amplitude of fEPSPs after tetanic stimulation were compared to those of baseline (Moghimi, Parvardeh, Zanjani, & Ghafghazi, 2016).

2.6. Measurement of antioxidant enzymes in the hippocampus

In order to explore the effect of minocycline on oxidative damage in the brain, the activity of antioxidant enzymes including SOD (superoxide dismutase), catalase, and GPx (glutathione peroxidase) was determined in the hippocampus. By the end of electrophysiological procedures, the rats were sacrificed under anesthesia and the whole hippocampus was dissected out of the brain. The activity of antioxidant enzymes in the hippocampal tissue was determined by using enzyme activity assay kits (ZellBio GmbH, Ulm, Germany). According to the guidance of manufacturer’s manual, the amount of hydrogen peroxide (H₂O₂) in the presence of 20 μl of samples was measured by using a spectrophotometer at 240 nm. GPx activity was evaluated spectrophotometrically at 412 nm based on NADPH oxidation to NADP⁺. SOD activity was determined based on the dismutation of supercarboxide radicals produced by hypoxanthine and xanthine oxidase which was detected by spectrophotometer at 420 nm (Khoshnazar, Bigdeli, Parvardeh, & Pouriran, 2019).

2.7. Statistical analysis

Obtained data were reported as mean ± SEM in each group. One-way ANOVA followed by post-hoc Tukey test were used to analyze the difference of means between groups. If P-value was obtained less than 0.05, the difference between groups was considered significant.

3. Results
3.1. Effect of minocycline on passive avoidance performance

Induction of cerebral ischemia-reperfusion in rats caused a significant reduction in step-through latency in control group while prolonged the duration of remaining of animals in dark chamber (P<0.001). In contrast, the treatment of rats with minocycline (20 and 40 mg/kg) once daily for 7 days resulted in a significant increase in the latency of entrance to dark chamber (P<0.05 and P<0.01, respectively). Furthermore, treatment of rats by minocycline (20 and 40 mg/kg) significantly shortened the duration of residence time of rats in dark chamber (P<0.01 and P<0.001, respectively, Figures 1A and 1B). In pretreatment group, the administration of maximum dosage of minocycline (40 mg/kg, single dose) could significantly prolong the latency of step-through (P<0.05, Figure 1C) and reduce the duration of spending in dark chamber (P<0.01; Figure 1D). The prolongation of step-through latency in the rats which were treated by minocycline (40 mg/kg) was more than the time of pretreatment group (143.3 ± 16.8 vs 107.5 ± 8.6; P=0.3253). Besides, the duration of remaining in the dark compartment in the rats which were treated by minocycline (40 mg/kg) was lower than the time of pretreated group (130.8 ± 10.2 vs 155.2 ± 12.1; P=0.4636).

3.2. Effect of minocycline on synaptic plasticity in the hippocampus

In the electrophysiological recording of LTP in sham group, tetanic stimulation of nerve fibers in the PP elicited a significant increase in the amplitude of fEPSPs in the DG region of the hippocampus. In contrast, no significant difference was obtained by comparing the amplitude of fEPSPs before and after tetanic stimulation in control rats (Figures 2 and 3). In the minocycline-treated rats (40 mg/kg, once daily for 7 days), tetanic stimulation of neurons successfully induced LTP in the hippocampus (Figure 2). As it is shown in Figures 3A and 3B, a significant increase was observed in the amplitude of fEPSPs which were recorded every 30 seconds during the first 5 min after tetanic stimulation (P<0.01, compared to baseline). A remarkable increase in the amplitude of fEPSPs was also obtained at the time intervals of 25 to 30 min (P<0.01), 55 to 60 min (P<0.05), and 115 to 120 min (P<0.01) after tetanic stimulation in the treatment group (Figures 3A and 3B). Furthermore, statistical analysis revealed a significant difference in the amplitude of fEPSPs between minocycline-treated rats and control group at the time intervals of 0 to 5 min, 25 to 30 min, and 55 to 60 min following high frequency stimulation (P<0.05; Figure 3B). The administration of lower doses of minocycline (10 and 20 mg/kg) once daily for 7 days following cerebral ischemia-reperfusion did not induce LTP in the hippocampus (data not shown). On the other hand, in the minocycline-pretreated group; tetanic stimulation did not succeed in raising the amplitude of fEPSPs (Figures 2 and 3).
3.3. Effect of minocycline on the activity of antioxidant enzymes in the hippocampus

The measurement of antioxidant enzyme activity revealed that the induction of cerebral ischemia-reperfusion in rats caused a significant reduction in the activity of the antioxidant enzyme including catalase, GPx, and SOD in the hippocampus (P<0.001 in Figures 4A–F, except for Figure 4D in which P<0.01; All control groups were compared to sham group). In contrast, a significant enhancement in the activity of catalase (P<0.01, Figure 4A), GPx (P<0.01, Figure 4B), and SOD (P<0.05, Figure 4C) was observed in the animals which were treated by minocycline with dose of 20 mg/kg. Besides, treatment with minocycline at the maximum dose of 40 mg/kg resulted in a significant increase in the activity of catalase (P<0.001, Figure 4A), GPx (P<0.001, Figure 4B), and SOD (P<0.01, Figure 4C). Treatment of rats with lower dose of minocycline (10 mg/kg) did not restore the function of antioxidant enzymes in the hippocampus (Figures 4A–C). Moreover, neither one of single doses of minocycline when administered prior to the induction of ischemia resulted in the enhancement of antioxidant enzyme activity in the hippocampus (Figures 4D–F).

4. Discussion

In this study, global cerebral ischemia-reperfusion was induced in rats through bilateral occlusion of common carotid arteries. By using this method, the function of memory in ischemic rats was successfully impaired which was evident in both the behavioral and electrophysiological experiments. The attained results indicated, for the first time, that treatment of rats with minocycline is capable of enhancing synaptic plasticity and restoring the function of antioxidant enzyme in the hippocampus.

The hippocampal formation which is believed to function as the fundamental structure in the brain for memory formation (Lynch, 2004) is highly vulnerable to brain ischemia and oxidative stress (Baron, Yamauchi, Fujioka, & Endres, 2014). Accordingly, oxidative stress plays a pivotal role to cause neuronal damage in the hippocampus and the resultant memory impairment following cerebral ischemia-reperfusion (Manzanero, Santro, & Arumugam, 2013).

Previous studies have shown that excessive amounts of ROS are generated during cerebral ischemia along with a remarkable decrease in antioxidant enzyme activity (Chen et al., 2011). In the present study, the induction of ischemia-reperfusion injury in the brain significantly reduced the activity of antioxidant enzymes in the hippocampus which indicates the progression of oxidative stress in the neural tissue. Convincing evidence indicates that oxidative stress-induced neuronal injury in the hippocampus causes apparent impairment in memory performance in behavioral tests including Morris water maze (Moghimi, Parvardeh, Zanjani, & Ghafghazi, 2016) and passive avoidance test (Arabian
et al., 2017). Consistently, the results obtained from the present study showed a remarkable impairment in passive avoidance task in the rats which were subjected to cerebral ischemia-reperfusion injury. This finding suggests that the reduction of blood flow to the brain and consequent oxidative stress particularly in the hippocampus is the main causative agent of memory deficit following cerebral ischemic events. The reason is that in sham-operated animals in those the induction of oxidative stress is unsuspected, memory impairment was not observed in the passive avoidance performance.

The present study showed for the first time that either pretreatment or treatment of rats with minocycline apparently improved the performance of animals in the passive avoidance test. In this regard, our findings showed that during memory retention task, the minocycline-treated rats presented a more desirable performance than that of pretreated animals. The reason could be possibly that, in the pretreated group single doses of minocycline was administered before the induction of cerebral ischemia and thus the animals did not receive enough doses of the drug. Accordingly, it is suggested that in future studies, multiple doses of minocycline rather than single ones could be administered for a long enough period prior to cerebral ischemia.

The results of behavioral tests indicated that the attenuating effect of minocycline on memory impairment was exerted in a dose-dependent manner. However, a significant improvement in the memory function was obtained in rats which were under treatment of maximum dosage of minocycline. A similar dose-dependent effect was also observed in minocycline-treated rats in biochemical assays of antioxidant enzyme activity in the hippocampus.

The passive avoidance test is one of the major methods which are used to investigate the learning and memory mechanisms. This test is widely used to identify chemicals which modify cognitive function. There is evidence suggesting that the function of the hippocampus plays a fundamental role in passive avoidance performance (Arabian et al., 2017). Indeed, this is the reason why the induction of ischemia in the hippocampus clearly disrupts passive avoidance performance. It has been shown that brain ischemia-reperfusion results in memory impairment through the disruption of synaptic plasticity (Xu et al., 2010; Moghimi, Parvardeh, Zanjani, & Ghafghazi, 2016).

In order to track the pathological changes in the structure and function of synapses, the properties of baseline synaptic neurotransmission and LTP are evaluated using electrophysiological methods (Nicoll & Schmitz, 2005). There is evidence showing that the formation of LTP phenomenon in rat hippocampus is impeded after transient cerebral ischemia which is induced by the occlusion of common carotid arteries (Xu et al., 2010; Moghimi, Parvardeh, Zanjani, & Ghafghazi, 2016). In line
with this evidence, the results obtained from the present study showed that in rats which were subjected
to cerebral ischemic-reperfusion injury, tetanic stimulation of nerve fibers in the PP area did not induce
LTP in the DG region of hippocampus. In contrast, the treatment by minocycline could restore the
formation of LTP in the hippocampus, which was persisted for two hours. These findings reinforce the
improving effect of minocycline on memory performance which was obtained from the passive
avoidance test. This indicates that the attenuating effect of minocycline on memory dysfunction
following cerebral ischemia is at least in part due to facilitating LTP formation in the hippocampus.
The enhancement of hippocampal LTP by minocycline was also reported by Hoshino, Hayakawa and
Morimoto (2017) in a mice model of septic shock. Similar studies have shown that minocycline
improve LTP by facilitating neuroplasticity and raising the expression of synapse-associated signaling
proteins in mice (Jiang et al., 2015). It should be noted that in the present work, minocycline had no
effect on the properties of baseline synaptic neurotransmission, such as fEPSP amplitude in the DG
area of the hippocampus. These data are consistent with the findings of recent work by Song, Liu and
Zhuo, (2015) in which they reported the lack of minocycline effect on baseline recording from neurons
in anterior cingulate cortex. In contrast to the results obtained from minocycline-treated rats,
pretreatment of animals with single doses of minocycline could not facilitate the synaptic
neurotransmission in the hippocampus.

In the process of neuronal damage after ischemia-reperfusion, several mechanisms are involved; the
most important are oxidative stress and apoptosis (Chen et al., 2011; Manzanero, Santro, &
Arumugam, 2013). Evidence suggests that the production of free oxygen radicals after cerebral
ischemia results in neuronal damage in the hippocampus which consequently leads to the impairment
of learning and memory (Chen et al., 2011). On the other hand, the pivotal role of antioxidant enzymes
including catalase, GPx, and SOD in the protection of neural cells against oxidative stress has been
established (Lalkovičová & Danielisová, 2016). Several studies have shown that during brain
ischemia, the function of these enzymes is remarkably disrupted and progressively result in neuronal
damage and death (Warner, Sheng, & Batinić-Haberle, 2004; Lalkovičová & Danielisová, 2016).
Consistently, the results obtained from the present work showed a considerable reduction in the activity
of catalase, GPx, and SOD in the hippocampus of rats which were subjected to cerebral ischemia-
reperfusion. Nevertheless, treatment (but not pretreatment) of rats with minocycline significantly
elevated the level of antioxidant enzyme activity in the hippocampus. These findings verify recent
study in which minocycline could restore the activity of GPx and SOD enzymes in rat spinal cord
following neuronal damage (Abbaszadeh et al., 2018). Additionally, there is evidence indicating that
minocycline exerts neuroprotective effects through attenuating lipid peroxidation (Naderi,
Sabetkasaei, Parvardeh, & Moini Zanjani, 2017) as well as reducing ROS formation (Réus et al., 2015; Dai et al., 2017). Furthermore, the direct radical scavenging effect of minocycline due to its phenolic structure has been reported (Kraus et al., 2005). These reports are similar to the current work and reinforce the role of antioxidant activity of minocycline in its neuroprotective effects. Accordingly, it can be concluded that the restoration of antioxidant enzyme activity and enhancement of synaptic plasticity in the hippocampus underlie the boosting effect of minocycline on memory performance following cerebral ischemia-reperfusion injury in rats.

Overall, it was attained that treatment by minocycline improves memory impairment after cerebral ischemia-reperfusion in rats. The attained results showed that these effects are mediated through the enhancement of enzymatic antioxidant capacity and facilitating synaptic plasticity in the hippocampal formation indeed.

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References


Kishida, K. T., & Klann, E. (2007). Sources and targets of reactive oxygen species in synaptic plasticity and memory. Antioxidants & Redox Signaling, 9(2), 233–244. [DOI:10.1089/ars.2007.9.ft-8] [PMID]


Figure captions:

Figure 1. The effect of minocycline on step-through latency and duration of spending in dark compartment in passive avoidance test. In the treatment protocol (A and B), control (normal saline) and minocycline were administered intraperitoneally (i.p.) once daily for 7 days after reperfusion, while in the pretreatment protocol (C and D) single doses of control and minocycline were injected i.p. to rats 60 min prior to ischemic injury. The columns represent mean ± SEM in each group (n=6). *P<0.05, **P<0.01, ***P<0.001 (in comparison with control group); +++P<0.001 (in comparison with sham group).

Figure 2. The effect of minocycline on long-term potentiation in the hippocampus. Each trace represents the mean of 5 fEPSPs (field excitatory postsynaptic potentials) recorded from hippocampal dentate gyrus area before and after tetanic stimulation in anesthetized rats. Control: normal saline.

Figure 3. The effect of minocycline on the amplitude of fEPSP recorded from the dentate gyrus area of rat hippocampus. In the treatment protocol (A and B), control (normal saline) and minocycline were injected intraperitoneally (i.p.) once daily for 7 days after reperfusion, while in the pretreatment protocol (C and D) single doses of control and minocycline were given i.p. to rats 60 min prior to ischemic injury. Each symbol indicates the mean ± SEM (n=6) (field excitatory postsynaptic potentials). *P<0.05, **P<0.01, and ***P<0.001 (in comparison with baseline); #P<0.05, ##P<0.01, and ###P<0.001 (compared to control group at the relevant time).

Figure 4. Effect of minocycline on the activity of antioxidant enzymes in the hippocampus. In the treatment protocol (A–C), control (normal saline) and minocycline were injected intraperitoneally (i.p.) once daily for 7 days starting after reperfusion, while in pretreatment protocol (D–F), a single dose of control or minocycline were administered i.p. to rats 60 min prior to ischemia. Each column indicates the mean ± SEM (n=6). ++P<0.01, +++P<0.001 (compared to sham group); **P<0.01 ***P<0.001 (compared to control group). GPx: glutathione peroxidase; SOD: superoxide dismutase.
Figure 1. The effect of minocycline on step-through latency and duration of spending in dark compartment in passive avoidance test.
Figure 2. The effect of minocycline on long-term potentiation in the hippocampus.
Figure 3. The effect of minocycline on the amplitude of fEPSP recorded from the dentate gyrus area of rat hippocampus.
Figure 4. Effect of minocycline on the activity of antioxidant enzymes in the hippocampus.
Author Contributions:

Conceptualization: Siavash Parvardeh; Methodology, Siavash Parvardeh, Mohammad Abbas Sheikholeslami; Investigation: Siavash Parvardeh, Mohammad Abbas Sheikholeslami, Shiva Ghafgahzi; Writing – Original Draft: Siavash Parvardeh, Seyed Erfan Mortazavi; Writing – Review & Editing: Siavash Parvardeh, Ramin Pouriran; Funding Acquisition: Siavash Parvardeh; Resources: Siavash Parvardeh, Mohammad Abbas Sheikholeslami; Supervision: Siavash Parvardeh.