The effect of minocycline on seizures induced by amygdala kindling in rats

Seyed Mehdi Beheshti Nasr a, Ali Moghimi b, Mohammad Mohammad-Zadeh c,*, Ali Shamsizadeh d, Seyed Mohammad Noorbakhsh e

a Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran.
b Department of Biology, Ferdowsi University of Mashhad, Mashhad, Iran.
c Department of Physiology and Pharmacology, Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran.
d Physiology and Pharmacology Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.
e ScienceBeam Institute, Tehran, Iran.

A R T I C L E   I N F O

Article history:
Received 20 December 2012
Received in revised form 2 May 2013
Accepted 3 May 2013

Keywords:
Minocycline
Seizures
Kindling
Rat

A B S T R A C T

Purpose: Minocycline is known as a chemical with neuroprotective, anti-inflammatory, and antimicrobial properties. In this study, the effects of minocycline on seizures induced by amygdala kindling in rats were studied.

Methods: Kindled Wistar rats were injected intraperitoneally with saline and, on the following day, with minocycline (50, 25, and 12.5 mg/kg for the three groups (1–3), respectively). The animals in groups 1–3 had similar protocols. Groups 4 and 5 were given for the rotational test and received 25 or 50 mg/kg minocycline, respectively, without any kindling stimulation. The animals in groups 6 and 7 (seven each) received 25 mg/kg minocycline or saline, respectively. All the injections were carried out 1 h before kindling stimulation. Seizure parameters, including after discharge duration (ADD), stage 4 latency (SdL), stage 5 duration (SdD), and seizure duration (SD), were recorded and compared with those of the saline groups.

Results: Minocycline (50 mg/kg) significantly decreased ADD, SdL, SdD, and SD (P < 0.001, P < 0.05, P < 0.001, and P < 0.001, respectively) in group 1. While the administration of 25 mg/kg of minocycline decreased the ADD and SdD (P < 0.05), in group 2. The injection of 12.5 mg/kg resulted in decreased SdD (P < 0.001) in group 3. The daily injection of minocycline (25 mg/kg) significantly decreased ADD, SdL, and SD (P < 0.001) in group 6.

Conclusion: The obtained results revealed that minocycline has anticonvulsant effect on seizures induced by amygdala kindling. Thus, it may be useful for epilepsy treatment.

© 2013 British Epilepsy Association. Published by Elsevier Ltd. All rights reserved.

1. Introduction

After stroke and Alzheimer’s disease, epilepsy is the most common neurological disorder.1 Epidemiological studies show that more than one percent of people in the world suffer from epilepsy.2 Kindling models of epilepsy are widely used to understand the neurobiology of this disorder and to discover new and effective treatments.3 Kindling refers to the gradual development of electrographic and behavioral seizure in response to repeated applications of brief, intermittent, and low-intensity trains of electrical stimulation.4 By means of this laboratory model, one can determine the effects of various drugs and chemicals on seizures created in a specific area.

Although extensive research has been carried out on epilepsy and seizures, its exact etiology is still not known.5 There are some reports on the development of inflammatory reaction during epilepsy attacks, including increased release of cytokines, chemokines, and prostaglandins in the brains of rodents.6–8 Therefore, anti-inflammatory agents such as minocycline could probably be an appropriate choice to decrease epileptic attacks. The anticonvulsant effects of tetracycline-class antibiotics minocycline, doxycycline, and tetracycline were demonstrated in mice using 6-Hz (minimal clonic seizure) test.9 Minocycline inhibits seizure-induced inflammation and also abolishes the increased susceptibility to the second seizure in kainic acid induced status epilepticus.10 Seizure frequency reduction during minocycline therapy has been reported in clinical studies.11

Minocycline is an antibiotic of tetracycline family, having both antimicrobial properties and anti-inflammatory effects. It can pass the blood-brain barrier, and affect the activities of brain cells.12,13 The systemic injection of minocycline reduces both the activity of microglia and the production of inflammatory cytokines in the central nervous system.14–16 It also protects neurons against traumatic brain damages.17,18 Thus, because of its anti-inflammatory

Please cite this article in press as: Beheshti Nasr SM, et al. The effect of minocycline on seizures induced by amygdala kindling in rats. Seizure: Eur J Epilepsy (2013), http://dx.doi.org/10.1016/j.seizure.2013.05.005
and protective effects, minocycline might be effective on epileptic seizures. Therefore, in the present study, an attempt was made to examine the effects of minocycline on amygdala kindling-induced seizures.

2. Materials and methods

2.1. Animals

Forty-five adult male Wistar rats (8–9 weeks old) were obtained from Razi Institute of Iran. The animals were kept in a colony room with a constant temperature on 12:12 light:dark schedule. The animals were individually housed in plastic cages with woodchip bedding and permitted free access to food and water. Procedures involving animals and their care were conducted in accordance with the “Guide to the Care and Use of Experimental Animals.”

All of the experiments were done during the same time of the day (8:00 a.m. to 2:00 p.m.) in the morning to avoid the bias of circadian rhythms.

2.2. Surgical and kindling procedure

For stereotaxic surgery, the animals were anesthetized by ketamine (100 mg/kg, i.p.). The rats were implanted with bipolar stimulating and monopolar recording electrodes (twisted into a tripolar configuration) terminating in the basolateral amygdala (coordinates: A, –2.5 mm; L 4.8 mm and 7.5 mm below dura) of the right hemisphere. The electrodes (stainless steel, Teflon-coated, 127 µm in diameter, AM-Systems, USA) were insulated except at the tips. Two other electrodes were connected to skull screws, placed above the left cortical surface as reference and differential electrodes. One week after surgery, after discharge (AD) threshold was determined in basolateral amygdala by a 2 s, 60 Hz monophasic square wave stimulus of 1 ms per wave (by Electromodule D3111, ScienceBeam Institute, Tehran, Iran). The stimulations were initially delivered at 10 µA and then at 5-min intervals, increasing stimulus intensity in increments of 10 µA until at least 5 s of ADs were recorded as described previously.

Evoked responses were amplified, filtered, and digitized (at 10 kHz) using a PC-based data acquisition system and recording software (Electromodule D3111 and NeuroTrace provided by Science Beam Institute, Tehran, Iran) and were continuously monitored and stored on computer hard drive.

The duration of epileptiform after discharges and the behavioral progression of kindling (stages 1–5 according to Racine’s scale) were monitored. Briefly, stage 1 involved chewing movements; stage 2, included head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus; and stage 5, rearing on the hindlimbs and loss of postural control. Then, the animals were stimulated twice daily (minimum interval 6 h) at the AD threshold intensity until five consecutive stage 5 seizures were elicited. The recorded parameters were: AD duration (ADD), the latency to the onset of bilateral forelimb clonus (S1), the duration of stage 5 (S5-D), and the seizure stage (SS). Seizure parameters according to Racine scales were as follows: S1 was measured from the start of the stimulation until the beginning of stage 4 seizure. The duration of stage 5 (S5-D) was measured from the start of stage 5 until the end of this stage. Seizure duration was measured from beginning until the end of seizure behavior. ADD was measured from the start of the stimulation until the end of ADs.

2.3. Drug administration

Minocycline (Sigma, St. Louis, MO) was dissolved in normal saline. The drug was injected intraperitoneally 60 min before kindling stimulation.

2.4. Experimental design

The animals were classified into seven groups: Groups 1–3 (seven each) were stimulated twice daily (with a minimum interval of 6 h) at the AD threshold intensity until five consecutive stage 5 seizures were elicited. These animals were considered fully kindled. Fully kindled rats were subjected to saline (1 ml/kg) injection and different minocycline doses (50, 25, and 12.5 mg/kg in groups 1–3, respectively) intraperitoneally on the following day.

The animals in groups 4 (n = 5) (receiving 25 mg/kg minocycline) and 5 (n = 5) (receiving 50 mg/kg minocycline) which did not receive any kindling stimulation, were used for the rotarod test. After the rotarod test (see Section 2.5), a dosage of 25 mg/kg was selected for the following experiments. The animals in groups 6 (n = 7) received 25 mg/kg minocycline, and those in group 7 (n = 7) received saline, 1 h before daily kindling stimulation. Kindling stimulation continued twice daily (with a minimum interval of 6 h) at the AD threshold intensity until stage 5 seizures were elicited, whereas minocycline or saline injection was performed once daily (1 h before the first kindling stimulation).

2.5. Rotarod

In view of the large number of daily injections in group 6, a rotarod test was used to determine the suitable dose of minocycline. It was considered likely that minocycline in high doses could have non-seizure behavioral effects in addition to its anticonvulsant effects. If non-seizure behavioral effects were not seen in the doses used, the highest dose administered to groups 1–3 was selected for group 6. In the rotarod assay, the animals were placed on a rotating rod that accelerated from 5 to 20 rpm over 30 s, and the latency to fall was manually recorded. Each animal had two 5-min training sessions. The following day, all animals were re-tested for their ability to complete a 3-min trial prior to minocycline administration. Two groups of the animals were injected with 25 and 50 mg/kg minocycline and tested on the Rotarod at 20-min intervals until two consecutive 3-min trials were completed. The length of time for which the animals are able to stay on the rotating rod is a measure of their coordination.

2.6. Histology

All the animals were sacrificed at the end of the procedure. Their brains were removed, sectioned, and examined under microscope for electrode positions and the presence of any tissue damage such as lesions. In case of any abnormality, the data from that particular animal were excluded for the final analysis.

2.7. Statistical analysis

The results are expressed as the means ± S.E.M., accompanied by the number of observations. The differences between the two dependent groups (Groups 1–5) were analyzed using paired Student’s t-test. A repeated measures ANOVA was used to determine changes in cumulative ADD, (the sum of after discharge durations recorded after 10 daily stimulations), cumulative S5-D, and cumulative SD (Groups 6–7). A P value less than 0.05 was considered to represent a significant difference.

3. Results

There was no significant difference in after discharge threshold between the different groups on the first day of stimulation. That is, all kindled rats responded with stable stage 5 seizures in either a non-infusion condition or after normal saline infusion. Histological assessment indicated that the electrodes were positioned in the...
amgdala. Six rats were removed from the study because of incorrect position of electrodes or disruption of their electrophysiological responses during freely moving records.

3.1. Effects of intraperitoneal injection of minocycline on amygdala-kindled seizures

In the first part of the experiments, the animals (groups 1–3) were stimulated 1 and 24 h after minocycline injection (50, 25, and 12.5 mg/kg for groups 1–3, respectively) and then the seizure parameters were measured.

3.1.1. Effects of minocycline on amygdala-kindled seizures after 1 h

One hour after minocycline injection (50 mg/kg), the values of ADD, 1/S4L, S3D, and SD significantly reduced compared to those of saline-treated animals (Fig 1A). The injection of 25 mg/kg of the drug caused a significant reduction in the values of ADD and S3D (P < 0.05) (Fig 1B). One hour after the injection of 12.5 mg/kg minocycline, S3D was significantly reduced compared to those of saline treatment (Fig 1C).

3.1.2. Effects of minocycline on amygdala-kindled seizures after 24 h

S3D was significantly reduced 24 h after injecting 12.5, 25, and 50 mg/kg of minocycline (P < 0.01, P < 0.01, and P < 0.001, respectively), but S4L and SD did not change significantly compared to those of groups with saline treatment (Fig 1).

Since 3 doses of minocycline were injected into all three groups of animals and the convulsive parameters were recorded twice for every injected dose (1 h and 24 h after injection), the dose-dependency and time-dependency features of the drug effect were studied. As for the ADD parameter, a repeated measures ANOVA on the obtained data revealed no significant effect of dose [F(2,15) = 0.8, P = 0.4], but a significant effect for time [F(2,30) = 104.1, P < 0.001] or interaction of time and dose [F(4,30) = 965, P < 0.001].

Repeated measures ANOVA for S4L showed that the drug effect is merely time-dependent [F(2,30) = 3.8, P < 0.05]. The same test for S3D showed that this effect is dose-dependent [F(2,15) = 6.9, P < 0.05], time-dependent [F(2,30) = 74.4, P < 0.001], and also dependent on the interaction of time and dose [F(4,30) = 5, P < 0.01]. The statistical test for the SD quantity showed that this effect is not dose-dependent [F(2,15) = 1.4, P < 0.02], but is time-dependent [F(2,30) = 80.5, P < 0.001] and time × dose dependent [F(4,30) = 84.2, P < 0.001].

3.2. Effect of minocycline on amygdala kindling acquisition

As observed in Section 3.1, minocycline mostly affected the convulsive parameters in a dose of 50 mg/kg. In addition, as stated in the previous section, the 50 mg/kg dose of the drug was injected to the kindled rats only once. The selected dose to study the kindling acquisition must have an anticonvulsant effect on the one hand, and should not affect the animal’s non-convulsive behavior on the other hand. Therefore, the rotarod behavioral test was used to select the appropriate dose (between 50, 25, and 12.5 mg/kg doses).

For this purpose, 25 and 50 mg/kg doses of minocycline were injected intraperitoneally into the animals in groups 4 and 5. One hour later, rats were placed in rotarod apparatus to record their balance duration. The results revealed that injecting the 50 mg/kg dose of minocycline (group 4) significantly reduced the balance duration compared to those of the saline group (P < 0.01). However, injecting the 25 mg/kg dose of the drug did not affect the animal behavior significantly in group 5 (Table 1). The animals in groups 4 and 5 were injected with saline on the day before. Therefore, the data for each group were compared with the results of saline injection on the previous day. Finally, the dose of 25 mg/kg was selected to study the effects of minocycline on kindling acquisition.

Given that the experimental groups of this section (groups 6 and 7) received daily minocycline injections and electrical stimulations for ten days, the convulsive parameters were analyzed statistically and cumulatively. The cumulative ADD (cADD) was significantly reduced in the animals receiving daily injections of the 25 mg/kg dose of minocycline (group 6) compared to the group receiving saline (group 7) [F(18,216) = 3.5; P < 0.001] (Fig 2). The cumulative S3D (cS3D) was also significantly reduced in the animals of group 6 (recipients of minocycline) compared to the group 7 (P < 0.001) (Fig 3).

A repeated measures ANOVA on the obtained data showed that cSD was significantly reduced in the animals receiving the 25 mg/kg dose of minocycline compared to the saline group [F(19,228) = 3.8; P < 0.001] (Fig 4).

3.2.1. Effect of minocycline on amygdala kindling rate

At the first day of the experiments, there was no significant difference between after discharge threshold of kindled + saline (50 ± 4.6 µA) and kindled + minocycline (48.5 ± 4 µA) groups. Thus, there was no difference in seizure susceptibility of different groups at the beginning of experiments. The application of minocycline (25 mg/kg) resulted in a significant retardation of kindling acquisition.
Repeated measures ANOVA test showed that the average number of stimulations required to reach the fully kindled state (stage 5) or other seizure stages was significantly increased in kindled + minocycline group compared with kindled + saline group ($P < 0.001$) (Fig. 5).

4. Discussion

The results of the present study showed that minocycline had anticonvulsant effect in fully kindled rats. Moreover, the injection of minocycline (25 mg/kg) for 10 days had antiepileptogenic effect during kindling acquisition and retarded kindling acquisition.

One of the most important characteristics of the temporal lobe epilepsy is the increase of glial production.\(^2\)\(^,\)\(^25\)\(^,\)\(^26\) It has been shown that the level of cytokvents, such as IL-1\(\beta\), TNF-\(\alpha\), and IL-6, in the brains of mice are rapidly (\(\leq 30\) min) increased at mRNA and protein levels after the induction of seizures (chemically or electrically), while they are expressed at very low levels in normal brain, and declined to basal levels within 48–72 h from the seizure onset.\(^7\) Moreover, it has been shown that 2 h following the last generalized convulsive seizure triggered from kindled amygdala, a significant up-regulation of IL-1\(\beta\), IL-1RI, TNF-\(\alpha\), and TGF-\(\beta\) mRNAs occurs in parietal and piriform cortices, amygdala, and hippocampus.\(^26\) On the other hand, the excessive release of the inflammatory cytokines leads to the neurotoxic effects of neurons and induces seizures.\(^27\)\(^,\)\(^28\) Many reports indicate that the inflammatory cytokines induce seizures.\(^8\)\(^,\)\(^29\)\(^,\)\(^30\)

It has been shown that in mice, in which exaggerated neuroinflammation was elicited by lipopolysaccharides, minocycline pretreatment was effective in reducing mRNA levels of IL-1\(\beta\) and TLR2. Also, minocycline decreased mRNA expression of inflammatory genes including IL-6, IL-1\(\beta\), MHCII, and TLR2 in in vitro study.\(^31\) Therefore, minocycline might be effective in the reduction of the epileptic seizure either by reducing the production of cytokines or by blocking the effects on the cell surface. The anticonvulsant effects observed in this study can be attributed to the anti-inflammatory effects of minocycline.

Pre-treatment with minocycline 1 h before stimulation in kindling acquisition group reduced the seizure parameters markedly. Based on 2–3 h half-life of minocycline in rodents, we
injected minocycline before kindling stimulation. The present results are consistent with a recent study showing anticonvulsant effects of minocycline in 6 Hz test models of epilepsy. In addition, another report has shown marked reduction in seizure frequency during minocycline therapy in some patients. The intraperitoneal injection of minocycline reduced the electrophysiological parameter of ADD significantly. ADD indicates the activity of the temporal circuits in the registration area, which depends on the nature of neurons and their circuits. It is also presumed that minocycline reduces this parameter during the kindling process by inhibiting the neuronal circuits of the amygdala area. In this study, it was observed that minocycline significantly reduced the duration of seizures.

In our study, minocycline delayed the kindling procedure and increased the number of stimulations required to reach different kindled seizure stages observed on acquisition of both focal (stage 3) and generalized (stage 4 and 5) seizures. In other words, it seems that minocycline prolongs the generalization stage of seizures in kindling, since S4s is the generalization rate index of the seizure attacks and is relevant to the involvement of the brain stem circuits leading to the seizure generalization. In addition, it was observed that minocycline reduced the S2-D significantly, indicating that the duration of tonic–clonic convulsions was reduced after minocycline. In accordance with our results, it has been shown that minocycline treatment in mice suppressed the increased susceptibility to the second seizure later in life. Yet it is not certainly clear whether epilepsy and induced seizures create the inflammatory agents, or the inflammatory agents themselves cause epilepsy.

5. Conclusion

According to the present study and other studies, it is postulated that minocycline can be effective in reducing epileptic seizures, probably through suppression of inflammation. Apart from a few case reports, there is no clear evidence of anticonvulsant effect of minocycline in human. Based on the results of this study, more studies seem necessary to investigate the possible benefits of minocycline in epileptic patients receiving minocycline as treatment for infections.

Conflict of interest

None of the authors has any conflict of interest to disclose.

Acknowledgment

This study was supported by a grant from Sabzevar University, Sabzevar, IRAN. We would like to thank Dr. Ali Meshkani for editing this manuscript. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References


