Research Article

Evaluation of antinociceptive activity of minocycline: An experimental study

Mangal Kishanrao Choure, Rakesh Ramratan Jadhav

Department of Pharmacology, Swami Ramanand Teerth Rural Government Medical College, Ambajogai, Maharashtra, India

Correspondence to: Mangal Kishanrao Choure, E-mail: drmangalchoure15@gmail.com

Received: December 29, 2017; **Accepted:** January 24, 2018

ABSTRACT

Background: Recently, some studies have shown that minocycline may have pleiotropic biologic activities besides its antimicrobial activity. Minocycline is also an inhibitor of microglial cell activation, an effect that may contribute to its antinociceptive activity, as these cells release several mediators, including cytokines and eicosanoids, which enhance synaptic transmission in the central nervous system. **Objectives:** The objective of this study is to evaluate antinociceptive activity of minocycline by tail-flick response and acetic acid-induced writhing methods. **Materials and Methods:** Wistar rats of either sex weighing 180–250 g and Swiss mice weighing 25–30 g were used. Analgesic activity of minocycline (100 mg/kg i.p.) was evaluated and compared with tramadol (10 mg/kg i.p.) and aspirin (100 mg/kg i.p.) using acetic acid-induced writhing method and tail-flick response method of analgesia. **Results:** In acetic acid-induced writhing model of analgesia, the action of minocycline (100 mg/kg i.p.) was significantly more than the control group, but it was less when compared to aspirin. Furthermore, in tail-flick model of analgesia, it showed significant analgesic activity but was less than that of aspirin and tramadol. **Conclusion:** Minocycline possesses analgesic activity. However, further studies need to be carried out to evaluate its analgesic activity.

KEY WORDS: Antinociception; Minocycline; Acetic Acid-induced Writhing; Tail-flick

INTRODUCTION

The International Association for the Study of Pain has defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."[1] It is not only a physical sensation but also an emotional experience and varies from person to person and in the same person from time to time.

Nociception is the neuronal processing of pain stimuli involving detection and transmission of noxious information from the peripheral to the central nervous system (CNS). It is a consequence of noxious stimuli causing the release of chemical mediators which activate nociceptors, defined as receptors that are capable of distinguishing between noxious and non-noxious stimuli in the tissue.[2]

The principal aim of effective pain control is to ameliorate nociception, to increase the pain threshold, and to maintain the quality of life.[3]

The tetracycline family of compounds is widely used as broad-spectrum antibiotics having antimicrobial activity against various bacteria, *Mycoplasma, Rickettsia,* and parasites.[4]

Minocycline is a second-generation semi-synthetic broad-spectrum bacteriostatic tetracycline antibiotic.

Ala'a Ahmed 2005 et al shows that co-administration of Minocycline to Indomethacin in hot plate method of

Access this article online	
Website: http://www.ijmsph.com	**Quick** Response code
DOI: 10.5455/ijmsph.2018.1234424012018	

analgesia and in monoarthritis model of inflammation, potentiates analgesic and anti-inflammatory effect of Indomethacin.[5]

Recently, some experimental studies have provided support to the potential benefit of tetracyclines in the treatment of inflammatory pain conditions such as rheumatoid arthritis, periodontitis, and neurodegenerative disease.[6]

Minocycline is also an inhibitor of microglial cell activation, an effect that may contribute to its antinociceptive activity, as these cells release several mediators, including cytokines and eicosanoids, which enhance synaptic transmission in the CNS.[7,8]

Drugs commonly used for suppression of pain are NSAIDs, and opioids provide only symptomatic relief. Long-term use of these drugs is associated with serious adverse effects. Hence, the search for a new, safe analgesic drug continues.

In case of inflammation caused by infection, it needs treatment not only with antimicrobial/antiparasitic drugs but also with analgesic agents. If the newer tetracycline possesses antinociceptive activity, they would be an useful addition to the existing analgesic drugs. Very few studies have been carried out till date to evaluate the antinociceptive activity of minocycline.

MATERIALS AND METHODS

Wistar rats of either sex weighing 180–250 g and Swiss Albino mice of either sex weighing 25–30 g were used. The study was conducted after approval from the Institutional Animal Ethics Committee.

The rats and mice were grouped in separate cages with six animals in each cage. They were maintained in a colony room at an ambient temperature of 23 ± 1°C with the help of air coolers and enough humidity on a 12 h light–dark cycle. They had free access to food and water. Care was taken to avoid coprophagy among animals by the use of net.

Chemicals

Aspirin, carboxymethyl cellulose (CMC), and minocycline pure powder form were obtained as gift samples from Medley and Cipla Pharmaceuticals, Mumbai.

Tramadol was obtained from Yarrow Chem Products and Unijules Life Sciences Ltd., respectively. Aspirin was suspended in normal saline using CMC.

Methods

Evaluation of antinociceptive (analgesic) activity

Acetic acid-induced writhing in mice[9]

Animals were divided into following 3 groups: (1) Control group: Normal saline dose: 0.2 ml (i.p.), (2) standard group: Aspirin dose: 100 mg/kg (i.p.), and (3) test group: Minocycline dose: 100 mg/kg (i.p.).

After 30 min of drug administration, 0.1 ml of 1% acetic acid solution was given to mice intraperitoneally (i.p.). The mice were placed individually into glass beakers and 5 min were allowed to elapse. The mice were then observed for 10 min, and a number of writhes were recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

The following formula was used to calculate percentage inhibition:

% Inhibition = (No. of writhes in control group - No. of writhes in test group × 100)/No. of writhes in control group

Tail-Flick Method in Rats[10]

Antinociceptive activity was assessed by tail-flick response method on analgesiometer, originally described by D'Amour and Smith in 1941. Animals were divided into following 4 groups: 1. Control group: Normal Saline Dose: 2ml/kg (i.p.) 2. Standard Group: Tramadol Dose: 10mg/kg (i.p.), 3. Standard Group: Aspirin Dose 100 mg/kg (i.p.) 4. Test Group: Minocycline Dose: 100mg/kg (i.p.).

Tail-flick latency was measured by the method originally described by D'Armour and Smith in 1941, using analgesiometer. This test was performed before and at the end of 30, 60, 90, and 120 min after drug administration.

Percentage analgesia was calculated using the formula:

$$\% \text{ Analgesia} = \text{M.P.E} = \frac{\text{T.L.-B.L.}}{\text{M.L.-B.L.}} \times 100$$

Where M.P.E = Maximum possible effect.
M.L = Maximum latency or cut-off time.
T.L = Test latency or latency at the end of particular period of time, B.L = Basal latency or control latency.

Statistical Analysis

Data were analyzed using Graph Pad Prism Software Version 5.01. Comparison between different groups was performed by one-way ANOVA followed by Bonferroni post-test for comparison between multiple groups. The $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 presents a total number of writhes in 10 min in acetic acid-induced writhing method in mice. As compared to control group, a number of writhes were inhibited significantly by both aspirin and minocycline groups.

However, minocycline compared to standard analgesic aspirin produced lesser reduction of writhes. This difference in inhibition of number of writhes is statistically significant when minocycline compared to aspirin.

The percentage analgesia was maximum in aspirin group 73.17 %, whereas it was in 59.36% minocycline group.

Table 2 summarizes that basal latency (basal mean reaction time) was comparable in all four groups in tail-flick model of analgesia in rats.

Minocycline at 30, 60, 90, and 120 min produced a statistically significant increase in latency as compared to control group.

Minocycline when compared to aspirin produced significantly less rise in latency at 30 and 60 min.

Minocycline compared to tramadol produced a lesser rise in latency at 30, 60, 90, and 120 min.

Tramadol produced an increase in latency as compared aspirin at all time intervals which was statistically significant at 90 and 120 min.

DISCUSSION

In acetic acid-induced writhing model of analgesia, the action of minocycline was significantly more than the control group, but it was less when compared to aspirin. Percentage analgesia of minocycline was 59.36% lower than that of aspirin, i.e., 73.17%. As the acetic acid-induced writhing method mainly evaluates peripherally acting analgesics, hence these two drugs appear to have a significant peripheral analgesic action.

Cho *et al.*[11] observed that minocycline effectively inhibits acetic acid-induced acute abdominal nociception by inhibitory action on the excitation of spinal neurons. Another action is by attenuating acetic acid-induced acute visceral pain by inhibition of phosphorylation, mediated by extracellular signal-regulated kinases (ERK) in spinal cord of mice.

The tail-flick method of analgesia is very effective in estimating the efficacy and potency of centrally acting analgesic drugs. In our study, the results showed that minocycline has analgesic activity which was statistically significant compared to control group.

This shows that the pain threshold increased significantly during the period of observation in each of the three drug-treated groups with the maximum effect observed in tramadol group at all observation times.

Abu-Ghefreh and Masocha[5] study has shown that minocycline increases the analgesic effect of indomethacin in hot plate method of analgesia than indomethacin alone.

Popiolek-Barczyk *et al*.[12] study showed that minocycline reduces neuropathic pain development and enhances the effect of nociceptin/orphanin.

In our study, we did not investigate the underlying mechanism by which minocycline inhibits nociception but work of other authors suggests that minocycline inhibits neuronal phosphorylation, mediated by ERK expression in the spinal cord, which has a substantial role in nociception.[11]

Table 1: Effect of different drugs in acetic acid-induced writhing models in mice

Groups (*n*=06 animals)	Number of writhes (in 10 min)	Percentage analgesia (%)
Control (normal saline 2 ml/kg i.p.)	20.50±0.7638	-
Aspirin (100 mg/kg i.p.)	5.500±0.5627*$^{\#}$	73.17
Minocycline (100 mg/kg i.p.)	8.333±0.6667*	59.36

Values are mean±SEM (standard error of mean), *n*=06 in each group. *P<0.001 as compared to control. $^{\#}P$<0.01 as compared to minocycline.

Table 2: Effects of different drugs on nociception in tail-flick model of analgesia in rats

Groups (*n*=06)	Basal latency (in s)	At 30 min (in s)	At 60 min (in s)	At 90 min (in s)	At 120 min (in s)
Control (normal saline 2 ml/kg i.p.)	3.767±0.1202	3.833±0.04216	3.983±0.04773	4.067±0.04944	3.950±0.09574
Aspirin (100 mg/kg i.p.)	3.883±0.04014	7.017±0.1167*$^{@}$	7.933±0.1764*$^{@@}$	7.117±0.1222*	6.933±0.2108*
Tramadol (10 mg/kg i.p.)	3.633±0.06667	7.500±0.1528*$^{@@}$	8.167±0.1202*$^{@@}$	8.700±0.07303*$^{@@\#\#}$	7.917±0.1973*$^{@@\#}$
Minocycline (100 mg/kg)	3.750±0.07638	6.100±0.3044*	6.833±0.2333*	6.800±0.2309*	6.600±0.1461*

Values are mean±SEM (standard error of mean), *n*=06 in each group. *P<0.001 as compared to control, $^{\#}P$<0.01 as compared to aspirin. $^{\#\#}P$<0.001 as compared to aspirin, $^{@}P$<0.05 as compared to minocycline, $^{@@}P$<0.001 as compared to minocycline

Furthermore, other authors suggest antinociceptive mechanism of tetracyclines may be by inhibiting the production of nitrous oxide, prostaglandin E2, interleukin (IL)-1β, IL-6, and tumor necrosis factor-α by different cell types *in vitro*.[13-15]

CONCLUSION

Minocycline in the dose 100 mg/kg possesses analgesic activity. However, further studies need to be carried out to evaluate its analgesic activity.

REFERENCES

1. Pain terms: A list with definitions and notes on usage. Recommended by the IASP subcommittee on taxonomy. Pain 1979;6:249.
2. Priya M, Narayanan VS, Mohapatra S, Rani RJ. Screening of cetirizine for analgesic activity in mice. Int J Basic Clin Pharmacol 2013;2:187-92.
3. Kilic FS, Sirmagul B, Yildirim E, Oner S, Erol K. Antinociceptive effects of gabapentin & its mechanism of action in experimental animal studies. Indian J Med Res 2012;135:630-5.
4. Tripathi KD. Essentials of Medical Pharmacology. 7th ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2013. p. 733-43.
5. Abu-Ghefreh AA, Masocha W. Enhancement of antinociception by coadminstration of minocycline and a non-steroidal anti-inflammatory drug indomethacin in naïve mice and murine models of LPS induced thermal hyperalgesia and monoarthritis. BMC Musculoskelet Disord 2010;11:276-86.
6. Choi Y, Kim HS, Shin KY, Kim EM, Kim M, Kim HS, *et al.* Minocycline attenuates neuronal cell death and improves cognitive impairment in Alzheimer's disease models. Neuropsychopharmacology 2007;32:2393-404.
7. Bastos LF, Angusti A, Vilaça MC, Merlo LA, Nascimento EB Jr., Rocha LT, *et al.* A novel non-antibacterial, non-chelating hydroxypyrazoline derivative of minocycline inhibits nociception and oedema in mice. Br J Pharmacol 2008;155:714-21.
8. Watkins LR, Maier SF. Glia: A novel drug discovery target for clinical pain. Nat Rev Drug Discov 2003;2:973-85.
9. Kolhe AM, Kale A. Evaluation of analgesic, anti-inflammatory, and antipyretic activity of leukotriene receptor antagonist-montelukast: An experimental study. Natl J Physiol Pharm Pharmacol 2017;7:32-7.
10. Parmar NS, Prakash S. Evaluation of analgesics, anti-inflammatory and anti- pyretic activity. In: Parmar NS, editor. Screening Methods in Pharmacology. New Delhi, India: Narosa Publishing House; 2006. p. 225-6.
11. Cho IH, Lee MJ, Jang M, Gwak NG, Lee KY, Jung HS, *et al.* Minocycline markedly reduces acute visceral nociception via inhibiting neuronal ERK phosphorylation. Mol Pain 2012;8:13.
12. Popiolek-Barczyk K, Rojewska E, Jurga AM, Makuch W, Zador F, Borsodi A, *et al*. Minocycline enhances the effectiveness of nociceptin/orphanin FQ during neuropathic pain. Biomed Res Int 2014;2014:762930.
13. Amin AR, Attur MG, Thakker GD, Patel PD, Vyas PR, Patel RN, *et al.* A novel mechanism of action of tetracyclines: Effects on nitric oxide synthases. Proc Natl Acad Sci U S A 1996;93:14014-9.
14. Célérier P, Litoux P, Dréno B. *In vitro* modulation of epidermal inflammatory cytokines (IL-1 alpha, IL-6, TNF alpha) by minocycline. Arch Dermatol Res 1996;288:411-4.
15. Sandler C, Ekokoski E, Lindstedt KA, Vainio PJ, Finel M, Sorsa T, *et al.* Chemically modified tetracycline (CMT)-3 inhibits histamine release and cytokine production in mast cells: Possible involvement of protein kinase C. Inflamm Res 2005;54:304-12.

How to cite this article: Choure MK, Jadhav RR. Evaluation of antinociceptive activity of minocycline: An experimental study. Int J Med Sci Public Health 2018;7(4):245-248.

Source of Support: Nil,**Conflict of Interest:** None declared.