International Journal of Biological Macromolecules 59 (2013) 20–28

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Evaluation of safety and efficacy of brain targeted chitosan nanoparticles of minocycline

Kalpana Nagpal*, Shailendra Kumar Singh, Dinanath Mishra

Department of Pharmaceutical Sciences, G.J. University of Science and Technology, Hisar 125001, India

ARTICLE INFO

Article history:
Received 20 March 2013
Accepted 7 April 2013
Available online 12 April 2013

Keywords:
Histopathology
Minocycline hydrochloride
Maximum tolerated dose
Chitosan nanoparticles

ABSTRACT

The aim of present study was to evaluate the antidepressant-like effects of minocycline hydrochloride (MH); enhance this effect using nanoparticulate drug delivery system; and further evaluate their safety by determining maximum tolerated dose (MTD). Pure drug MH, MH loaded nanoparticles (MHNP) and Tween 80® coated MH encapsulated nanoparticles (cMHNP) were explored for antidepressant-like activity in terms of immobility period using despair swim test (DST) and tail suspension test (TST) in mice (dose equivalent to 100 mg/kg MH, *i.p.*). For MTD determination, Wistar rats were treated with gradual increasing doses of MH and cMHNP orally for 28 consecutive days and observed for body weight, weight indices (WI), behavioral, biochemical and histopathological changes until MTD was found. In mice, MH treatment showed antidepressant-like activity and cMHNP treatment significantly improved this effect. On the other hand, no significant effect was observed for MHNP treated group. However, administration of MH in any case did not produce locomotor activation, suggesting that the antidepressant-like effects of MH may not be attributed to the enhanced locomotion. The MTD was found to be 319 mg/kg for MH and 350 mg/kg for cMHNP (350 mg/kg). Thus surface modified nanoparticles (cMHNP) improved the therapeutic efficacy as well as safety of MH.

1. Introduction

Neuropsychiatric disorders are expected to rise sharply in the current decade [1,2]. Depression is one of the serious mental disorders with major symptoms like consistently depressed mood and inability to take pleasure from normally enjoyable events, a condition termed anhedonia [1,2]. Several authors have reported that minocycline (MH), a tetracycline antibiotic, may serve as an effective antidepressant [3,4]. Although among the tetracyclines, the MH level in the brain was reported as almost threefold than that of doxycycline [5], still MH poorly crosses the blood–brain barrier (BBB) due to its low lipophilicity and half-life [6,7]. MH is routinely administered to humans for the treatment of infectious and inflammatory diseases, but the dose used is much lower (3 mg/kg/day) than that used for its neuroprotectant activity (22–100 mg/kg multiple times daily; *p.o.* or *i.p.*) in most experimental models [6,7]. Also, at higher dose of MH, peripheral side effects limit its use for neuroprotectant activity. Therefore, there is a need to lower the dose of MH for neuroprotectant activity and to overcome the peripheral side effects.

The Tween 80® coated nanoparticulate drug delivery system is one of the promising techniques to improve the transport of drugs to brain by overcoming the blood–brain barrier (BBB) [8,9]. For this purpose, nanoparticulate drug delivery system is utilized to maximize its BBB permeability. Since these nanoparticles may alter the body distribution of drugs [8,9], the safety investigations of the nanoparticulate formulations are of paramount importance. The objective of present investigation was to prepare, study the preclinical efficacy and safety of Tween 80® coated nanoparticulate drug delivery system when loaded with MH as compared to free MH treatment.

2. Materials and methods

2.1. Drugs and chemicals

MH and chitosan were obtained as gift samples from Ranbaxy Laboratories Ltd., Gurgaon (Haryana), India and Central Institute of Fisheries Technology, Kochi, India, respectively. Other chemicals used during the study were of suitable analytical grade and were used as received.

2.2. Method for preparation of nanoparticles

Tween 80® coated MH loaded chitosan nanoparticles (cMHNP) were prepared with chitosan (0.07%, w/v) and Tween 80®

* Corresponding author at: Department of Pharmaceutical Sciences, G.J. University of Science and Technology, Hisar 125001, Haryana, India. Tel.: +91 94167 29190; fax: +91 16622 76240.
E-mail address: kalpananagpal@gmail.com (K. Nagpal).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.04.011

(2.00%, w/v) using ionotropic gelation method [8]. The formulated nanoparticles were then characterized for particle size, zeta potential, polydispersity index, percent drug entrapment efficiency (DEE), drug polymer interaction and *in vitro* drug release. The average particle size, zeta potential and DEE was found to be 155.16 ± 14.57 nm, 33.5 ± 1.04 mV and 88.61 ± 0.81%, respectively in our previous studies [8]. In vitro release revealed initial burst release followed by a sustained release of 72.73 ± 0.98% after 24 h in phosphate buffer saline as dissolution media following Korsmeyer–Peppas model [8].

2.3. Animals and diet

For behavioral study, Swiss male albino mice (3 months old, weighing around 20–25 g) and for safety study, the male Wistar rats weighing 100–150 g were procured from Disease Free Small Animal House, LLRUVAS, Hisar (Haryana, India). The study design for behavioral study and safety study are shown in Tables 1 and 2, respectively. Estrogens have been reported to affect antidepressant-like activity, therefore, only male mice were used for the study, excluding female mice [10]. A total of nine groups (containing 6 animals each) were taken for tail suspension test (Table 1) and the same animals were reused after 7 days (more than 7 half life, $t_{1/2}$ = 18–24 h [8]) for despair swim test. The MHNP and cMHNP batch was freshly prepared at the beginning of each experiment. For safety study, the dose to be administered was gradually increased by 10% starting from 319 mg/kg as the initial dose. The animals had free access to drinking water and the food was supplied *ad libitum* throughout the experiment. All the experiments were carried out under an institutionally approved protocol by Institutional Animals Ethics Committee (IAEC). The care of animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Registration No. 0436) and all possible efforts were made during the experiment period. The test animals were acclimatized for 2 weeks prior to experimentation.

Groups for despair swim test (DST)

Group 1: Control group; normal saline (*i.p.*) was administered.
Groups 2 and 3: Fluoxetine (10 mg/kg, *i.p.*) [2], an antidepressant drug was administered as the standard drug (positive control) and tested after 1 and 2 h of dose administration, respectively.
Groups 4 and 5: Pure drug (MH) solution (100 mg/kg, *i.p.*) [8] was administered and tested after 1 and 2 h of dose administration, respectively.
Groups 6 and 7: MHNP (equivalent to 100 mg/kg MH, *i.p.*) was administered and tested after 1 and 2 h of dose administration, respectively.
Groups 8 and 9: cMHNP (equivalent to 100 mg/kg MH, *i.p.*) was administered and tested after 1 and 2 h of dose administration, respectively.

Groups for tail suspension test (TST)

Group 10: Control group; normal saline was administered.
Groups 11–18: These were same as groups 2–5, and immobility period was measured in TST model.

Groups for biochemical estimations

Groups 10–18: After behavioral testing on TST, the animals were sacrificed and monoamine oxidase-A (MAO-A) activity was measured.

2.4. Evaluation of antidepressant-like activity

2.4.1. Despair swim test (DST)

In this test, the mouse was individually forced to swim in open chamber of glass (dimensions: 25 cm × 15 cm × 25 cm) containing fresh water whose temperature was maintained at 26 ± 1 °C. The height of water level was 15 cm [11]. Every time after subjecting each animal to this test, water in the chamber was replaced because "used water" may change the behavior of experimental animals [12]. When the mouse was placed in the chamber for the first time, it was initially highly active and this activity began to subside after 2 min and interspersed with phases of immobility or floating of increasing length. The immobility period was recorded 1 and 2 h after treatment during the next 4 min. The total testing period was 6 min. The mouse was considered "immobile" when it stopped struggling and remained floating in water, making only those movements necessary to keep their head above water. After the testing period, each mouse was towel dried and returned to its housing conditions.

2.4.2. Tail suspension test (TST)

In this test, the mouse was suspended on the edge of a table which was 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail [13]. In order to avoid disturbances to animals, the test was conducted under calm conditions by isolating each animal under test from other animals both acoustically as well as visually. The immobility period was recorded 1 and 2 h after treatment recorded for a testing period of 6 min. The animal was considered "immobile", when it did not show any body movement and hung completely motionless.

2.4.3. Measurement of locomotor activity

Photoactometer (INCO, Ambala, India) was used to study the effects of various treatments on locomotor activity. The horizontal locomotor activities of all the groups were recorded for a period of 5 min.

2.4.4. Measurement of MAO-A activity

After the behavioral testing, the mice were sacrificed by decapitation. Their brains were isolated, washed with cold 0.25 M sucrose–0.1 M Tris–0.02 M EDTA buffer (pH 7.4); weighed; and treated as described by Nagpal et al. [2]. Briefly, the samples were homogenized in 9 volumes of cold 0.25 M sucrose–0.1 M Tris–0.02 M EDTA buffer (pH 7.4) and centrifuged twice (800 × *g*, 10 min, 4 °C) using cooling centrifuge (C24, Remi instruments, Mumbai, India). The supernatant thus obtained was centrifuged (12,000 × *g*, 20 min, 4 °C) and the precipitate was washed twice with sucrose–Tris–EDTA buffer; suspended in 9 volumes of 10 mM cold sodium phosphate buffer pH 7.4 (containing 320 mM sucrose); mixed well for 20 min and centrifuged (15,000 × *g*, 30 min, 0 °C). The pellets were suspended again in cold sodium phosphate buffer. 2.75 ml sodium phosphate buffer (100 mM, pH 7.4) and 100 μl of 4 mM 5-hydroxytryptamine were mixed and the absorbance was recorded using UV–vis spectrophotometer (Varian Cary-5000; Christ, The Netherlands) at 280 nm. This was then followed by the addition of 150 μl solution of precipitate in order to initiate the enzymatic reaction. The change in absorbance at 280 nm before and 5 min after the addition of precipitate was recorded for 5 min. The blank used was the mixture of sodium phosphate buffer and 5-HT.

2.5. Safety study

According to IUPAC Compendium of Chemical Terminology, MTD is the highest dose used in chronic toxicity testing that is