Pharmacological Reports 67 (2015) 934–942

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

5-HT_6 receptor antagonist attenuates the memory deficits associated with neuropathic pain and improves the efficacy of gabapentinoids

Pradeep Jayarajan [a,b], Ramakrishna Nirogi [a,*], Anil Shinde [a], Venkatesh Goura [a], Vuyyuru Arun Babu [a], Sumanth Yathavakilla [a], Gopinadh Bhyrapuneni [a]

[a] Discovery Research, Suven Life Sciences Ltd., Hyderabad - 500 055, India
[b] Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, India

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 9 March 2015
Accepted 25 March 2015
Available online 8 April 2015

Keywords:
Gabapentinoids
Neuropathy
Memory
Rotarod
5-HT6 receptor antagonist

ABSTRACT

Background: Memory deficit is a co-morbid disorder in patients suffering from neuropathic pain. Gabapentin and pregabalin (gabapentinoids) are among the widely prescribed medications for the treatment of neuropathic pain. Memory loss and sedation are the commonly reported side effects with gabapentinoids. Improving the cognitive functions and attenuating drug-induced side effects may play a crucial role in the management of pain.
Methods: We evaluated the effects of 5-HT_6 receptor antagonists on the memory deficits associated with neuropathy. We also studied the effects of 5-HT_6 receptor antagonists on the side effects, and the analgesic effects of gabapentinoids.
Results: 5-HT_6 receptor antagonists attenuated the cognitive deficits in neuropathic rats. Neuropathic rats co-treated with 5-HT_6 receptor antagonist and gabapentinoids showed improvement in memory. 5-HT_6 receptor antagonists enhanced the analgesic effects of gabapentinoids but had no effect on the motor side effects. The observed effects may not be due to pharmacokinetic interactions.
Conclusions: 5-HT_6 receptor antagonist attenuate the cognitive deficits associated with neuropathy, and this effect is also seen when co-treated with gabapentinoids. Since, 5-HT_6 antagonists improved the effectiveness of gabapentinoids, reduction in the dosage and frequency of gabapentinoids treatment may reduce the side effects. Combining 5-HT_6 receptor antagonist with gabapentinoids may offer a novel treatment strategy for neuropathic pain.

Introduction

Pain is one of the principal complaints of individuals seeking frequent medical attention. Neuropathic pain is one among them, and approximately 1% of the population suffers from neuropathic pain. Memory deficit is co-morbid disorder in patients suffering from neuropathic pain [1]. Cognitive deficits are also reported in animal models of neuropathic pain [2–4]. Gabapentin and pregabalin, the most commonly prescribed medications for the treatment of neuropathic pain also cause memory deficits. In addition, gabapentinoids cause side effects such as dizziness, sedation, and weight gain [5–10]. These side effects could be because of the dosage and frequency of administration. Side effects associated with the treatments negatively affect the daily functions and overall quality of life [1]. For this reason, attenuating the memory deficits and drug-induced side effects may aid the management of pain disorder by reducing passivity, dependency, and disability.

5-Hydroxytryptamine 6 (5-HT_6) receptor is a member of serotonin receptor family. It is exclusively localized in the central nervous system and plays an important role in cognitive functions [11–13]. Blocking the functions of the 5-HT_6 receptor improves learning and memory [14–16]. To date, the effect of 5-HT_6 antagonists on the cognitive deficits associated with neuropathy and gabapentinoids is not known.

5-HT_6 receptors also modulate the pain behavior. It has a pronociceptive role in formalin-induced pain [17], and administration of 5HT_6 receptor antagonist reduced the nociceptive behavior [18]. Treatment with 5-HT_6 receptor antagonist also alleviated cold allodynia [19]. Thus, blockade of 5-HT_6 receptor causes analgesia.

Based on the pharmacological effects of 5-HT_6 receptor antagonist, we hypothesized that blockade of 5-HT_6 receptors

* Corresponding author.
E-mail addresses: nvsrk@suven.com, ramakrsisha_nirogi@yahoo.co.in (R. Nirogi).

http://dx.doi.org/10.1016/j.pharep.2015.03.013
1734-1140/

may attenuate the cognitive deficits associated with neuropathic pain. Hence, this study was designed to evaluate the effect of 5-HT_6 antagonists *i.e.* SB-742457 [16] and Compound A (compound 12ab, [20]) on the cognitive deficits seen in neuropathic rats. Since the gabapentinoids also compromise memory, we also studied the effect of co-treating 5-HT_6 receptor antagonists with gabapentinoids.

Materials and methods

Chemicals

Compound A (compound 12ab, [20]), SB-742457 and gabapentin were synthesized at Suven Life Sciences Ltd., Hyderabad, India. Pregabalin was isolated from commercially available tablets. Compound A is a selective 5-HT_6 receptor antagonist (K_b – 0.02 nM and exhibits >10,000 fold selectivity over closely related GPCRs (compound 12ab, [20]). SB-742457 blocks 5-HT_6 and 5-HT_{2A} receptors. SB-742457 was chosen because its drug-like properties is well documented [16].

Treatments

Compound A, SB-742457, pregabalin, and gabapentin were prepared using sterile water (2 or 5 mL/kg). Treatments were co-administered. Compound A and SB-742457 were administered orally, and gabapentinoids were administered intraperitoneally.

Animals

Male Wistar rats (7–10 weeks old) were used. Animals had free access to food and water (except during experimentation). Rats were housed in an air-conditioned, pathogen-free and temperature-controlled room (21 ± 3 °C and 30–70% relative humidity), maintained on a regulated 12 h light/dark cycle (lights on at 7.00 am). Naïve animals were used for all the experiments. All animal care and experiments were carried out according to guidelines set by local ethics committee (India).

Pharmacokinetics

Blood samples (approximately 0.30 mL) were collected from the lateral tail vein. Plasma was harvested by centrifuging (Eppendorf AG 22331, Hamburg, Germany) the blood at 4000 rpm for 10 min at 4 °C and stored frozen at −70 °C until bioanalysis. Concentration of Compound A, SB-742457, pregabalin, and gabapentin were estimated using a validated LC–MS/MS method (supplementary material).

Partial sciatic nerve ligation

Rats were subjected to partial sciatic nerve ligation as per the method described by Seltzer et al. [21]. Briefly, rats were anesthetized using a mixture of xylazine (5 mg/kg, *ip*) and ketamine (75 mg/kg, *ip*). The sciatic nerve was partially ligated (∼1/3rd to 1/2) using silk thread (6.0). Sham animals were subjected to the same procedure as described above, except for the ligation. The animals were allowed to recover for at least 2 weeks.

Rats were brought to the laboratory at least 1 h prior to evaluation. Prior to evaluation, rats were allowed to acclimatize for ∼15 min to a modular animal enclosure. Mechanical allodynia was evaluated as per Dixon up–down method using a series of monofilaments (0.4, 0.6, 1, 1.4, 2, 4, 6, 8, 10 and 15 g; Aesthesio®, USA). Monofilaments were applied perpendicularly to the glabrous skin of the hind paw, and pressed against the target site until the filament bowed. Filament was held in place for 6–8 s. A flinch or flick of the paw was considered as a positive response. The 50% g threshold was calculated as described by Chaplan et al. [22].

Rats with paw withdrawal threshold of ≤4 g were considered neuropathic [23]. Neuropathic rats were evaluated for the allodynia at 1, 2, 4, 6 and 8 h post treatment. The experimenter was blinded to treatment.

Contextual fear conditioning

Contextual fear conditioning was performed in operant conditioning chamber (model 259900-HOU-SK-RAT, TSE Systems, Germany) placed in a sound and light attenuating cubicle equipped with a house light, and grid floor connected to a shocker. On day 1, rats were placed in the operant chamber with house light switched on, and allowed to acclimatize for 2 min. Rats received a conditioned stimulus (CS) (tone for 10 s) followed by an unavoidable foot shock (unconditioned stimulus (US): electric shock of 0.5 mA for 1 s). Following a 1 min interval between each administration, tone and shock were repeated to deliver a total of three CS–US pairings. All stimuli were configured and delivered through the software and control unit. One minute after the last CS–US pairing, rats were removed from the chamber, and treatments were administered. Rats were then transferred to their home cages. The freezing behavior was recorded for 1 min after the last CS–US pairing. On day 2, rats were placed in the operant chamber (house light turned on), and total freezing time (complete immobility of the animal, with the absence of vibrissae movements) was scored for a period of 5 min. Once the trial was over, the rat was removed, and the chamber was cleaned using 70% alcohol. The experimenter was blinded to treatment.

In a separate group of animals, freezing behavior of the neuropathic rats was evaluated 6 h post-conditioning. No treatment was administered. The procedure described above was followed.

Rotarod

Rats were trained to stay on the rotarod (4 rpm) for 60 s. Rats that learned the task within four trials were selected. Next day, rats were subjected to a test session by placing on the rotarod (4 rpm) for 60 s. Rats, which completed this trial, were included for testing. Rats were administered respective treatments and subjected to testing by placing on the rotarod (4 rpm) for 60 s. Duration of stay on the rotating bar was recorded. The experimenter was blinded to treatment.

Data analysis

Data from the neuropathic pain (time course), rotarod, and pharmacokinetics experiments were analyzed and compared using two-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison *post hoc* tests. Results from the neuropathic pain (AUC) and fear conditioning experiments were analyzed and compared using one-way ANOVA followed by Bonferroni multiple comparison *post hoc* tests or Students '*t*' test. All data were calculated as mean ± SEM.

Results

Pharmacokinetics

Gabapentin in combination with Compound A

Co-treatment of gabapentin (300 mg/kg) and Compound A significantly altered the exposure of gabapentin. Two-way ANOVA indicated a significant effect of exposure ($F_{3,75}$ = 443.7, $p < 0.0001$),