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Anti-nociceptive and anti-allodynic activity of aliskiren in various pain models

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ABSTRACT

In the present study, we have investigated the anti-nociceptive and anti-allodynic activity of the renin inhibitor, aliskiren, in various pain models. The anti-nociceptive activity of aliskiren was investigated in chemically-induced pain, orofacial pain and centrally mediated pain models. Anti-allodynic activity was evaluated in post-operative and neuropathic pain models. The levels of TNF-α and IL-6 were measured in homogenates of hind paw as markers of inflammation in formalin injected mice. Intraperitoneal administration of aliskiren (1–50 mg/kg) showed anti-nociceptive activity in the writhing test, formalin hind paw test, capsaicin induced pain, and orofacial pain tests in ICR mice in a dose dependent manner. Aliskiren (50 mg/kg, i.p.) reduced levels of TNF-α and IL-6 in hind paw homogenates of formalin-injected mice. Aliskiren (50 mg/kg, i.p.) did not show any analgesic activity in hot-plate and tail-flick tests, indicating the absence of centrally mediated anti-nociceptive effects. On the other hand, intra-plantar administration of aliskiren (0.1, 0.5 and 1 mg) showed analgesic activity in rat formalin tests, indicating a locally mediated effect. Aliskiren (30–100 mg/kg, i.p.) showed anti-allodynic activity in post-operative pain and chronic constriction injury-induced neuropathic pain in Sprague Dawley rats. This data suggests that aliskiren may have the potential to be used as an anti-nociceptive and anti-allodynic agent.

1. Introduction

Pain is an essential sensation that usually signals tissue injury inflicted by external or internal damaging events (Sawynok, 2003). Due to nerve injury or inflammation of injured tissue, a tonic input from nociceptive fibers produces a hyperexcitable state among central nociceptive pathways, resulting in hyperalgesia, allodynia and spontaneous pain (Price, 1996). Despite the availability of a large number of therapeutic options, side effect potential and modest efficacy limits the clinical usefulness of available analgesics agents (Nakamura-Craig and Follenfant, 1995).

The renin–angiotensin system (RAS) is known to play an important role in regulating blood pressure, fluid volume and electrolyte balance. However, inhibition of the renin–angiotensin system has also been reported to be beneficial in various painful conditions such as migraine pain, nociceptive pain and pancreatic pain (Takai et al., 1996; Motta et al., 2002; Tronvik et al., 2006; Oruc et al., 2010; Kurihara et al., 2012). Aliskiren inhibits the renin angiotensin system by directly inhibiting renin and subsequently decreases the production of angiotensin II. Aliskiren has been found to be beneficial in hypertension, chronic kidney disease, cardiac hypertrophy atherosclerosis and vascular inflammation (Wood et al., 1994; Ino et al., 2005; Lu et al., 2008; Campos et al., 2011). Various studies have also shown anti-cytokine and antioxidant activities of aliskiren (Bassim, 2010; Schmerbach et al., 2010; Tang et al., 2012). Recently, aliskiren was reported to ameliorate development of neuropathic pain in rats upon repeated dose administration (Kukkar et al., 2012). Our study has been undertaken to evaluate the anti-nociceptive and anti-allodynic potential of aliskiren using different kind of pain models.

2. Materials and methods

2.1. Animals

Six to nine weeks old healthy ICR mice and Sprague Dawley rats of either sex were used in the experiments. Animals were bred in-house at Zydus Research Center, Ahmedabad, India, and housed in individually-ventilated cages under controlled temperature (18–25 °C), humidity (30–70% relative humidity) and normal light/dark (12 h/12 h) cycle conditions. Food and water were provided ad libitum. Animals were acclimatized to testing environment for at least 1–2 h before the experiments. Experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

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2.2. Drugs and chemicals

Aliskiren (Zhejiang Apeloa Jiayuan Pharma. Ltd., Zhejiang, China) was dissolved in normal saline. Morphine procured from the Opium and Alkaloid Center, India, and gabapentin obtained from Cadila Healthcare Ltd., India, were dissolved in normal saline. Diclofenac (Troikaa Ltd., India) and diazepam (Ranbaxy Ltd., India) were procured as parenteral formulations. Formalin (Fisher Scientific, India) was prepared by diluting 37% v/v formaldehyde solution with normal saline to obtain the concentration of 0.4% and 2% v/v. Acetic acid (Merck, India) was diluted with normal saline to get concentration of 1% v/v. Capsaicin (Fluka, USA) was prepared in normal saline and 1% v/v Tween-80 (Merck, India). Mouse TNF-α and IL-6 BD OptEIA ELISA kits were procured from BD Biosciences, USA.

2.3. Assessment of motor coordination by rota-rod in rats and mice.

Motor coordination in animals was measured using the rota-rod apparatus for mice (Ugo Basile, USA) and for rat (INCO, India). Animals were trained for 2 days on rotating rod at 16 rotations per minute. Animals that stayed on rotating rods for 300 s were selected for the experiment. Latency to stay on the rotating rod was recorded 0, 30 and 60 min after treatment of the vehicle, diazepam (10 mg/kg, p.o.) or aliskiren (10, 30 and 50 mg/kg, i.p.) in mice and after treatment of the vehicle, diazepam (15 mg/kg, p.o.) or aliskiren (30 and 100 mg/kg, i.p.) in case of rats.

2.4. Acetic acid-induced writhing test in mice

Anti-nociceptive effect of aliskiren was assessed using a modified acetic acid-induced writhing test (Ulugol et al., 2006). Briefly, a vehicle, diclofenac or aliskiren, was administered intraperitoneally and 30 min later the writhing response was elicited by intraperitoneal (i.p.) injection of 1% v/v acetic acid (10 mL/kg). Numbers of writhes were counted for 15 min.

2.5. Formalin induced hind paw test in rats and mice

In rats, hind paw test was performed using modification of the method described by Vissers et al. (2006). Briefly, a vehicle (50 μL, i.pl.) or aliskiren (0.1, 0.5 and 1 mg, i.pl.) was injected 15 min before intraplantar (i.pl.) injection of formalin (50 μL of 0.4% v/v) into the right hind-paw. The animal was immediately returned to the plexiglass chamber and nociceptive behavior was observed. Numbers of flinching responses by the animal in consecutive 5 min periods were recorded for 40 min following formalin injection

In mice, formalin hind paw test was carried out as per the method described by Luchese et al. (2010), with a few modifications. Briefly, a vehicle (10 mL/kg, i.p.), morphine (5 mg/kg, i.p.) or aliskiren (10, 30 and 50 mg/kg, i.p.) were injected 30 min before injection of formalin (25 μL of 2% v/v) into the plantar surface of the right hind paw. Animals were observed from 0 to 5 min (early phase/neurogenic phase) and 15–40 min (late phase/inflammatory phase). The total time spent in licking and biting of the injected paw was recorded and considered as indicative of nociception. After completion of observation, animals were sacrificed by using CO_2 and right hind paws were collected in dry ice. The paw was homogenized in phosphate buffer saline buffer (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride and homogenates were centrifuged at 5000 × *g* at 4 °C for 15 min. Supernatants were stored at −80 °C until analysis. TNF-α and IL-6 were estimated in supernatant using mouse ELISA kits. Protein was estimated in supernatant by the method described by Lowry et al. (1951).

2.6. Orofacial formalin test in mice

The orofacial formalin test was carried out using the method described previously by Luccarini et al. (2006). Briefly, animals were treated with vehicle (10 mL/kg, i.p.), morphine (5 mg/kg, i.p.) or aliskiren (3, 10, 30 and 50 mg/kg, i.p.), followed 30 min later by subcutaneous (s.c.) injection of formalin (25 μL, 2% v/v) into the upper right lip of each mouse. Animals were observed for 0–5 min (early phase/neurogenic phase) and 15–40 min (late phase/inflammatory phase) after formalin injection and the total time spent in rubbing its lip with one of its extremities was recorded and considered as an indicator of nociception.

2.7. Capsaicin induced hyperalgesia in mice

Vehicle (10 mL/kg, i.p.) or aliskiren (10, 30 and 50 mg/kg, i.p.) or morphine (5 mg/kg, i.p.) were injected 30 min before administration of capsaicin (2 μg/25 μL, i.pl.) in the plantar surface of the right hind paw of each mouse. The total time spent in licking the injected paw by mice for 5 min after capsaicin injection was recorded and considered as an indication of nociception (Spindola et al., 2010).

2.8. Hot plate method

The hot plate test was performed with a modification of method described by Garcia et al. (2011). Briefly, mice were kept on hot plate (55 ± 1 °C). Hot plate latency was recorded at 0 and 30 min after vehicle (10 mL/kg, i.p.), morphine (5 mg/kg, i.p.) or aliskiren (50 mg/kg, i.p.) treatment. The cut-off time was fixed at 20 s to avoid skin damage. Licking of hind paw and/or jumping off were used as parameters for determining hot plate latency, indicative of nociception.

2.9. Tail flick method

The tail flick method was performed with a modification of the method described by Garcia et al. (2011). Analgesiometer (IITC Life Sciences, USA) was used to measure tail flick latency. The light beam was focused on the animal's tail about 3 cm away from the tip and tail flick latency was recorded at active intensity (AI: 20%). The cut-off time was fixed at 20 s to avoid tail damage. Tail flick latency was recorded at 0 and 30 min after vehicle (10 mL/kg, i.p.), morphine (5 mg/kg, i.p.) or aliskiren (50 mg/kg, i.p.) treatment.

2.10. Effect of aliskiren on post-operative pain in rats

Evaluation of post-operative pain was performed using a method described by Brennan et al. (1996). Rats were anesthetized with ketamine and xylazine and a 1 cm longitudinal incision was made with a scalpel, through skin and fascia of the plantar aspect of the left paw, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally. Following hemostasis with gentle pressure, the skin was opposed with two single interrupted sutures using a 3-0 silk suture. After 24 h of surgery, animals were tested for development of mechanical allodynia. The allodynic responses were assessed by the withdrawal thresholds to mechanical stimuli induced by calibrated von Frey filaments (Touch Test sensory evaluators, USA) with logarithmically incremental stiffness from 0.4 to 15 g. Beginning with the 2 g filament, the filament was applied on ipsilateral mid plantar area of paw encircled by tori/footpads (5–10 mm from site of incision) for 6–8 s while the filament was bent. Brisk withdrawal or flinching of paw was considered as a positive response. If a positive response was observed, the filament with the next lower force was applied; otherwise, the next stiffer filament was used. The stimulus producing a 50% likelihood withdrawal was determined using the Dixon "up–down" method, as described by Chaplan et al. (1994). Rats