European Journal of Pharmacology 737 (2014) 70–76

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Immunopharmacology and inflammation

Ameliorative potential of aliskiren in experimental colitis in mice

Rakesh B. Patel, Kanaiyalal D. Prajapati, Bhavin M. Sonara, Manoranjan M. Sharma, Hiren M. Patel, Vishwanath D. Pawar*, Mukul R. Jain

Department of Pharmacology, Zydus Research Centre, Sarkhej-Bavla National Highway-8A, Moraiya, Ahmedabad 382213, Gujarat, India

ARTICLE INFO

Article history:
Received 27 February 2014
Received in revised form
2 May 2014
Accepted 7 May 2014
Available online 21 May 2014

Keywords:
Aliskiren
Renin
m-RNA
DSS
Colitis

ABSTRACT

In the present study, we investigated the ameliorative potential of aliskiren in dextran sulfate sodium (DSS) induced colitis in mice. Aliskiren (3 and 10 mg/kg, i.p.) was administered for 10 days from the day of DSS administration. The severity of colitis in mice was assessed using body weight loss, colon and spleen weight, hematological parameters, food intake, stool consistency, rectal bleeding and colon shortening. Colonic malondialdehyde (MDA), myeloperoxidase (MPO) and renin mRNA levels were also estimated. Furthermore, TNF-α and IL-6 in plasma and colon were analyzed. The results showed that aliskiren (10 mg/kg, i.p.) significantly improved the severity of colitis by, decrease in weight loss, improvement in food intake and stool consistency, decrease in rectal bleeding, decrease in relative colon and spleen weight and improvement in colonic shortening. Aliskiren (10 mg/kg, i.p.) improved blood hemoglobin, red blood cells (RBC) and hematocrit. Colonic malondialdehyde (MDA), MPO and histolopathological score were significantly diminished by aliskiren (10 mg/kg, i.p.). Furthermore, aliskiren (10 mg/kg, i.p.) significantly diminished the elevated levels of TNF-α, IL-6 and renin mRNA in inflammed colon. These results indicate involvement of renin in colitis and inhibition of renin by aliskiren ameliorates colitis.

1. Introduction

Inflammatory bowel disease (IBD) is chronic and relapsing gastrointestinal (GI) disease with unknown etiology characterized by inflammation of the intestine and colon (Blumberg et al., 1999; Russel, 2000; Strober et al., 2002). Despite availability of a large number of therapeutic options, side effect potential and modest efficacy limits the clinical usefulness of available agents (5-aminosalicylic acid and corticosteroids). Current research focuses on novel therapeutic options to treat IBD. The role of RAS (renin angiotensin system) in inflammatory process is gaining widespread interest in recent times.

Angiotensin II (Ang II) is a proinflammatory peptide that has been shown to be involved in several pathological processes (Ruiz-Ortega et al., 2001). It modulates the production of adhesion molecules, cytokines (IL-6 and TNF-α), chemokines and the responses of immune and inflammatory cells, such as chemotaxis, proliferation and differentiation (Ferreri et al., 1998; Nakamura et al., 1999; Moriyama et al., 1995).

In addition to its vasoconstrictor activities, Ang II can promote tissue inflammation, enhancing neutrophil infiltration, raising the possibility of its contribution to intestinal ulceration (Bregonzio et al., 2003). Moreover, Ang II is known to regulate motility in the intestine, as well as ion and water absorption via receptors in mucosa and muscle (Johansson et al., 2001). Colonic mucosal levels of both angiotensin- I and II are greater in patients with Crohn's disease and appear to correlate with the degree of inflammation (Jaszewski et al., 1990). 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 (Lu et al., 2008; Campos et al., 2011; Ino et al., 2009; Wood et al., 1994). Various studies have also shown the anti-inflammatory and antioxidant activities of aliskiren (Bassim, 2010; Del Fiorentino et al., 2010; Higashikuni et al., 2012; Ino et al., 2009; Schmerbach et al., 2010; Tang et al., 2012). Moreover, in our previous study we have demonstrated the anti-inflammatory activity of aliskiren in formalin induced pain (Patel et al., 2013).

It was demonstrated that ACE (angiotensin converting enzyme) inhibitors, captopril and lisinopril are beneficial in an animal model of colitis (Jahovic et al., 2005; Wengrower et al., 2004). Moreover, losartan and valsartan (Angiotensin type 1(AT_1) receptor blockers) were found to have beneficial effects in colitic mice (Santiago et al., 2008; Inokuchi et al., 2005). Since both ACE

* Correspondence to: Department of Pharmacology, Zydus Research Centre, Ahmedabad 382213, Gujarat, India. Tel.: +91 2717 665555; fax: +91 2717 665355.
E-mail address: vishwanathpawar@zyduscadila.com (V.D. Pawar).

http://dx.doi.org/10.1016/j.ejphar.2014.05.009
0014-2999/

enzyme and AT_1 receptors are part of the renin–angiotensin system, it is hypothesized that blocking the renin–angiotensin system at a very early stage by directly inhibiting renin with aliskiren may also attenuate colitis. Therefore, in the present study, we used aliskiren, a renin inhibitor as a possible anti-inflammatory and antioxidant drug to investigate therapeutic potential in experimental model of colitis induced by dextran sulfate sodium (DSS) in mice.

2. Materials and methods

2.1. Animals

Eight to nine weeks old healthy female C57BL/6 mice weighing 21–24 g were employed in the study. Animals were bred in-house at Zydus Research Center, Ahmedabad, India accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Animals were housed in individually ventilated cages under controlled temperature (18–25 °C), humidity (30–70% RH) and normal light/dark (12 h/12 h) cycle conditions. Food and water were provided *ad labium*. 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.

2.2. Drugs and chemicals

Aliskiren (Novartis, India) was dissolved in normal saline. Dextran sulfate sodium (MW; 36,000–50,000, MP Biomedical, OH, USA) was dissolved in drinking water. Marketed formulation of cyclosporine A (Panimun Boral®, Panacea Biotech Ltd., New Delhi, India) was used. Mouse TNF-α and IL-6 (BD OptEIA ELISA) kits were procured from BD Biosciences, USA. Analytical grade chemicals/reagents were used in the study. Hematology analyzer-Cell-Dyn-3700 (Abbott, USA) was used for hematology.

2.3. Induction of colitis

Animals were randomized into five groups based on body weight. Experimental colitis was induced by feeding mice with 3% w/v DSS for 5 days in drinking water. Control mice received drinking water without DSS. Mice received DSS were treated with vehicle (normal saline, 10 ml/kg, i.p.), Aliskiren (3 and 10 mg/kg, i.p.) and cyclosporine A (25 mg/kg, p.o.) for 10 days from the day of DSS treatment. Cyclosporine A was used as positive control to ensure responsiveness of model. Doses of aliskiren were selected based on anti-cytokine activity of aliskiren in LPS induced inflammation (data not shown) in our pilot study.

2.4. Assessment of DSS induced colitis

Body weight, food intake, rectal bleeding and stool consistency for each mouse were recorded daily. The disease activity index (DAI) was calculated for each mouse by daily grading on a scale of 0 to 4 using the following parameters: loss of body weight (0=normal; 1=0–5%; 2=5–10%; 3=10–20%; 4=>20%), stool consistency (0=normal; 2=loose stools; 4=watery diarrhea) and rectal bleeding (0=none; 2=presence of hemoccult; 4=severe bleeding). The mean DAI score (i.e., combined scores divided by 3) was determined as described by Siegmund et al. (2001).

On 10th day of treatment, all mice were anaesthetized with isoflurane and sacrificed by cardiac puncture and exsanguination. Blood was sampled in EDTA-containing tubes for hemoglobin (Hb), red blood cells (RBC), hematocrit, TNF-α and IL-6 estimation. Plasma was obtained by centrifugation at 2000 g for 20 min at 4 °C and stored at −80 °C until analysis. Midline incision was made and colons from the colocecal junction to the anus were removed. Colon length and weight as indirect markers of inflammation were measured after removal of fecal matter and washed with cold PBS (phosphate buffer saline). The spleens were also dissected out and their weights were measured. For cytokines and mRNA expression, colon samples were snap frozen and stored at −80 °C until homogenization.

2.5. Measurement of myeloperoxidase activity

Colons were homogenized in the assay buffer provided with the kit and centrifuged to remove the supernatants. The pellets were suspended in 0.5% w/v hexadecyltrimethylammonium bromide, homogenized and sonicated for 30 s. The samples were subjected to two cycles of freeze–thaw and then centrifuged at 11,000 g for 15 min at 4 °C to collect the supernatants. Myeloperoxidase was assayed in the supernatant using fluorometric detection kit (Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions. The kit utilizes a non-fluorescent detection reagent, which is oxidized in presence of hydrogen peroxide and MPO to produce its fluorescent analog. The fluorescence was measured with an excitation wavelength of 530–570 nm and an emission wavelength of 590–600 nm in a fluorescent plate reader.

2.6. Measurement of cytokines and malondialdehyde

Colons were homogenized in phosphate buffer saline buffer (pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride and centrifuged at 11,000 g for 15 min at 4 °C to collect the supernatants. Supernatants were stored at −80 °C until analysis of cytokines and malondialdehyde (MDA).

TNF-α and IL-6 were estimated in supernatants (colon homogenates) and plasma by enzyme-linked immuno-sorbent assay (ELISA) kits according to the manufacturer's instructions (BD Biosciences, USA).

MDA, an indicator of mucosal injury induced by reactive oxygen species were measured as described by Ohkawa et al. (1979). Briefly, supernatant was mixed with TBA (thiobarbituric acid), SDS (sodium dodecyl sulfate) and acetic acid. Mixture was heated for 1 h at 95 °C. The color complex was estimated at 532 nm. MDA level was calculated by using molar extinction coefficient and expressed as nmol/mg of protein.

Total protein was estimated in supernatants by method described by Lowry et al. (1951).

2.7. Histopathology

Segments of colon were fixed in 10% formalin and after routine processing; sections were stained with hematoxylin and eosin. Sections were evaluated by an expert pathologist who was blinded to the treatment allocation of the animals. Score was given as per severity of pathology, 0=normal colon mucosa, 1=shortening of basal crypts with slight edema and infiltration of inflammatory cells, 2=loss of basal crypts with moderate inflammation in lamina propria, 3=total loss of basal crypts with severe inflammation in lamina propria but with surface epithelium still remaining, 4=loss of all crypts and surface epithelium with severe inflammation in the mucosa, muscularis propria and submucosa.