Chemico-Biological Interactions xxx (2016) 1–10

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Inhibiting renin angiotensin system in rate limiting step by aliskiren as a new approach for preventing indomethacin induced gastric ulcers

Zekai Halici [a], Beyzagul Polat [b], Elif Cadirci [a, *], Atilla Topcu [c], Emre Karakus [d], Duygu Kose [a], Abdulmecit Albayrak [a], Yasin Bayir [e]

[a] Department of Pharmacology, Ataturk University, Faculty of Medicine, Erzurum, Turkey
[b] Department of Pharmacology, Ataturk University, Faculty of Pharmacy, Erzurum, Turkey
[c] Department of Pharmacology, Recep Tayyip Erdoğan University, Faculty of Medicine, Rize, Turkey
[d] Department of Pharmacology and Toxicology, Ataturk University, Faculty of Veterinary Medicine, Erzurum, Turkey
[e] Department of Biochemistry, Ataturk University, Faculty of Pharmacy, Erzurum, Turkey

ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form
7 September 2016
Accepted 15 September 2016
Available online xxx

Keywords:
Aliskiren
Cytokine
Indomethacin
Renin angiotensin aldestron system
Ulcer

ABSTRACT

Purpose: Previously blocking the renin angiotensin system (RAAS) has been effective in the prevention of gastric damage. Therefore, the aim of this study was to investigate the effects of aliskiren, and thus, direct renin blockage, in indomethacin-induced gastric damage model.
Methods: Effects of aliskiren were evaluated in indomethacin-induced gastric damage model on Albino Wistar rats. Effects of famotidine has been investigated as standard antiulcer agent. Stereological analyses for ulcer area determination, biochemical analyses for oxidative status determination and molecular analyses for tissue cytokine and cyclooxygenase determination were performed on stomach tissues. In addition, to clarify antiulcer effect mechanism of aliskiren pylorus ligation-induced gastric acid secretion model was applied on rats.
Results: Aliskiren was able to inhibit indomethacin-induced ulcer formation. It also inhibited renin, and thus, decreased over-produced Angiotensin-II during ulcer formation. Aliskiren improved the oxidative status and cytokine profile of the stomach, which was most probably impaired by increased Angiotensin II concentration. Aliskiren also increased gastroprotective prostaglandin E2 concentration. Finally, aliskiren did not change the gastric acidity in pylorus ligation model.
Conclusion: Aliskiren exerted its protective effects on stomach tissue by decreasing inflammatory cytokines and oxidative stress as a result of inhibiting the RAAS, at a rate-limiting step, as well as its end product, angiotensin II. Aliskiren also significantly increased protective factors such as PGE2, but not affect aggressive factors such as gastric acidity.

1. Introduction

Gastric ulcer is a multi-etiologic chronic disease. Various factors, such as impairment of the balance between aggressive (increased acid secretions) and protective factors, stress, trauma, sepsis, hemorrhagic shock, burns, pulmonary and liver diseases, helicobacter pylori, use of cigarettes and alcohol, and steroidal and nonsteroidal anti-inflammatory drugs, have been shown to play a role in gastric ulcerogenesis [1,2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs worldwide due to their analgesic, anti-inflammatory and antipyretic therapeutic properties [3]. More than 30 million people use NSAIDs every day, and they account for 60% of the US over-the-counter analgesic market [3,4]. However, NSAIDs are associated with a broad spectrum of side effects, including gastrointestinal side effects [5]. Digestive adverse effects of NSAIDs includes gastric erosions, ulceration, bleeding, perforation, as well as increased risk of complications from pre-existing ulcers [6]. Among NSAIDs indomethacin is commonly used as a reference drug for ulcer modeling in rats because of its high gastric toxicity [7–9]. Many different mechanisms have been mentioned for indomethacin-induced gastric damage. It has been suggested that indomethacin induces gastric damage by inhibiting the release of protective factors, such as cyclooxygenase-1 (COX-1), prostaglandin E2 (PGE2),

* Corresponding author. Ataturk University, Faculty of Medicine, Department of Pharmacology, 25240, Erzurum, Turkey.
E-mail address: ecadirci@atauni.edu.tr (E. Cadirci).

http://dx.doi.org/10.1016/j.cbi.2016.09.011
0009-2797/

bicarbonate, and mucus; by increasing aggressive factors such as acid; and by increasing oxidant parameters while decreasing antioxidant parameters [7]. Current standard antiulcer treatment protocols include drugs decreasing gastric acid secretion such as histamine H_2 receptor blockers and proton pump inhibitors, antacids such as magnesium, aluminum, calcium derivatives and gastro protective agents such as PGE2 analogues, sucralfate etc. [10]. Some studies have also suggested a relationship between indomethacin-induced gastric ulcers and the renin angiotensin aldosterone system (RAAS) [11–13].

In the RAAS, angiotensin II is an important end-product that induces oxidative stress and inflammation [14] and constricts the gastric vasculature [15] through angiotensin II receptor stimulation. It has been reported that in the gastrointestinal tract, the RAAS was accompanied with functional effects in the stomach [16], intestine [17], and colon [18]. Drugs affecting the RAAS, both angiotensin-converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) have been investigated in different ulcer models. Captopril, the first used ACEi in hypertension treatment, has shown antioxidant properties during aspirin-induced gastric lesions [19]. ARBs have been reported to protect gastric blood flow by partially inhibiting sympathoadrenal discharge and angiotensin II-mediated vasoconstriction [20,21]. Nakagiri et al. reported that angiotensin AT1 receptor blockers suppress ischemia/reperfusion-induced gastric injury in rats [22]. As a physiological antagonist of angiotensin II, angiotensin (1–7) (Ang (1–7)), has also been effective against stress-induced gastric lesions in rats [23,24]. These data suggest that inhibition of the RAAS in different steps can be effective during ulcer treatment; however, they mainly focused on potential anti-inflammatory, anti-oxidative and/or gastro-protective effects of RAAS inhibition in different ulcer models. However, there is no study investigating direct renin inhibition and stomach RAAS parameters such as renin and angiotensin II levels for ulcer prevention.

Aliskiren, the first orally active direct renin inhibitor, was approved by the US Food and Drug Administration in March 2007 [25]. It has demonstrated effective blood pressure control and is generally well tolerated as a monotherapy or in combination with other antihypertensive drugs [26]. Aliskiren links to the site within renin that is responsible for the hydrolysis of angiotensinogen, which leads to the generation of the decapeptide fragment angiotensin I (Ang I), the initial and rate-limiting step of the RAAS [27]. However, there have been no studies on the effects of aliskiren in gastric ulcers.

In the light of previous literature suggesting potential role of the renin angiotensin system (RAAS) in the gastric ulcers, we aimed to investigate the effects of aliskiren, and thus, direct renin blockage, in indomethacin-induced gastric damage model and compare with that of standard antiulcer agent famotidine. We also aimed to clarify the antiulcer effect mechanism of aliskiren by evaluating stomach renin and angiotensin levels and contributing pathways by molecular and biochemical analyses and pylorus ligation-induced gastric acid secretion studies.

2. Materials and methods

2.1. Animals

A total of 60 male albino Wistar rats were used in the experiments. Each rat weighed 250–280 g, and all were obtained from Ataturk University's Experimental Animal Laboratory at the Medicinal and Experimental Application and Research Centre. The animal experiments and procedures were performed in accordance with national guidelines for the use and care of laboratory animals and approved by Ataturk University's local animal care committee (2013/11). The rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22 ± 1 °C. Standard rat food and tap water were given ad libitum.

2.2. Chemicals

All of the chemicals used in our laboratory experiments were purchased from Sigma Chemical Co. (Munich, Germany). Aliskiren (Rasilez 300 mg tab) was obtained from Novartis Drug Company (Basel, Switzerland); indomethacin (Endol 25 mg; 25 cap.) was obtained from DEVA Holding A.S. (Istanbul, Turkey); famotidine (Famodin 40 mg; 30 tab) was obtained from Sandoz Drug Company (Istanbul, Turkey); and thiopental sodium was obtained from IE Ulagay A.S. (Istanbul, Turkey).

2.3. Experimental design of indomethacin-induced ulcer model

The rats were separated into five groups, each composed of six individual rats:

Group 1: Healthy control
Group 2: Indomethacin 25 mg/kg control
Group 3: Indomethacin 25 mg/kg + famotidine 40 mg/kg administered orally
Group 4: Indomethacin 25 mg/kg + aliskiren 100 mg/kg administered orally
Group 5: Indomethacin 25 mg/kg + aliskiren 200 mg/kg administered orally

2.4. Experimental design of pylorus-ligation-induced acid secretion model

The rats were separated into five groups, each composed of six individual rats:

Group 1: Healthy control
Group 2: Pylorus ligation (PL) control
Group 3: PL + famotidine 40 mg/kg administered orally
Group 4: PL + aliskiren 100 mg/kg administered orally
Group 5: PL + aliskiren 200 mg/kg administered orally

2.5. Ulcer model

The aliskiren groups received 100 and 200 mg/kg doses of drugs as a pretreatment, after which the rats were fasted for 24 h. At the end of this period, famotidine and aliskiren were administered to the corresponding rat groups in the above-mentioned doses. One hour after the drug treatments, a dose of 25 mg/kg of indomethacin was suspended in isotonic saline and applied by oral gavage to all groups except the healthy intact rats, who received only isotonic saline as vehicle. Six hours after the administration of indomethacin, all of the rats were euthanized with a high dose (50 mg/kg) of thiopental. The stomachs were removed, and the ulcer focus on the gastric surface was assessed macroscopically. Ulcer areas were evaluated by stereo-investigator, and numerical densities of ulcerated areas were calculated by software, as described below. After this assessment, the stomachs were transported to the laboratory for determination of the biochemical and molecular analyses.

2.6. Stereological analyses

In this study, an unbiased point-counting grid was used to evaluate ulcer hematoma areas in the rats' stomach tissues. Images

Please cite this article in press as: Z. Halici, et al., Inhibiting renin angiotensin system in rate limiting step by aliskiren as a new approach for preventing indomethacin induced gastric ulcers, Chemico-Biological Interactions (2016), http://dx.doi.org/10.1016/j.cbi.2016.09.011