

SHORT COMMUNICATION

ANTIULCER ACTIVITY OF LEVCROMAKALIM AND NICORANDIL IN ALBINO RATS: A COMPARATIVE STUDY

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Antiulcer activity of levcromakalim and nicorandil in albino rats: a comparative study. M. BOSE, V.M. MOTGHARE, G.N. DAKHALE, A.V. TURANKAR. Pol. J. Pharmacol., 2003, 55, 91–95.

The objective of the present study was to investigate and compare the antiulcer effect of potassium channel openers, nicorandil and levcromakalim in the models of ulcer induced by pylorus ligation, aspirin and water immersion plus restraint stress in albino rats. Levcromakalim (250 µg/kg) and nicorandil (10 mg/kg) were administered intraduodenally immediately after pylorus ligation. Ulcer index was determined and gastric juice was subjected to analysis of total acid output (TAO) and pH. In aspirin-induced gastric ulcer model, the drugs were administered orally 30 min prior to noxious challenge, and in water immersion restraint stress model, the drugs were administered orally and ulcer index was determined. A significant reduction in ulcer index was observed after treatment with both potassium channel openers in all the gastric ulcer models. In pylorus-ligated rats, a significant decrease in TAO was noted. The conclusion is that potassium channel openers possess antiulcer activity. Antiulcer activity of levcromakalim is better than nicorandil but comparable to that of cimetidine. The antiulcer action of potassium channel openers is mediated partially by a decrease in gastric acid secretion, increase in gastric mucosal resistance and improvement in gastric mucosal blood flow.

Key words: *levcromakalim, nicorandil, antiulcer activity, potassium channel openers*

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Abbreviations: CGRP – calcitonin gene related peptide, CMC – carboxymethylcellulose, TAO – total acid output

INTRODUCTION

Peptic ulcer is one of the common diseases in human population. Its incidence is increasing due to rapid development and civilizational constraints. The estimates of incidence of peptic ulcer vary ranging between 3–10% [14]. Although exact etiology of the disease is not known, an imbalance between defensive mechanisms and aggressive factors result in peptic ulcer. Available information supports a central role of *Helicobacter pylori* [11]. Some epidemiological studies revealed the co-occurrence of both peptic ulcer and arterial hypertension in humans [16]. Therefore, it is important to examine the antiulcer activity of antihypertensive drugs. Recent advances in this field revealed contribution of different types of ion channels like potassium and calcium channels. Potassium channel openers, levcromakalim, nicorandil and pinacidil were found to be effective in the treatment of hypertension and angina pectoris. Cromakalim showed protective effect on some experimentally induced ulcers, which suggests its potential role in ulcer therapy [7].

Uptill now, very few and incomplete studies have been conducted on antiulcer activity of potassium channel openers. No study is available comparing antiulcer activity of levcromakalim and nicorandil. Hence, it was considered worth to investigate and compare the effect of two potassium channel openers, levcromakalim and nicorandil, on gastric ulcers induced by water immersion plus restraint stress, pylorus ligation and aspirin in albino rats and to some extent to explore the mechanism of their antiulcer activity.

MATERIALS and METHODS

Animals

Wistar albino rats of either sex weighing 150–200 g were maintained in animal house. They were acclimatized to housing conditions for at least one week prior to use. Animals were housed individually to avoid coprophagy with a 12 h light/12 h dark cycle. Prior to the experimentation, they were fasted for a period of 24–48 h, allowing them free

access to drinking water. In order to avoid the influence of diurnal variation, all the experiments were carried out at same time of the day i.e. between 10 a.m. to 5 p.m. Twelve animals were used for each test drug and control group in each ulcer model. Ethical Committee approval was obtained before carrying out this experiment.

Drugs

Levcromakalim was obtained as gift sample from SmithKline Beecham Pharmaceutical, Sussex, UK. Nicorandil and cimetidine were obtained from Cadila Healthcare Limited, Ahmedabad, and aspirin was received from Adroit Pharmaceutical, Nagpur. All drugs were dissolved in 1% sodium carboxymethylcellulose (CMC) solution in water and administered orally in the models of aspirin and water immersion plus restraint stress-induced ulcers, whereas in pylorus-ligated rat model, the drugs were given intraduodenally after pylorus ligation. Levcromakalim (250 µg/kg), nicorandil (10 mg/kg) and cimetidine (50 mg/kg) were given in different models of ulcers. The treatment with CMC at the dose of 1 ml/kg was considered a control for all the groups.

Experimental models

Pylorus ligation-induced gastric ulceration

Albino rats were fasted for 48 h but water was allowed *ad libitum* prior to pylorus ligation. Under light ether anesthesia, abdomen was opened by a small midline incision below the xiphoid process. The stomach was placed carefully and the abdominal wall was closed by interrupted sutures. The drugs were administered intraduodenally immediately after pylorus ligation. The 19 h after pylorus ligation, the rats were killed with overdose of ether. The stomach was then taken out and cut open along the greater curvature and ulcer index was determined [1]. Gastric contents were drained into the tubes and subjected to the biochemical analysis for total acid output (TAO) and pH.

Aspirin-induced gastric ulceration [13]

Animals were fasted for 36 h but water was allowed *ad libitum*. Aspirin suspended in 1% CMC in water was administered orally at a dose of 500 mg/kg, and the animals were killed 4 h later with overdose of ether. The drugs were administered 1 h prior to noxious challenge. The stomach was dis-

sected out. Its inner surface was cleaned with physiological saline and stomach lesions were examined under a magnifying hand lens and ulcer index was determined.

Water immersion plus restraint stress-induced gastric ulceration

Rats were fasted for 24 h before inducing the ulcer. They were kept immobilized in special cages and then immersed to the level of xiphoid process in a water bath (23°C) for 16 h. The animals were killed by overdose of ether. The stomach was removed and cut open along the greater curvature and pinned on wooden board after washing lightly with running water. Ulcers were examined with hand magnifying lens and the ulcer index was calculated.

Statistics

Results were expressed as means \pm SEM and were analyzed for statistically significant difference using one-way analysis of variance (ANOVA) and Turkey's post-hoc test; p value less than 0.05 was considered significant. Graph Pad Prism Version 3.02 was used for statistical calculations.

RESULTS

Effect of the drugs on pylorus ligation-induced gastric ulceration

In pylorus-ligated rats, intraduodenally administered nicorandil and levromakalim showed highly

significant ($p < 0.001$) reduction in ulcer index as compared to control group. Activity of levromakalim preventing the formation of lesions corresponded with that of cimetidine. Volume of gastric contents was reduced significantly ($p < 0.05$) by levromakalim but not by nicorandil when compared to control group. Also levromakalim and nicorandil significantly reduced total acidity ($p < 0.001$) as compared to control group. pH was increased significantly by levromakalim ($p < 0.001$) and nicorandil ($p < 0.05$) when compared to control group (Tab. 1). Significant difference in pH was observed between levromakalim- and nicorandil-treated group ($p < 0.01$).

Effect of the drugs on aspirin-induced gastric ulceration

Aspirin treatment resulted in the production of gastric lesions mainly in the glandular segments of the stomach. Nicorandil and levromakalim caused highly significant ($p < 0.001$) reduction in ulcer index as compared to control group (Fig. 1). When levromakalim was compared with nicorandil and cimetidine, no significant difference was found.

Effect of the drugs on water immersion plus restraint stress-induced gastric ulceration

A highly significant ($p < 0.001$) gastroprotective action of the treatment of nicorandil and levromakalim was evidenced by reduction in ulcer index as compared to control group. The areas of

Table 1. Effect of levromakalim, nicorandil and cimetidine (intraduodenally) on secretory parameters and ulcer index after pylorus ligation

Group	Dose	pH	Volume of gastric content (ml/kg)	Total acidity (meq/l)	Ulcer index
Control		3.2 \pm 0.09	20.2 \pm 1.4	142.30 \pm 11.40	5.90 \pm 0.376
Levromakalim	250 μ g/kg	4.3 \pm 0.22***	13.6 \pm 1.62* [@]	54.12 \pm 9.23***	0.47 \pm 0.048***
Nicorandil	10 mg/kg	3.5 \pm 0.15*	17.3 \pm 1.7	83.22 \pm 7.76***	0.65 \pm 0.054***
Cimetidine	50 mg/kg	4.7 \pm 0.12***	11.4 \pm 1.5**	42.10 \pm 10.12***	0.41 \pm 0.039***
ANOVA					
F ratio		72.66	6.264	21.17	196.4
p value		< 0.001	< 0.01	< 0.001	< 0.001

The values are expressed as means \pm SEM, n = 12 animals in each group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control group, [@] $p < 0.01$ vs nicorandil treated group

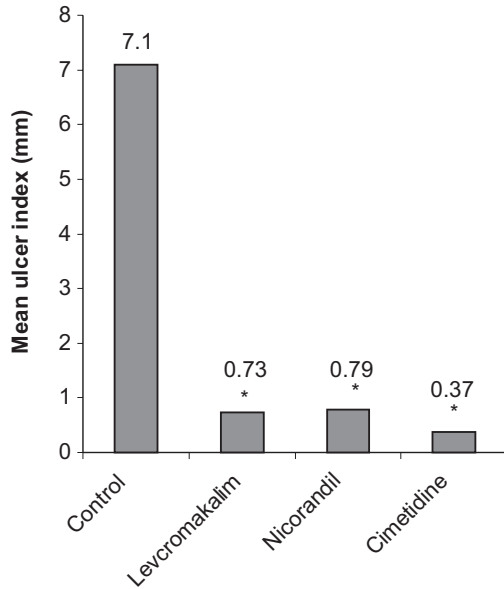


Fig. 1. Effect of levromakalim, nicorandil and cimetidine on aspirin-induced gastric ulceration in rats; * $p < 0.001$ vs control group; $n = 12$ animals in each group

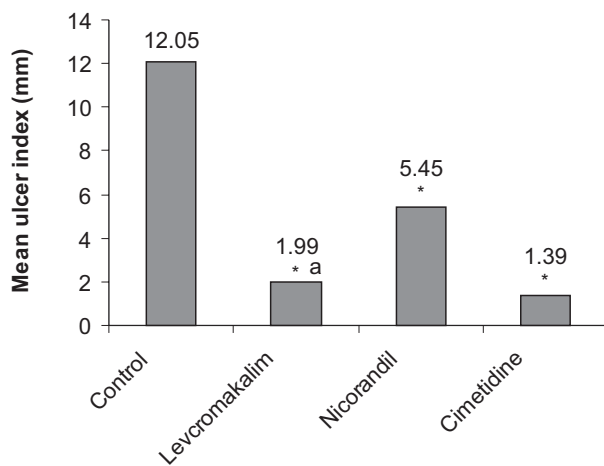


Fig. 2. Effect of levromakalim, nicorandil and cimetidine on water immersion and restraint stress-induced gastric ulcers in rats; * $p < 0.001$ vs control group; ^a $p < 0.001$ vs nicorandil group; $n = 12$ animals in each group

involvement in all the rats were confined to glandular region of stomach. Activity of levromakalim preventing the ulcer formation was significant ($p < 0.001$) compared to nicorandil but only comparable ($p > 0.05$) with that of cimetidine (Fig. 2).

DISCUSSION

Present study demonstrates antiulcer activity of potassium channel openers, nicorandil and levro-

makalim, in all three experimental models of gastric ulceration, which was evidenced by significant decrease in ulcer index, total acidity, and volume of gastric content. Various mechanisms are thought to be involved in the ulcer production in different experimental models [13]. Hence, it is not possible to propose a single mechanism for antiulcer effect of a particular drug.

Nicorandil and levromakalim produced significant decrease in ulcer index in pylorus ligation ulcer model. Digestive effect of the accumulated gastric juice is believed to be responsible for producing ulcers in the pylorus-ligated rats. In addition to gastric acid secretion, reflex or neurogenic effect has also been suggested to play an important role in the formation of gastric ulcer in this model [7].

Levromakalim and nicorandil increased pH significantly. Whereas total acidity was reduced significantly by levromakalim and nicorandil, only levromakalim significantly decreased volume of gastric content. Calcitonin gene related peptide (CGRP) [15] and nitric oxide [12] play an important role in mucosal protection. CGRP and adrenomedullin are endogenous vasodilators acting partly through opening of K_{ATP} channels [15]. CGRP is present in the afferent capsaicin-sensitive nerve fibres innervating rats gastric mucosa [17] and released from isolated rat stomach by capsaicin. All these findings suggest that both peptides are involved in pathophysiological regulation of gastric functions such as acid secretion and mucosal blood flow, thereby protecting gastric mucosa against noxious stimuli [6].

Aspirin-induced damage to the gastric mucosa is associated with breakage of gastric mucosal barrier [3]. Nicorandil and levromakalim produced significant reduction in gastric lesions in aspirin-induced gastric ulcer model. Therefore, nicorandil and levromakalim may act through a mechanism, which increases the gastric mucosal resistance and further supports the potential antiulcer activity of potassium channel openers.

In water immersion plus restraint stress-induced model, ulcers are formed as a result of disturbance of gastric secretion, alteration in microcirculation of gastric mucosa and abnormal gastric motility [10]. Central mechanism including vagal overactivity has also been considered for pathogenesis of stress-induced ulcers [9]. Nicorandil and levromakalim produced significant reduction in the mean ulcer index in this model, which points to

a possible mechanism of action by increasing the gastric mucosal blood flow and by inhibition of gastric acid secretion [5]. Levromakalim dilated gastric blood vessels and increased blood flow to the heart and small intestine in anesthetized rabbits [2]. It is also well known that levromakalim reduces contractility of smooth muscles because of its vasodilatory or hyperpolarizing properties [8]. Therefore, inhibition of gastric motility might play a role in the mechanism of antiulcer action of potassium channel openers. Potassium channel openers interfere with calcium influx [4], and calcium influx plays an important role in gastric secretion, which explains one of the mechanisms of the antiulcer activity of these drugs.

The findings of the present study corroborate the data presented in earlier report [7], but no previous studies have used all the three experimental models for testing antiulcer activity of potassium channel openers. At the same time, no data is available comparing antiulcer activity of levromakalim with nicorandil. The average mean values in this study suggested that levromakalim had better antiulcer activity than nicorandil in all three experimental methods for ulcer induction whereas cimetidine was the best among the three drugs used. Also this study included twelve animals in each group. Therefore, the results of the present study confirm the protective activity of potassium channel openers against gastric ulceration in rats. The mechanism of antiulcer activity of potassium channel openers is attributed partially to a decrease in acid secretion, increase in gastric mucosal resistance and improvement of gastric mucosal blood flow. However, further studies are indicated to elucidate the precise mechanism and actual doses of potassium channel openers necessary for prevention of peptic ulcer.

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Received: September 17, 2002; in revised form: November 19, 2002.