EFFECT OF SR 58611A, A BETA-3 RECEPTOR AGONIST, AGAINST EXPERIMENTAL GASTRO-DUODENAL ULCERS

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Abstract : The present study was designed to study the effect of SR 58611A, a selective β_3-adrenoceptor agonist against gastric ulcers: pylorus ligation, water immersion plus restraint stress (WIRS), ethanol, aspirin-induced and on cysteamine-induced duodenal ulcers, in rats. SR 58611A (10 mg/kg, p.o.) was found to be effective in attenuating gastric ulceration and the results were comparable with those from standard cimetidine-treated group.

Apart from reducing ulcer index, SR 58611A significantly decreased total acidity and thereby exhibited antisecretory activity in pylorus ligation model. SR 58611A showed significant reduction in ulcer index alongwith significant rise in the gastric wall mucus content in WIRS model. Further it showed significant cytoprotective activity against ethanol insult, that was evident from significant reduction in ulcer index. It showed significant reduction in gastric ulceration in aspirin-treated rats. The drug was found to be ineffective in inhibiting the cysteamine-induced duodenal ulcers as evident from the ulcer index and total lesion area parameters.

It is concluded that SR 586111A possesses significant gastroprotective activity. This activity could be attributed to the inhibition of gastric acidity, increase in gastric wall mucus content and the reversal of gastric microvascular injury resulting into protection of the vascular integrity.

Key words :

INTRODUCTION

β-agonist such as isoprenaline salbutamol and salmeterol provide mucosal protection and also inhibit gastric acid secretion (1). It is reported that isoprenaline markedly decreases pentagastrin-induced acid secretion (2).

β_3-adrenoceptors are also located within the gastrointestinal tract besides adipose tissue (3). Stimulation of bicarbonate secretion was also observed in rat caecum by isoprenaline and SR 58611A (4). Recently it has been shown that β_3-adrenoceptor agonists confer protection against indomethacin-induced gastric

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antral ulcers (5, 6) and jejunal ulcers (7).

In view of these findings, the effects of SR 58611A, a selective β_3-adrenoceptor agonist against different experimental models of gastric and duodenal ulcers was studied.

METHODS

Animals: Albino rats of either sex weighing between 150–250 g were used. They were fed with standard chow diet. Coprophagy was prevented by placing the animals in cages with grating on its floor. Throughout the experiment, the animals were housed at $25 \pm 1°C$ and $55 \pm 10\%$ humidity.

Drugs: SR 58611A [ethyl [[(7S) 7-((2R)2-(3-chlorophenyl)-2-hydroxyethyl)amino]-5,6,7, 8tetrahydronapthyl-2-yl] oxyacetate hydrochloride, Sanofi Research, Milan, Italy], aspirin [acetyl salicyclic acid, Loba Chemie, India], cimetidine [Cadila Lab. Ltd., Ahmedabad], cysteamine hydrochloride [Kemphasol, Mumbai, India].

Methods: SR 58611A (10 mg/kg, p.o.) and cimetidine (75 mg/kg, p.o.) were administered in different experiment models as mentioned below. The drug solutions were freshly prepared by dissolving in distilled water. Control group received vehicle alone.

Gastric ulcer models

Pylorus ligation-induced gastric ulcers in rats (8) : 36 h fasted rats were anesthetized with ether and abdomen was opened by a small midline incision below the xiphoid process. Pylorus portion was lifted and ligated avoiding traction to the pylorus or damage to its blood supply. The drug was administered (p.o.) immediately after the pylorus ligation. Six hours later animals were killed, stomachs removed and opened along the greater curvature. The contents were removed in test tubes and subjected to analysis. Ulcer index was determined as :

$$\text{Ulcer Index} = 10/X$$

$$\text{Where, } X = \frac{\text{Total area of stomach mucosa}}{\text{Total area of ulcerated mucosa}}$$

Water immersion plus restraint stress-induced gastric ulcer in rats (9) : Rats previously fasted for 36 h were placed into specially designed restrainers made up of acrylic polymer. They were then kept submerged in water at 24°C up to the xiphoid process and the animals were sacrificed after seven hours. Ulcer index and the severity of ulcer bleeding were measured. Stomach tissue was subjected to the measurement of gastric wall mucus content. Drug was given 30 min prior to ulcerogenic procedure. Severity of ulcer bleeding was measured according to the method of the Hayase and Takeuchi (10).

Ethanol-induced gastric ulcers in rats (11) : Animals were fasted for 24 h before experiment. 1 ml of 80% ethanol was administered p.o. Two hours after ethanol administration animals were killed, stomachs removed and opened along the greater curvature and subjected to

measurement of ulcer index. The drug was given 1 h before the administration of ethanol to test group. The stomach tissue was subjected to lipid peroxidation for measurement of malondialdehyde (MDA) content.

Aspirin-induced gastric ulcers in rats (12): Aspirin was suspended in 1% carboxymethyl cellulose in water and administered orally at the dose of 500 mg/kg to 36 h fasted rats. Six hours later, the animals were killed, stomachs were removed and opened alongwith greater curvature for the determination of ulcer index. Drug was given 30 min before the administration of aspirin.

Duodenal ulcer model

Cysteamine-induced duodenal ulcers in rats (13): Rats were administered p.o. total dose of 700 mg/kg cysteamine HC1. To avoid acute toxicity, cysteamine HC1 was administered in two doses each of 350 mg/kg in 10% aqueous solution at an interval of four hours. SR 58611A is administered 30 min before first dose of cysteamine. Animals were killed 24 h after the first dose of cysteamine administration. Ulcers were seen at the wall of duodenum close to pylorus region. The duodenal ulcers were scored for their intensity using a scale of 1 to 3 as follows: 0, no ulcer; 1, superficial mucosal erosion; 2, deep ulcer or transmucosal necrosis; 3, Perforated or penetrated ulcer. Ulcer index was calculated as the sum of the arithmetic mean of the intensity in a group and the ratio of positive/total multiplied by 2. e.g. 2.1 + (9/10*2).

Parameters under investigations

Total acidity (14): Gastric contents were assayed for total acidity against 0.01 N NaOH to pH 8.0 using phenolpthalein as indicator. It was expressed as mEq/L per six h.

Pepsin activity: The determination of pepsin activity was carried out by the modified method of Debnath et al (15). The pepsin output was calculated in terms of µg/ml of Tyrosine liberated per six hours of gastric juice.

Gastric wall mucus content (GWMC): A procedure of Corne et al (16) was used for estimating GWMC and that was expressed as mg alcian blue/g wet glandular tissue.

Dissolved mucosubstances

Total carbohydrates (TC) was estimated as described by Nair (17) and was expressed in terms of µg/ml of gastric juice.

Protein content (PR) was estimated according to the method of Lowry et al (18) and was expressed in terms of µg/ml of gastric juice.

Thiobarbituric acid reactive substances assay (19) and expressed as M moles/g malondialdehyde content (MDA) of glandular tissue.

Statistical analysis: The results were expressed in terms of Mean ± S.E.M. The significance of difference between mean values for the various treatments were tested using the unpaired students 't' test. The nonparametric data were subjected to Wilcoxon's rank sum test.

RESULTS

Pylorus ligated rats : Hemorrhagic lesions were observed in the glandular portion of the stomach. The parameters studied in this model include ulcer index, volume of gastric secretion, total acidity, total acid output, pepsin activity, total carbohyderates, protein content and total carbohydrates to protein (TC/PR) ratio.

SR 58611A caused significant reduction in ulcer index. Total acidity was also significantly reduced by the SR 58611A treatment (Table I). However, volume of gastric secretion, pepsin activity were not significantly altered. Cimetidine treatment showed significant reduction in ulcer index, total acidity, total acid output, and pepsin activity.

In case of biochemical parameters, SR 58611A did not significantly alter the total carbohydrates (summation of total hexose, hexosamine, fucose and sialic acid) when compared with control group (Table II). But there was a significant reduction in total protein content in treatment group (68.66 ± 5.56, P<0.05) when compared with control group (78.83 ± 4.66). Based upon the results of TC and PR content of gastric juice, TC: PR ratio was derived. SR 58611A showed insignificant increase in mucin activity i.e. TC: PR ratio. Similar results were observed with cimetidine treated rats.

TABLE I : Effect of SR 58611A on ulcer index and acid secretory parameters in pylorus ligation ulcer model.

Parameters	Control	SR 58611A (10 mg/kg)	Cimetidine (75 mg/kg)
Ulcer index	0.89±0.15	0.37±0.06*	0.25±0.04*
Volume of gastric secretion (ml 100 g/b.w.)	1.7±0.21	1.98±0.19	1.75±0.20
Total acidity (m Eq/L)	22.0±1.18	5.83±1.11**	4.79±0.90*
Total acid output (μ Eq/100 g)	37.58±4.48	12.74±2.4**	9.23±1.70**
Pepsin output (μg/ml)	41.0±4.44	29.1±3.12	18.50±2.50*

*P<0.01; **P<0.001; when compared with control group.
All values represent mean ± SEM
n = 8 in each group.

TABLE II : Effect of SR 58611A on biochemical parameters in pylorus ligation ulcer model.

Parameters	Control	SR 58611A (10 mg/kg)	Cimetidine (75 mg/kg)
Total carbohydrates (μg/ml)	340.0±32.78	359.1±38.15	389.41±30.5
Total protein (μg/ml)	78.83±4.66	68.66±5.56*	72.54±5.55
Mucin activity (TC/PR)	4.28±0.22	5.42±0.52	5.59±0.49

*P<0.05; Compared with control group.
All values represent mean ± SEM
n = 8 in each group.

Water immersion plus restraint stress-induced gastric ulcers in rats : Haemorrhagic ulcers were observed along the rugae of glandular portion of the stomach. The lumen of the stomach was found to be filled with blood. The parameters studied in this model included ulcer index, score for intensity of intraluminal bleeding (SI) and gastric wall mucus content. SR 58611A pretreatment showed a marked reduction in ulcer index but the score for intraluminal bleeding remained unaltered (Table III). Further the drug showed a marked increase in gastric wall mucus content (Table IV).

Ethanol-induced gastric ulcer model : Linear haemorrhagic gastric mucosal erosions were observed in the glandular stomach. The parameters studied were ulcer index and stomach thiobarbituric acid reactive substances (TBARs). In both SR 58611A and cimetidine treated animals, there was significant decrease in ulcer index (Table V). As ethanol is known to increase the free radical formation, SR 58611A was also studied for TBARs assay. SR 58611A and cimetidine showed reduction in the stomach TBARs but it was not found to be statistically significant (Table V).

TABLE III : Effect of SR 58611A on water immersion plus restraint stress-induced gastric ulcers.

Treatment	Dose, p.o.	Ulcer index	Score for intensity of intraluminal bleeding
Control	3 ml/kg	1.42±0.29 (7)	1.78±0.15 (7)
SR 58611A	10 mg/kg	0.49±0.09* (6)	1.21±0.27 (6)
Cimetidine	75 mg/kg	0.51±0.06* (6)	1.29±0.31 (6)

*$P<0.02$; when compared with control group.
All values represent mean ± SEM.

TABLE IV : Effect of SR 58611A on gastric wall mucus content against water immersion plus restraint stress-induced gastric ulcers.

Treatment	Number of animals	Dose, p.o.	Gastric wall mucus content (mg alcian blue/g wet glandular tissue)
Control	6	2 ml/kg	0.49±0.05
SR 58611A	6	10 mg/kg	0.89±0.06*
Cimetidine	6	75 mg/kg	0.69±0.04

*$P<0.001$; when compared with control group.
All values represent mean ± SEM.

TABLE V : Effect of SR 58611A on ulcer index and malondialdehyde (MDA) content against ethanol-induced gastric ulcers.

Treatment	Number of animals	Dose, p.o.	Ulcer index	Malondialdehyde (M modes/g)
Control	7	2 ml/kg	1.75±0.09	51.99±4.44
SR 58611A	8	10 mg/kg	0.40±0.12*	35.30±5.93
Cimetidine	6	75 mg/kg	0.64±0.08	39.60±4.10

*$P<0.001$; when compared with control group.
All values represent mean ± SEM.

TABLE VI : Effect of SR 58611A on ulcer index in aspirin-induced gastric ulcers.

Treatment	No. of animals	Dose, p.o.	Ulcer index
Control	6	2 ml/kg	1.04±0.095
SR 58611A	6	10 mg/kg	0.41±0.07*
Cimetidine	6	75 mg/kg	0.29±0.03*

*P<0.001; when compared with control group.
All values represent mean ± SEM.

TABLE VII : Effect of SR 58611A on cysteamine-induced duodenal ulcers.

Treatment	Dose, p.o. (b.i.d.)	No. of animals	Ulcer incidence	Score for intensity	Total lesion area (mm^2)
Control	350 mg/kg	7	100%	4.78	17.3±1.8
SR 58611A	10 mg/kg	6	100%	4.75	17.0±1.6
Cimetidine	75 mg/kg	6	100%	2.89⁺	9.42±0.55*

*P<0.05; when compared with control group.
⁺P<0.05; when compared with control group; Wilcoxon's rank sum test.

Aspirin-induced gastric ulcer model :
Haemorrhagic and non-haemorrhagic lesion were observed in the glandular region of the stomach. Ulcer index was studied in this model. SR 58611A showed significant reduction in ulcer index (Table VI). The results of SR 58611A were comparable with that of cimetidine treated group.

Cysteamine-induced duodenal ulcer model :
Two kissing craters developed on the wall of the duodenum and usually the anterior ulcer progressed to transmural necrosis and perforation. The parameters studied were score for intensity of duodenal ulcers and total lesion area. SR 58611A did not show any alteration in duodenal lesions as evident from the unaltered SI and total lesion area (Table VII). Cimetidine showed significant reduction in SI and total lesion area.

DISCUSSION

The protection afforded by the SR 58611A against gastric ulcers induced by pylorus ligation appears to be produced, at least in part, by the suppression of acid secretion, since the acid concentration of the gastric content in the SR 58611A treated animals was profoundly reduced. The antisecretory effect of the β-adrenoceptor agonists has been well reported in pylorus-ligated rats (20, 21) as well as in other species (22, 23).

Although pepsin plays a major role in the genesis of gastric erosions, in the present study SR 58611A was found to be devoid of any activity on pepsin secretion. This suggests that inhibition of pepsin secretion is not involved in the protective

effect of SR 58611A. In addition, SR 58611A did not show significant rise in the mucin activity (TC/PR ratio). It could be suggested that inhibition of acid concentration could be the possible mechanism through which the protection is afforded in the pylorus ligation model.

The specific pathophysiologic mechanism involved in the stress-induced ulcers could be due to multifactorial impairment of mucosal defense system. Stress in animals is known to increase gastric motility and acidity which could lead to ulceration manifested by severe mucosal damage and hemorrhage (24). Gastric wall mucus content is one of the important gastric mucosal barriers which maintains the pH gradient between the lumen and the mucosa and prevents the back diffusion of H^+ ions and later consequences. It is believed that the visible mucus adhering to the wall, rather than the mucus dissolved in gastric secretion plays an important role in protection against autodigestion of the gastric mucosa. Hence, significant rise in the gastric wall mucus content by SR 56118A might contribute to the protection against stress ulcers.

Ethanol and several NSAIDs such as aspirin, irritate the gastrointestinal mucosa in both human and animals and may therefore cause injury and bleeding. Studies in rat showed that oxygen derived free radicals are directly implicated in the mechanism of acute and chronic gastroduodenal ulceration (25). However, the MDA content was not significantly affected in the lipid peroxidation process under study.

Kuratani et al (6) have reported that β_3-adrenergic receptor agonists improves gastric mucosal blood flow. Further, Anthony et al (8) reported that β_3-adrenoceptor agonist SR 58611A inhibits indomethacin induced jejunal ulceration by reversing both villous shortening and vascular injury. Hence it can be suggested that protective effect of SR 58611A against ethanol and aspirin induced gastric ulceration might be due to increase in gastric mucosal blood flow and reversal of vascular injury thereby maintaining the integrity of vascular mucosa.

Cysteamine ulcers are considered to be due to a long lasting hypersecretion of the gastric acid which may partly be due to decrease in buffering capacity of the duodenum or due to increased plasma levels of gastrin (26). Infact hypersecretion of acid, disturbed gastroduodenal motility, hypergastrinaemia and decreased mucosal resistance have all been implicated in the pathogenesis of the cysteamine-induced duodenal ulcers (25, 26).

Our observations shows that SR 58611A is devoid of any effect on experimentally induced duodenal ulcers. Although the drug under study has shown profound decrease in gastric acid secretion, no reason can be attributed at this stage for the ineffectiveness of SR 58611A against duodenal lesions.

In conclusion, the drug SR 58611A possesses significant antiulcer property against different experimental gastric ulcer models. The mechanisms of their

antiulcer activity could be attributed to the profound reduction in gastric acid concentration, strengthening of gastric mucosal barrier by increasing the gastric wall mucus content, reversal of vascular injury and thereby maintaining integrity of vascular mucosa and enhancement of gastric mucosal blood flow.

The drug is devoid of any effect on duodenal ulcer.

ACKNOWLEDGMENT

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