Gastroprotective effects of β₃-adrenoceptor agonists on water immersion plus restraint stress-induced gastric ulcer in rats

A. Paul,* S. Goswami, D. Santani

ABSTRACT

Objective: To evaluate the gastroprotective effects of β₃-adrenoceptor agonists CGP 12177A and SR 58611A, on water immersion plus restraint stress (WIRS)-induced gastric ulceration in rats.

Material and Methods: Drugs were administered (5, 10 and 15 mg/kg, p.o.) 30 min prior to the ulcerogenic procedure. Ulcer index and the score for intensity of intraluminal bleeding were determined. Gastric wall mucus content (GWMC) and mast cell counts were determined in the glandular portion of the stomach.

Results: A dose-dependent reduction in the ulcer index was observed with both the drugs. A significant rise in the GWMC in the glandular tissue at 15 mg/kg dose was caused by the β₃-adrenoceptors agonists. In the glandular tissue the mast cell count was significantly decreased at 10 and 15 mg/kg dose with both drugs.

Conclusion: The present study shows the gastroprotective effect of β₃-adrenoceptor agonists CGP 12177A and SR 58611A against WIRS-induced gastric ulceration in rats. The gastroprotective effect may be mediated by the enhancement of mucin activity and the decrease in mast cell degranulation.

KEY WORDS: CGP 12177A, SR 58611A, β₃ stimulants, stress ulcer, mucoprotectives

Introduction

Recent localization of β₃-adrenoceptors using immunohistochemical studies has confirmed the presence of β₃-adrenoceptors in human vascular and non-vascular smooth muscles of the gastrointestinal tract.¹ The presence of β₃-adrenoceptors has been well described in mast cells and basophils and the stimulation of these receptors results in the inhibition of immune-stimulated histamine release.²³

Espluges et al.⁴ observed significant antiulcer activity with β-adrenergic drugs such as salbutamol, salmeterol and isoprenaline against polymixin-B-induced gastric ulcers involving histamine release. However, propranolol could only partially antagonize the isoprenaline-induced inhibitory effect on histamine release. This shows that besides β₂-adrenoceptor stimulation, these agonists inhibit histamine release through some additional mechanism other than beta-receptor stimulation. The involvement of β₁-adrenoceptors in the histamine release mechanism was ruled out by these studies. Isoprenaline was reported to be as potent as SR 58611A, a β₃-adrenoceptor agonist, in stimulating β₃-adrenoceptors in isolated rat colon.⁵ β₃-adrenoceptor agonists inhibit gastric ulcer-induced by indomethacin,⁶ pylorus ligation and ethanol⁷ in rats.

Hence, this study was undertaken to evaluate the antiulcer effect of β₃-adrenoceptor agonists on water immersion plus restraint stress (WIRS)-induced gastric ulcer in rats. The study was also directed towards the elucidation of the mechanism of the antiulcer activity of β₃-adrenoceptor agonists.

Material and Methods

Wistar albino rats of either sex weighing 200-250 g were selected. Rats were fed with standard chow diet and water ad libitum till the end of the experimental period. Distributions of the animals in-group, sequence of trials and treatment aspects were randomized. This experiment complied with the guidelines of our laboratory for animal experimentation. The Animal Ethics Committee of the institute cleared the experimental protocols.

CGP 12177A [(±)-4-(3-t-butylamino-2-hydroxy-propoxy) benzimidazol-2-one] and SR58611A [ethyl ((7S)-7-{(2R)-2-(3-
chlorophenyl]-2-hydroxyethylamino[ 5.6.7.8-tetrahydro-naphthalene-2-oyoxy) acetate hydrochloride] were obtained as gift samples from Novartis, Switzerland and Sanofi Recherche, France respectively. Drugs dissolved in distilled water were administered orally to rats in doses of 5, 10 and 15 mg/kg. Saline treated (0.5 ml/100 g, p.o.) rats served as controls. The dose of β1-adrenoceptor agonists were selected on the basis of ED50 values of BRL 35135, a β1-adrenoceptor agonist on indomethacin-induced ulceration and total acid-output in pylorus-ligated rats.5

Water immersion plus restraint stress-induced gastric ulceration

The method described by Takagi and Okabe8 was employed with slight modification. Rats were fasted for 12 h, care being taken to avoid coprophagy. The rats were immobilized in a restrainer and subsequently they were immersed in water up to xiphoid process for 7 h. The temperature of the water was maintained at 24±1°C. Drugs were given orally 30 min prior to the restraint procedure. After 7 h of immobilization and water immersion the animals were taken out and killed with high-dose anesthetic ether. The stomach was removed and the severity of intraluminal bleeding was examined and expressed as score for intensity (SI) of intraluminal bleeding according to the following scale: 0, no blood detectable; 1, thin blood follows the rugae; 2, thick blood follows the rugae; 3, thick blood follows the rugae with blood clots in certain areas; 4, extensive covering of the whole of mucosal surface with thick blood.9 After wiping the blood, the ulcer index was determined and the stomach tissue was subjected to mast cell examination and analysed for gastric wall mucus content (GWMC).

Measurement of gastric wall mucus

The modified procedure of Corne et al11 was used for the determination of gastric wall mucus. One half of the glandular portion of the stomach, opened along the greater curvature, was carefully separated from the rumenal part and transferred into 10 ml Alcian blue (8GX, Sigma) 0.1% (w/v) solution (Alcian blue was dissolved in 0.16 M sucrose buffered with sodium acetate 0.05 M, and finally adjusted to pH 5.8 with HCl IM). The tissue was stained for 2 h in Alcian blue solution; excess dye was removed by 2 successive rinses, soaking the tissue each time in 10 ml sucrose 0.25 M, first for 15 min and then for 45 min. Dye complexed with gastric wall mucus was then extracted with 10 ml magnesium chloride at 30 min intervals for 2 h. Four ml of the extract was shaken with an equal volume of ether until an emulsion was formed. This was centrifuged at 3600 rpm for 10 min. Ether was pipetted out and discarded, and the concentration of Alcian blue was determined in the aqueous layer. Color absorbance was recorded using a spectrophotometer (Shimadzu) at 598 nm. The quantity of Alcian blue extract per g wet glandular tissue was calculated from freshly prepared standard curves.

Examination of mast cells

Mast cells in the glandular mucosal layer were stained and measured by the method described by Cho and Ogle.12 Following ulcer measurement, one half of the glandular stomach was fixed in freshly prepared lead acetate 4% (w/v) solution for 2 days. The tissues were then dehydrated by 1 h immersion in each of progressively increasing concentrations of 70, 95 and 100 % v/v ethanol, and cleared in xylene for 30 min. The specimens were immersed in melted paraffin at 60°C for 3 h, and finally embedded in paraffin block. 7 µm thick sections were made with an “820” Spencer microtome and were transferred to slides, using the water floatation method. The sections were oven-dried at 60°C for 3 h before deparaffinisation with 3 changes of xylene, hydrated by passing the sections at 2 min intervals through solutions of ethyl alcohol 100, 100, 95 and 70% and finally water. Sections were then stained with an aqueous solution of 0.5% w/v toluidine blue for 0.5 min, dehydrated through 3 changes of tertiary butanol over a period of 1.5 min, cleared with xylene (3 changes over 3 min) and mounted in di-phenyl xylol (DPX). The mast cell count was expressed as the number of granulated metachromatically stained cells seen in 42 adjacent oil immersion fields (o.i.f., magnification 100x) covering an area of 1 mm². Cells that were partially stained (i.e., partly degranulated) were also counted.

Statistical analysis

The results were expressed as mean ± SEM and analyzed for statistical significance by the two-tailed Student’s ‘t’ test and by one-way ANOVA followed by Dunnett’s test. P values < 0.05 were considered significant. The ED50 values for anti-ulcer activity (ulcer index) were calculated using ED50 plus v 1.0 software.

Results

Severe hemorrhagic gastric glandular mucosal ulcers were observed in stress-induced control animals (Table 1). Significant change in the ulcer index, GWMC and mast cell count were observed in WIRS-stress as compared with non-stressed controls (Table 1).

Both the β1-adrenoceptor agonists (CGP 12177A and SR 58611A) reduced the ulcer index in a dose-dependent manner (10 and 15 mg/kg, Table 2). ED50 values for anti-ulcer activity (ulcer index) of CGP 12177A and SR 58611A in WIRS-induced gastric ulcer model were found to be 10.25 and 10.48 mg/kg respectively.

Gastric wall mucus content was significantly higher in the CGP 12177A and SR 58611A treated group at 15 mg/kg dose as compared to controls. At 10 and 15 mg/kg doses, a significant rise in the mast cell count was observed with both the compounds (Table 2).

Discussion

The experimental stress ulcer may be considered equivalent to clinical stress ulcer which occurs after surgery, head injury or shock. An acute gastric hemorrhagic lesion in the glandular stomach characterizes a stress ulcer.13 The present study shows anti-ulcer activity of β1-adrenoceptor agonists (CGP 12177A and SR 58611A) which was evident from a significant decrease in the ulcer index at 10 and 15 mg/kg doses in a WIRS-induced gastric ulcer model.

The centrally-induced vascular disturbance of mucosal capillaries is being implicated in restraint-induced gastric
bleeding. β3-adrenoceptor agonists can cause enhancement in antral gastric mucosal blood flow (GMBF) in rats. The insignificant decrease in score of intensity of intraluminal bleeding caused by β3-adrenoceptor agonists in the present study can be partly attributed to their ability to enhance antral GMBF. The specific pathophysiologic mechanism involved in stress-induced ulcers could be ultimate multifactorial impairment of mucosal defense system. An increase in gastric acid secretion, reduction of gastric mucus and alteration in the microvasculature of the gastric mucosa play a major role in the pathogenesis of stress-induced ulcers. Our earlier study has shown that the mechanism of the anti-ulcer action of β3-adrenoceptor agonists in the pylorus ligation model is partly attributed to a decrease in acid secretion. β-adrenoceptor agonists are known to inhibit the release of histamine. Histamine has been known to induce gastric acid secretion mainly through H2-receptor activation. Gastrin-stimulated and cholinergically-mediated acid secretions require a background release of histamine from mast cells for their maximal effects. Thus any agent that reduces the release of histamine from mast cells should suppress acid secretion. Therefore, the effect of β3-adrenoceptor agonists on mucin activity and mast cell counts was also studied in the present study. In the WIRS-induced gastric ulcer model, enhanced mucin activity (GWMC) and increase in mast cell counts (i.e., decrease in mast cell degranulation) caused by CGP 12177A and SR 58611A may explain the antiulcer action of β3-adrenoceptor agonists.

In conclusion, our study shows significant gastroprotective activity of β3-adrenoceptor agonists against WIRS-induced gastric ulcer model. The mechanism for antiulcer action is attributed to the enhancement of mucin activity and a decrease in mast cell degranulation.

Acknowledgements

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References

7. Paul A, Santani DD. Preliminary study on antiulcer effect of β3-adrenoceptor agonists.

Table 1

Effect of water immersion plus restraint stress (WIRS) on ulcer index, gastric wall mucus content (GWMC) and mucosal mast cell count in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index</th>
<th>GWMC (mg Alcian blue/ g wt. of glandular tissue)</th>
<th>Mast cell counts per 42 o.i.f.</th>
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<tr>
<td>No stress</td>
<td>0.02 ± 0.02</td>
<td>1.85 ± 0.14</td>
<td>74.13 ± 5.04</td>
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<tr>
<td>WIRS- stress</td>
<td>1.42 ± 0.22*</td>
<td>0.48 ± 0.07*</td>
<td>23.38 ± 3.14*</td>
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</table>

*P<0.001 when compared with the No stress group (Student’s ‘t’ test). The values are mean ± SEM, n=8 in each group.

Table 2

Effect of CGP 12177A and SR 58611A on WIRS-induced gastric ulceration in rats

<table>
<thead>
<tr>
<th>Pretreatment (p.o.)</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>GWMC (mg Alcian blue/ g wt. of glandular tissue)</th>
<th>Mast cell counts per 42 o.i.f.</th>
<th>SI of intraluminal bleeding</th>
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<tr>
<td>Control</td>
<td>Saline 0.5 ml/100 g</td>
<td>1.42 ± 0.22</td>
<td>0.48 ± 0.07</td>
<td>23.38 ± 3.05</td>
<td>2.13 ± 0.29</td>
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<td>CGP 12177A</td>
<td>5</td>
<td>0.92 ± 0.13</td>
<td>0.58 ± 0.07</td>
<td>35.38 ± 3.20</td>
<td>1.63 ± 0.26</td>
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<td>CGP 12177A</td>
<td>10</td>
<td>0.69 ± 0.12*</td>
<td>0.68 ± 0.08</td>
<td>49.25 ± 2.96*</td>
<td>1.50 ± 0.19</td>
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<tr>
<td>CGP 12177A</td>
<td>15</td>
<td>0.48 ± 0.10*</td>
<td>0.89 ± 0.07*</td>
<td>59.50 ± 3.26*</td>
<td>1.50 ± 0.19</td>
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<tr>
<td>SR 58611A</td>
<td>5</td>
<td>0.99 ± 0.15</td>
<td>0.62 ± 0.09</td>
<td>36.25 ± 3.70</td>
<td>1.50 ± 0.27</td>
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<tr>
<td>SR 58611A</td>
<td>10</td>
<td>0.68 ± 0.11*</td>
<td>0.75 ± 0.08</td>
<td>51.88 ± 3.32*</td>
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<td>SR 58611A</td>
<td>15</td>
<td>0.49 ± 0.10*</td>
<td>1.07 ± 0.10*</td>
<td>56.50 ± 4.28*</td>
<td>1.50 ± 0.19</td>
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One-way ANOVA

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<tr>
<th>F</th>
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<td>5.95</td>
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<tr>
<td>6.49</td>
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<td>6.49</td>
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*P<0.05 when compared with the control group (Dunnett’s test). The values are mean ± SEM, n=8 in each group.

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