**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

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**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. 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.

Espluges et al. 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. β₃-adrenoceptor agonists inhibit gastric ulcer-induced by indomethacin, pylorus ligation and ethanol 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 as-pects 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-
cholorphenyl)-2-hydroxyethylamino[ 5.6.7.8-tetrahydro-
naphthalene-2-olxy] 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 β₂-adrenoceptor agonists were selected on the basis of
ED₅₀ values of BRL 35135, a β₂-adrenoceptor agonist on
indomethacin-induced ulceration and total acid-output in py-
lorus-ligated rats.³

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.² After wiping the blood, the ulcer index was determined⁹
and the stomach tissue was subjected to mast cell examina-
tion 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 buffer 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 vol-
ume of ether until an emulsion was formed. This was centri-
fuged at 3600 rpm for 10 min. Ether was pipetted out and
discarded, and the concentration of Alcian blue was deter-
mined in the aqueous layer. Color absorbance was recorded
using a spectrophotometer (Shimadzu) at 598 nm. The quan-
tity of Alcian blue extract per g wet glandular tissue was cal-
culated 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 Follow-
ing 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 spec-
imens 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, dehy-
drated 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 v1.0
software.

Results

Severe hemorrhagic gastric glandular mucosal ulcers were
observed in stress-induced control animals (Table 1). Signifi-
cant 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 β₂-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 antiulcer 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 signifi-
cant rise in the mast cell count was observed with both the
compounds (Table 2).

Discussion

The experimental stress ulcer may be considered equiva-
ient 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 β₂-adrenoceptor agonists
(CGP 12177A and SR 58611A) which was evident from a sig-
nificant 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 significant 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 antulcer 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 antulcer 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 ant ulcer effect of β₃-adrenoceptor

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index (g wt. of glandular tissue)</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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<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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*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

<table>
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<tr>
<th>Pretreatment (p.o.)</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index (g wt. of glandular tissue)</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>
</tr>
</thead>
<tbody>
<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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<tr>
<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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<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>
<td>1.75 ± 0.25</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

*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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