Antiulcer and Gastric Acid Inhibitory Activity of ZD 7114, A β_3-Adrenoceptor Agonist in Rats

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The antiulcer activity of ZD 7114, a β_3-adrenoceptor agonist was studied in aspirin plus pylorus ligation-induced gastric ulcer in rats and its effect on continuous acid secretion was also studied against *in vivo* as well as *in vitro* models. For comparison, the effect of isoprenaline and salbutamol was studied simultaneously in all experimental models. In gastric ulcer model, β-adrenoceptor agonists at all doses were found to be highly effective in affording gastroprotection. ZD 7114 treatment caused significant reduction in acid secretion in *in vivo* model whereas no significant change was observed in *in vitro* model of continuous acid secretion. In contrary to this no significant change in acid secretion was observed by treatment with isoprenaline and salbutamol in *in vivo* model and a significant rise in acid secretion in *in vitro* model.

Several lines of evidence suggests that vasodilator activity of isoproterenol, in addition to gastric acid secretory inhibition due to beta-adrenergic stimulation protects the gastric mucosa from experimental ulcerations[1]. β-Adrenoceptor agonists have been reported to inhibit gastric mucosal lesions in rats induced by immobilisation and indomethacin, salicylates or reserpine administration[2]. A range of β_3-adrenoceptor agonists were found to inhibit indomethacin-induced gastric antral ulcer in rats[3]. β-Adrenoceptor activation in many experimental models has been reported to increase[4] as well as decrease[5-7] gastric acid secretion. A range of β_3-Adrenoceptor agonists were found to inhibit gastric acid secretion[8,9]. It has been unclear as to why stimulation of β-adrenoceptors should give such divergent response on gastric acid secretion. Although authentic reports are available for selective β_3-adrenoceptor agonists as gastroprotective agents, the importance of alteration of acid secretion in gastroprotection by β_3-adrenoceptor

agonists remains unclear. The present study was undertaken to study the role of β_3-adrenoceptors on gastric acid secretion and in protection against experimentally induced-gastric ulcer models.

MATERIALS AND METHODS

Aspirin plus pylorus ligation-induced gastric ulcer model[10]:

Wistar rats of either sex weighing about 200-250 g were used. Aspirin was suspended in 1% CMC in water and administered orally at a dose of 200 mg/kg in non-fasted rats once daily for five days. Drug treatment was carried out 30 min prior to aspirin treatment on each day. On the sixth day pylorus ligation was carried out on 12 h fasted rats under light ether anaesthesia. The sixth dose of drug was given 30 min prior to pylorus ligation. Four hours after the pylorus ligation, animals were killed with a high dose of anesthetic ether. Ulcer Index was determined[11]. Gastric juice was subjected to analysis of total acidity, total acid output, pepsin activity[12], total carbohydrates (TC)[13] and protein content (PR)[14]. Finally the ratio

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of total carbohydrates to protein content (TC/PR) was derived.

Continuous recording of gastric acid secretion in rat:

Gastric acid secretion was measured continuously in rats as described by Ghosh and Schild[15]. The stomach was perfused continuously at a uniform rate of 0.2 ml/min with N/10000 NaOH by using a continuous flow injector, NO ME 5855, INCO. Total acid output was determined in 30 min fractions of gastric perfusate. Drugs were administered by slow intravenous infusion through cannulated external jugular vein.

Continuous gastric acid secretion in isolated lumen-perfused stomach of rats:

Gastric acid secretion was measured in isolated lumen-perfused stomach of rats as described previously for mice[16]. The total acid output was determined in 15 min fractions of gastric perfusate. The nutrient solutions were prepared as described by Szelenyi[17]. Test drugs were added to serosal nutrient solution. ZD 7114 and isoprenaline were added to the serosal solution so that final concentration in organ bath was 1 µM and salbutamol at 10 µM. Before addition of a test drug a period of 30 min was allowed for basal acid secretion.

Analysis of results:

The results were expressed as mean±SEM. Results of drug treatment groups were compared with those of control group and were analysed for significant statistical difference by using one-way ANOVA followed by Dunnett's test at a level of significance $P<0.05$. The results of SR 59320A pretreatment group (SR 59320A+ZD 7114) was compared with group receiving ZD 7114 treatment alone.

RESULTS

Aspirin plus pylorus ligation-induced gastric ulcer model:

Isoprenaline, salbutamol and ZD 7114 showed

TABLE 1: EFFECT OF β-ADRENOCEPTOR AGONISTS ON ULCER INDEX AND ACID SECRETORY PARAMETERS

Treatment	Dose (p.o.) mg/kg	Ulcer Index	Volume of Gastric Content (ml/100 g/4 h)	Total Acidity (mEq/1/4 h)	Total Acid Output (µEq/100 g/4 h)	Pepsin Activity (µg/ml/4 h)
Control	—	0.75±0.06	2.45±0.24	56.28±6.27	128.41±10.23	41.36±3.74
Isoprenaline	5	0.40±0.06*	1.6±0.12	37.0±3.02	58.75±5.55*	28.13±4.20
Isoprenaline	10	0.35±0.04*	1.73±0.24	36.22±4.30	64.03±13.04*	28.69±3.09
Isoprenaline	15	0.27±0.04*	1.6±0.16	46.43±2.25	74.43±9.16*	31.27±3.71
Salbutamol	5	0.51±0.07	1.72±0.12	55.78±7.21	95.67±13.32	32.11±4.26
Salbutamol	10	0.42±0.07*	2.37±0.23	36.3±2.74	84.66±9.86*	25.53±2.02
Salbutamol	15	0.37±0.05*	1.98±0.15	38.27±3.64	75.11±7.44*	28.57±3.85
ZD 7114	5	0.40±0.06*	1.90±0.22	52.73±7.01	95.26±13.5	39.09±4.65
ZD 7114	10	0.24±0.04*	1.92±0.27	39.53±3.21	72.73±8.47*	33.33±4.77
ZD 7114	15	0.23±0.04*	1.97±0.26	40.97±2.65	76.77±5.35*	34.64±3.23
SR 59320A + ZD 7114	5+10	0.59±0.06$	2.15±0.12	67.25±3.41$	144.01±9.61$	32.33±3.82

Six rats were used in each group and all values are in mean±SEM. *represents significant difference when compared with control group at $P<0.05$ and$ represents significant difference when compared with ZD 7114 (10 mg/kg) treatment group at $P<0.05$. Ulcer index and acid secretory parameters were determined in aspirin plus pylorus ligation-induced gastric ulcer model in rats.

significant reduction in ulcer index at all doses except salbutamol at 5 mg/kg. Treatment with isoprenaline caused a decrease in total acid output at all doses tested when compared to control group. In comparison to that of isoprenaline treatment, salbutamol and ZD 7114 both were found to be less potent as these drugs caused significant reduction total acid output only at 10 and 15 mg/kg. Isoprenaline, salbutamol and ZD 7114 caused an apparent reduction in volume of gastric content, total acidity and pepsin activity all doses but these were not found to be statistically significant when compared with control group (Table 1). Although at all doses total carbohydrate content remained unaltered in all drug treatment groups, the significant reduction in protein content at higher doses, i.e., 10 and 15 mg/kg in isoprenaline and ZD 7114 treated group lead to significant increase in TC/PR ratio at same dose levels when compared with control group (Table 2).

In this model the gastroprotective effect of ZD 7114 was studied in the presence SR 59320A (β_3-adrenoceptor antagonist) pretreatment. Administration of SR 59320A at 5 mg/kg, po, 30 min prior to ZD 7114 treatment prevented the gastroprotective effects of ZD 7114 (10 mg/kg). Pretreatment of SR 59320A significantly inhibited the ZD 7114-induced reduction in ulcer index and total acid output when compared with ZD 7114 treated group (Table 1). Although SR 59320A pretreatment antagonized ZD 7114-induced reduction in protein content of gastric juice, it was not found to be statistically significant ($P>0.05$). Similarly, reversal of ZD 7114-induced rise in TC/PR ratio caused by SR 59320A pretreatment was also not found to be significant when compared with ZD 7114 treated group (Table 2).

**Continuous recording of gastric acid secretion in rats:**

Under resting condition, acid secretions were stable and remained at plateau level. ZD 7114 (5 mg/kg) caused significant decrease in total acid output (8.17 ± 1.35 μEq/100 g/30 min) rapidly within 30 min of drug administration when compared with control group (16.75 ± 1.12 μEq/100 g/30 min). It was also observed that the significant decrease in total acid output in these treatment groups remained significant till the end of experiment, i.e, 90 min. In contrast to this, isoprenaline (5 mg/kg) and salbutamol (10 mg/kg) treatment caused a slight increase in total acid output. However, it was not found to be

TABLE 2: EFFECT OF β-ADRENOCEPTOR AGONISTS ON MUCOPROTECTIVE PARAMETERS

Treatment	Dose (p.o.) mg/kg	Total Carbohydrates (μg/ml/4 h)	Protein Content (μg/ml/4 h)	TC/PR Ratio
Control	—	340.23±11.27	83.15±2.07	4.10±0.35
Isoprenaline	5	337.25±10.33	71.9±3.45	4.76±0.26
Isoprenaline	10	349.33±12.60	65.68±3.44*	5.39±0.31*
Isoprenaline	15	353.98±9.93	65.72±3.25*	5.48±0.34*
Salbutamol	5	333.88±19.31	73.27±4.86	4.62±0.25
Salbutamol	10	347.54±15.59	70.35±5.74	5.04±0.23
Salbutamol	15	335.43±14.68	68.51±3.39	4.91±0.13
ZD 7114	5	345.04±12.42	70.71±3.31	4.94±0.27
ZD 7114	10	339.75±15.22	65.44±3.92*	5.22±0.13*
ZD 7114	15	343.11±14.04	63.17±3.13*	5.46±0.18*
SR 59320A + ZD 7114	5+10	329.21±3.82	78.15±4.87	2.26±0.18

Six rats were used in each group and all values are in mean±SEM. *represents significant difference when compared with control group at $P<0.05$. Total carbohydrates, protein content and total carbohydrates to protein content (TC/PR) ratio were determined in aspirin plus pylorus ligation-induced gastric ulcer model in rats.

statistically significant at all time intervals (fig. 1), SR 59320A pretreatment at dose of 5 mg/kg was found to be effective in reversing the ZD 7114-induced reduction in total acid output significantly (16.5±1.98 µEq/100 g/30 min) within 30 min of neutral red administration when compared with ZD 7114 treated group (8.17±1.35 µEq/100 g/ 30 min). The antagonistic effect of SR 59320A was not abolished till the end of experiment (fig. 1).
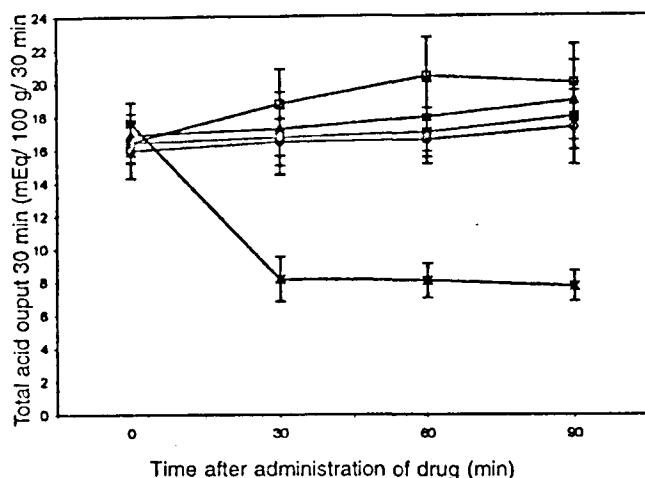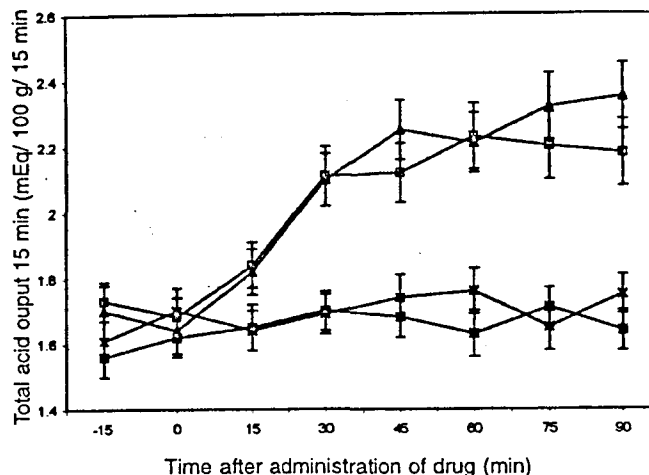

Fig. 1: The effect of beta-adrenoceptor agonists on total acid output *in vivo*

Six rats were used in each group and all values are in Mean±SEM.* represents significant difference when compared with control group at P<0.05. Total acid output was determined in 30 min-fractions of gastric perfusate collected in continuous acid secretion *in vivo* model in rats. Key: ◻ Control, x ZD 7114 (5 mg/kg), ▲ Isoprenaline (5 mg/kg) (◻) Salbutamol (10 mg/kg), (○) SR 59320A (5 mg/ kg) + ZD 7114 (5 mg/kg)

Continuous acid secretion in lumen-perfused isolated stomach of rats:

In control group the basal acid secretion remained apparently at a plateau level. Treatment with ZD 7114 caused no significant change (P>0.05) in total acid output even after 90 min of drug treatment. Whereas in contrast to this, isoprenaline and salbutamol treatment caused a significant rise in total acid output after 30 min of drug administration which gradually reached at a plateau level, viz., isoprenaline 1µM (2.35±0.10 µEq/15 min) and salbutamol 10 µM (2.23±0.10 µEq/15 min), control value being 1.64±0.06 µEq/15 min) (fig. 2).

Time after administration of drug (min)

Fig. 2: The effect of beta-adrenoceptor agonists on total acid output *in vitro*

Six rats were used in each group and all values are in Mean±SEM.* represents significant difference when compared with control group at P<0.05. Total acid output was determined in 15 min-fractions of gastric perfusate collected in lumen perfused isolated stomach *in vitro* model in rats. Key: ◻ Control, x ZD 7114 (1µM), ▲ Isoprenaline (1 µM), ◻ Salbutamol (10 µM).

DISCUSSION

Gastric mucosa is highly innervated with adrenergic fibres and it has been postulated that adrenergic system plays an important function in gastrointestinal tract[18]. Among the aggressive factors, it is generally accepted that increased gastric acid secretion is one of the important factors causing gastric erosion.

Aspirin plus pylorus ligation-induced gastric ulcer model was selected in order to evaluate the β-adrenoceptor agonists gastric secretion as well as gastric ulcers because aspirin alone does not allow to study the effect of drugs on gastric acid secretion. Moreover, in this model the gastric ulcers appeared rapidly in the stomach wall. All β-adrenoceptor agonists have showed significant antiulcer activity in this model. Similar gastroprotective effect with treatment of β-adrenoceptor agonists such as isoprenaline and salbutamol was also shown by studies of Espluges et al[1]. It was also shown that isoprenaline afford gastroprotection against ethanol-induced gastric damage via stimulation of β_2-adrenoceptors[1]. The effect of β_1-adrenoceptor agonist was not studied in the present study, as the involvement of β_1-adrenoceptors in affording protection against gastric ulceration was ruled out[3].

In the present study isoprenaline was found to be more potent in inhibiting the total acid output although all β-adrenoceptor agonists caused significant reduction in total acid output in aspirin plus pylorus ligation-induced gastric ulcer model. Normally, it is observed that the drugs in the same way affect pepsin and gastric acid secretion[19]. But in contrary to it all β-adrenoceptor agonists showed alteration in acid secretion without affecting the pepsin activity in this model. On the basis of this observation, it appears that β-adrenoceptors are involved only in acid secretory activity of GIT but not in pepsin activity. In earlier studies both stimulant[20] and inhibitory effect[19] on pepsin activity was observed by treatment of β-adrenoceptor agonists. Therefore, the antiulcer effect of β-adrenoceptor agonists appears to be produced at least in part by the suppression of acid secretion. The antisecretory role of β$_3$-adrenoceptors in affording gastroprotection was also substantiated by the observation that β$_3$-adrenoceptor antagonist, SR 59320A blocked the ZD 7114-induced gastric acid inhibitory and antiulcer actions in aspirin plus pylorus ligation-induced gastric ulcer model. Role of β$_3$-adrenoceptors in secretion of gastric acid was also suggested by earlier studies, where β$_3$-adrenoceptor agonists were found to inhibit gastric acid secretion[9,10].

In aspirin plus pylorus ligation-induced gastric ulcer model, the mechanism for the protective effect may not be solely through the inhibition in acid output as a number of other mechanisms are also responsible for the development of gastric mucosal damage. One of such essential criteria is the state of mucus secretion to the status of mucosal resistance/barrier[21]. Therefore, the effect of β-adrenoceptor agonists on soluble mucosubstances such as total carbohydrates and proteins in gastric juice was also studied. The mechanism for decrease in protein content found in our study appears to be atleast partly, due to prevention of leakage of proteins from serum to gastric juice[22]. Based upon the results of TC and PR content of gastric juice, the TC/PR ratio was estimated. The effect of an agent on mucin activity is reflected by its effect on TC/PR ratio[13]. In the present model, increased mucin activity caused by β$_3$-adrenoceptor agonists may explain the antiulcer action of these drugs. However, the inability of antagonising the ZD 7114 induced rise in TC/PR ratio by SR 59320A pretreatment shows non-involvement of β$_3$-adrenoceptors in improvement of gastric mucus.

In contrary to no significant change in acid secretion caused by isoprenaline and salbutamol in continuous acid secretion *in vivo* model, it was found that these drugs caused significant rise in acid secretion in *in vitro* model. At the same time ZD 7114 caused significant reduction in acid secretion in *in vivo* model, whereas, no significant change in *in vitro* model. This observation suggests that the direct effect of β-adrenoceptor stimulation on the parietal cells of the stomach is that of acid stimulation, whereas the acid inhibition is indirect. Therefore, it can be said that the β$_3$-adrenoceptor agonists is devoid of any direct effect on parietal cells to cause a increase in gastric acid secretion and the resultant effect is profound decrease in gastric acid secretion through an indirect action on stomach. Whereas the insignificant change in gastric acid secretion caused by isoprenaline and salbutamol treatment in intact rat model is due to resultant net effect of these direct and indirect effects on stomach. This observation was found to be in agreement with earlier studies[4,9]. Further, this was also substantiated by significant antagonistic effect of ZD 7114-induced reduction in total acid output by SR 59320A pretreatment found in our study.

In conclusion, our study shows a strong evidence for the presence of β$_3$-adrenoceptors in the microvasculature of the stomach and the antiulcer effect of β$_3$-adrenoceptor agonists in gastric ulcers is mediated through these receptors. The mechanism of action of β$_3$-adrenoceptor agonists can be attributed to the inhibition of acid secretion.

REFERENCES

1. ‘ Howard, T.J., Passaro, E. Jr. and Guth, P.H., **Dig. Dis. Sci.,** 1993, 38, 1201.
2. Kekes-Szabo, A., In; Mozsik, G. and Javer, T. Eds., Progress in Peptic Ulcer, Akademia Kiado, Budapest, 1976, 433.
3. Bahl, A.K., Clayton, N.M., Coates, J., Martin, D.P., Ookley, I.G., Strong, P. and Trevethick, M.A., **Brit. J. Pharmacol.,** 1996, 117, 580.
4. Yokotani, K., Park, J., DelValle, J. and Yamada, T., **Digestion,** 1994, 55, 148.
5. Bass, P. and Patterson, M.A., **J. Pharmacol. Exp. Ther.** 1967, 156, 142.
6. Curwain, B.P. and Holton, P., **Brit. J. Pharmacol.,** 1972, 46, 225.
7. Daly, M.J., Long, J.M. and Stables, R., **Brit. J. Pharmacol.,** 1978, 63, 376.
8. Kuratani, K., Kodama, H. and Yamaguchi, I., **J. Pharmacol. Exp. Ther.** 1994, 270, 559.

9. Coruzzi, G. and Bertaccinni, G., **Naunyn Schmiedebergs Arch. Pharmacol.,** 1997, 356, 263.
10. Goel, R.K., Chakrabarti, A. and Sanyal A.K., **Planta Med.,** 1985, 2, 85.
11. Ganguli, A.K. and Bhatnagar, O.P., **Can. J. Physiol. Pharmacol.,** 1973, 51, 748.
12. Debnath, P.K., Gode, K.D., Das, G. and Sanyal, A.K., **Brit. J. Pharmacol.,** 1974, 51, 213.
13. Sanyal, A.K., Mitra, P.K. and Goel, R.K., **Indian J. Exp. Biol.,** 1983, 21, 78.
14. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., **J. Biol. Chem.,** 1951, 193, 265.
15. Ghosh, M.N. and Schild, H.O., **Brit. J. Pharmacol.**

16. Black, J.W. and Shankley, N.P., **Brit. J. Pharmacol.,** 1985, 86, 571.
17. Szelenyi, I., **Arzneim. Forsch - Drug Res.,** 1981, 31, 998.
18. Sanders, J.J., **Agents Actions,** 1976, 6, 385.
19. Espluges, J., Lloris, J.M., Marti-Bonmati, E. and Morcillo, E.J., **Brit. J. Pharmacol.,** 1982, 76, 587.
20. Holton, P., In; International Encyclopaedia of Pharmacology & Therapeutics, Oxford, Pergamon, 1973, Section 39a, 1.
21. Blum, A.L., **Amer. J. Med.,** 1985, 79, 8.
22. Wallace, J.L. and Whittle, B.J.R., **Gastroenterology,** 1986, 91, 603.

Chemother., 1958, 13, 54.