Prevention of duodenal ulcers in the rat using a combination of ranitidine and sucralfate in subtherapeutic doses

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SUMMARY This study investigated whether ulcer prevention would be greater with the combined use of an acid-inhibiting agent, ranitidine, given together with a mucosal-protective agent, sucralfate. Duodenal ulcers were induced in rats with the secretagogues pentagastrin and bethanechol. Subtherapeutic doses of ranitidine (5 mg/kg/6 hours) and sucralfate (50 mg/6 hour) yielded an ulcer index of 4.0 and 4.1 respectively, not significantly different from the control (untreated) ulcer index of 4.3. Therapeutic doses of ranitidine (20 mg/kg) and sucralfate (200 mg/animal) gave an ulcer index of 0.4 and 0.5 respectively. Subtherapeutic doses of ranitidine and sucralfate given in combination yielded an ulcer index of 0.7. Thus, subtherapeutic doses of ranitidine and sucralfate given in combination had a synergistic effect equal to therapeutic doses of each of these drugs given alone. The therapeutic implications of combined acid inhibiting drugs with mucosal protective drugs is discussed.

The time honoured equation of ulcer disease acid-pepsin aggression vs mucosal resistance remains the mainstay of the therapeutic approach to ulcer disease. H₂ histamine receptor antagonists and the mucosa-protective, or cytoprotective agents have been shown to promote ulcer healing in man. They also prevent ulcer formation by a large variety of ulcer-inducing techniques in animals.¹⁻³ There have been few investigations whether ulcer prevention would be greater with the combined use of an acid-inhibiting agent with a mucosal-protective agent. The effect of ranitidine, a histamine H₂ receptor antagonist and sucralfate, a mucosal-protective agent, were investigated alone to establish therapeutic and subtherapeutic levels in an ulcer model; the combination of these drugs at subtherapeutic levels was then studied in the same animal model.

Methods

ANIMALS

Seventy six female Wistar rats weighing between
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170-200 g were fasted for 24 hours before the experiment. Duodenal ulceration was induced by a method previously described.⁴ Briefly, this consisted of the subcutaneous infusion of pentagastrin 2.5 µg/kg/min, and bethanechol 12 µg/kg/min, for 24 hours through a needle inserted between the shoulder blades. The rats were allowed reasonable mobility in cages 25×5×5 cm. Five sets of experiments were done.

1 Pilot study

Six rats were used to test the stress related ulcerogenic effect of the experimental system. These animals received a 24 hour subcutaneous infusion of saline in place of pentagastrin or bethanechol and received no oral medication during the time they were placed in the restraining cages.

2 Control group

Nine rats received 24 hour infusion of pentagastrin and bethanechol and were given saline via intragastric feeding tube at the start of the infusion and at three subsequent occasions six hours apart. This tested the efficacy of the ulcerogenic protocol.

3 Sucralphate group

Twenty seven rats were divided into three groups of nine. All the groups received sucralphate at the start of the infusion and every six hours thereafter. Sucralphate was crushed and given as a suspension in saline *via* an intragastric feeding tube. Group one received 50 mg per dose as a 10% suspension. Group two received 100 mg per dose as a 10% suspension. Group three received 200 mg per dose as a 20% suspension (20% suspension was used to decrease the total volume administered).

4 Ranitidine group

Twenty seven rats were divided into three groups of nine. Each of these groups received ranitidine at the start of the infusion and every six hours thereafter *via* an intragastric feeding tube. The first group received ranitidine at a dose of 5 mg/kg as a 0.25% suspension in saline. Second group received 10 mg/kg as 0.5% suspension and the third received 20 mg/kg as a 1% suspension.

5 Sucralphate and ranitidine group

Nine rats received 50 mg of sucralphate as a 10% suspension at the start of the infusion and every six hours thereafter. In addition, ranitidine was given at 5 mg/kg as a 0.25% suspension in saline one hour after each of the sucralphate doses. Both were given by intragastric feeding tubes.

At the end of the 24 hours the rats were killed with intracardiac pentobarbitol and the stomach and duodenum dissected; the duodenal mucosa was examined with a 10 $\times$ magnifying hand lens by three observers (of which one (SB) was blinded) for the presence of ulceration. The severity of the ulcer was scored as follows: 0=no ulcer, 1=superficial erosions, 2=black based ulcer, 3=penetrating, or perforating ulcer. The ulcer index was calculated by the following formula: Index=mean severity+(incidence $\times$ 2.)⁵ The severity of ulceration was averaged between the three observers, there was little difference between observers as the grading was distinct. The ulcer index scores were analysed by the two tailed Mann Whitney test. The gastric pH was estimated with a mini-surface electrode (Beckman pH meter, model 3500) but formal acid and pepsin studies were not done as this was not the intention of this study.

Results

1 Pilot study

No ulcers, or erosions were found in any of the six rats used in the pilot study. These results verify that the drug free experimental protocol was itself not ulcerogenic.

2 Control group

All nine control rats developed duodenal ulcers of marked severity, with an ulcer index of 4.3. The pH range was 1.2–1.7.

3 Sucralphate group

The effect of varying doses of sucralphate on the incidence of ulcers and ulcer index is shown in Figure 1. The 50 mg dose was almost ineffective in preventing ulceration, eight of the nine rats having severe ulcers, not significantly different from controls ($p<0.1$) with a mean ulcer index of 4.1. The gastric mucosa had a pH range of 1.0–1.7.

In contrast with the 50 mg dose, the 100 mg, and 200 mg doses had an increasing protective effect on ulcer formation.

Only one of nine animals had an erosion after the 200 mg dose. This was significantly different from controls ($p<0.02$). The mucosa had a pH range of 1.0–3.0.

4 Ranitidine group

All rats at the 5 mg/kg dose developed ulceration (Fig. 2) with the mean ulcer index of 4.0, not significantly different ($p<0.1$) from the control group. The mucosal pH with the 5 mg/kg dose was virtually unchanged from the control group (with a pH range of 1.2–1.7).

In contrast with the 5 mg/kg dose, the 10, and 20 mg/kg doses had an increasingly inhibitory effect on ulcer formation. Only two animals had ulceration after the 20 mg/kg dose, with an ulcer index of 0.4.

Fig. 1 Effect of sucralphate 50 mg, 100 mg, and 200 mg four times a day on the incidence and ulcer index of secretagogue-induced duodenal ulcer. (Sucralphate vs control, 200 mg and 100 mg $p<0.001$ and 50 mg=NS).

Fig. 2 Effect of ranitidine 5 mg/kg, 10 mg/kg and 20 mg/kg four times a day on the incidence and ulcer index of secretagogue-induced duodenal ulcer. (Ranitidine vs control, 20 mg/kg $p < 0.001$ 10 mg/kg $p < 0.01$, and 5 mg = NS).

Fig. 3 Effect of subtherapeutic doses – that is 50 mg sucralfate four times a day and 5 mg/kg ranitidine four times a day, on the incidence and ulcer index of secretagogue-induced duodenal ulcer. (Pentagastrin/bethanechol vs sucralfate NS; vs combined sucralfate/ranitidine $p < 0.001$.)

This was significantly different ($p < 0.002$) from control. The gastric mucosa had a pH range of 3–7.

5 Sucralfate and ranitidine group

Doses of 50 mg sucralfate and 5 mg/kg ranitidine were subtherapeutic, in that they were associated with near 100% ulcer incidence and an ulcer index not significantly different from controls. When given in combination, the incidence of ulcer was decreased to 22% (two of nine rats), with an ulcer index of 0.7. This was significantly less than controls ($p < 0.002$). In fact, of the two animals showing ulceration the latter were no more than erosions. The data are presented in Figure 3. The mucosal pH with this combination of drugs was only slightly higher, pH range 1.9–3.1, when compared with controls, or when the drugs were given individually.

Discussion

This study showed that histamine H_2 antagonists and sucralfate prevent the formation of pentagastrin-bethanechol induced duodenal ulceration in the rat, and that the protective effect was dose dependent. Virtually complete protection from ulceration was recorded when the drugs were given together in doses that failed to prevent ulcer formation when they were given alone. Thus, 'subtherapeutic' doses given in combination had a

synergistic protective effect equal to therapeutic doses of each of the drugs when they were given separately.

The mechanism of ulcer prevention with the two drugs differs. Ranitidine protects the duodenum from the acid load induced by pentagastrin and bethanechol by inhibiting acid output.⁶ Sucralfate protects the mucosa by forming a mucosal protective layer, or sealing micro-ulcers by binding with juxtamucosal, or ulcer proteins;^{3,7} although the data⁷ refer to gastric mucosa, and not to duodenal mucosa as in the present study. Sucralfate has little effect on intragastric pH. It is therefore reasonable to conclude that the drugs act at the two ends of the ulcer equation, ranitidine on the aggressive factors and sucralfate on the defensive side. Although both drugs affect peptic activity, ranitidine by raising the intragastric pH and sucralfate by a binding to pepsin, the inactivation of peptic activity was ineffective in preventing ulcer formation when the drugs were given alone at the subtherapeutic doses. The mechanism of the synergism is unclear as the study was not designed to include acid-pepsin measurements.

Whether the findings in this experimental study bear any relevance to therapy in man is uncertain. This study, however, appears to present the first experimental evidence of the possible beneficial effect of combining acid-inhibitory and mucosal protective agents. At present there is no good

evidence to show that combining such agents in therapeutic doses increases ulcer healing or preventive potential above that of the single agents in established therapeutic doses. As the toxicity and adverse effects of the many ulcer preparations presently being used are clearly related to potency and dose,⁸ the combined use of subtherapeutic doses may be of therapeutic importance.

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