Gastrocytoprotection by colloidal bismuth subcitrate (De-Nol) and sucralfate. Role of endogenous prostaglandins

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SUMMARY This study compares the gastroprotective effects of colloidal bismuth subcitrate (De-Nol) with those of sucralfate and a methylated analogue of prostaglandin E₂ (PGE₂) against acute gastric lesions induced by acidified aspirin and absolute ethanol in rats. Both De-Nol and sucralfate given orally prevented dose dependently the formation of gastric lesions by these ulcerogens, De-Nol being, respectively, twice and seven times more potent, on a weight basis, than sucralfate. As the gastroprotective activities of both De-Nol and sucralfate on ethanol lesions can be reversed by pretreatment with indomethacin and as De-Nol and sucralfate increase the mucosal generation and luminal release of PGE₂, we postulate that mucosal prostaglandins may be involved in the mechanism of action of these drugs on the gastric mucosa.

Bismuth compounds have been used for over two centuries for the treatment of various gastrointestinal disorders because of their local protective, demulcent, and antacid properties. The more recently developed colloidal bismuth subcitrate (De-Nol), known also as CBS, offered a new approach in peptic ulcer therapy because of its selective binding to the ulcer base, the protection against acid-pepsin attack and its activity against pyloric campylobacter.^{1–5}

De-Nol was reported to protect the gastric mucosa against various ulcerogens in animals^{1,2} and to be effective in promoting the healing of gastric⁶ and duodenal ulcers⁷ in man. Moreover, ulcer relapse has been shown to be reduced after healing with De-Nol compared with healing with H₂-antagonists.^{8,9} The mechanism of these effects has not been fully elucidated. Because prostaglandins are known to exhibit both anti-ulcer and protective activities¹⁰ and sucralfate is considered to be a standard protective agent in experimental animals^{11,12} and effective drug in the healing and the reduction of the recurrency of peptic ulcer in man,^{13,14} we decided to compare the gastroprotective effects of De-Nol and sucralfate with those

of prostaglandins and to determine the influence of these agents on mucosal generation and release of prostaglandin.

Methods

GASTRIC SECRETORY STUDIES

The effect of De-Nol, sucralfate and methylated prostaglandin analogue on gastric secretion were studied in conscious rats prepared with a gastric fistula about one month earlier. They were fasted for about 24 hours and then placed in Bollman cages. The cannulas of the fistulas were opened and then the stomachs were washed out with saline. The basal gastric juice was collected for five 30 minute periods. After collecting three control 30 minute samples De-Nol (80 mg/kg), sucralfate (400 mg/kg) or 16,16 dimethyl PGE₂ (10 µg/kg) dissolved in 1 ml water was introduced intragastrically for a 30 minute period, the gastric fistula being closed for 30 minutes. After this interruption the fistula was opened again, the stomach drained for five minutes and this collection discarded. The collection was continued for two 30 minute periods with saline infused sc at a rate of 4 ml/h throughout. Acid and pepsin secretion was measured in each 30 minute sample as described¹⁵ and expressed as outputs/60 minute period after

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Received for publication 3 June 1986.

administration of tested drugs or 1 ml of water (control). All secretory tests were done on the same 10 gastric fistula rats weighing about 250 g.

PRODUCTION OF GASTRIC MUCOSAL LESIONS

Acute gastric ulcerations were induced in Wistar rats (180–200 g bw) by intragastric administration of absolute ethanol or acidified ASA.

Absolute ethanol was administered in 24 hour fasted rats in a volume of 1 ml using a metal orogastric tube.¹⁶ One hour later the animals were killed by a blow to the head, the stomach was removed and the number and area of gastric necrotic lesions were measured planimetrically (Morphomat, Carl Zeiss, Berlin, Germany).

Acidified aspirin was administered in 24 hour fasted rats in a bolus dose of 60 mg/kg followed by a dose of 42 mg/kg/h for a three hour period. Aspirin was dissolved in 0.15 M HCl and instilled intragastrically (through a plastic tube inserted surgically) into the stomach two hours before the start of the experiment as previously described.^{15,17} After three hours of aspirin administration, the animals were killed and the area of all gastric ulcerations was measured planimetrically.

De-Nol (gift from Dr D W R Hall, Medical Department, Gist-brocades, Delft, The Netherlands) was dissolved in water and administered po in doses ranging from 2.5–80 mg/kg about 30 minutes before the start of the administration of absolute ethanol or acidified aspirin. Sucralfate (gift from Dr E Gehrls, E Merck, Darmstadt, GFR) was suspended in water and used in doses ranging from 12.5–400 mg/kg. For comparison, 16,16 dimethyl PGE₂ (gift from Dr J Pike, The Upjohn Co, Kalamazoo, Michigan) was used po in a standard protective doses (10 µg/kg).

MEASUREMENT OF MUCOSAL GENERATION OF PROSTAGLANDIN

The role of endogenous prostaglandin in the protection by De-Nol and sucralfate was examined in two ways: (1) by reversal of their gastroprotective effects by pretreatment with indomethacin (5 mg/kg po) and (2) by directly measuring mucosal generation and luminal release of PGE₂ using radioimmunoassay of PGE₂. Immediately after killing the animals, the abdomen was opened and the stomach was clamped at the cardia and the pylorus. One millilitre of saline was then injected into the stomach and its contents collected for the measurement of luminal release of PGE₂ using commercially available kits (New England Nuclear NEN, Dreieich, Germany). A large biopsy (about 50 mg) of the fundic mucosa was then obtained to determine the capability of the mucosa to generate PGE₂ as previously described.^{15,17}

All experiments were carried out on Wistar rats

Table 1 Effects of De-Nol (80 mg/kg), sucralfate (400 mg/kg) or 16,16 dimethyl PGE₂ (10 µg/kg) given po on gastric acid and pepsin secretion in chronic gastric fistula rats. Mean ± SEM of results obtained from 10 rats

Treatment	Acid output µmol/60 min	Pepsin output mg/60 min
Vehicle control	416 ± 48	3.12 ± 0.52
De-Nol	386 ± 62	2.84 ± 0.42
Sucralfate	406 ± 82	2.48 ± 0.68
16,16 dm PGE ₂	348 ± 64	3.22 ± 0.60

fasted for 24 hours. Each series of experiments was repeated and each experimental group included eight to 10 animals.

STATISTICAL ANALYSIS

All values reported are the means (±SEM). These means were used in the *t* test for paired values to evaluate the significance of differences in gastric secretory output, ulcer area and prostaglandin generation.

Results

EFFECTS OF DE-NOL, SUCRALFATE AND A METHYLATED PROSTAGLANDIN ANALOGUE ON GASTRIC SECRETION

Table 1 shows the effects of the highest dose of De-Nol, sucralfate and 16,16-dimethyl PGE₂, used in the gastroprotective study, on gastric acid and pepsin

Fig. 1 Effects of De-Nol or sucralfate given po in various doses on mean lesion area induced by absolute ethanol. For comparison, the effect of po administration of a standard dose of 16,16 dimethyl PGE₂ on ethanol-induced lesions area is presented. In this and subsequent figures, each point represents mean ± SEM of results obtained from at least 10 rats. Asterisks indicate statistically significant (*p* < 0.05) differences from the control value obtained with vehicle (1 ml water) given po.

Fig. 2 Effects of De-Nol, sucralfate of 16,16 dimethyl PGE_2 administered po 30, 60, 120, 240, 360 min before absolute ethanol on mean ulcer area induced by this ethanol.

secretion. None of these agents significantly affected acid or pepsin outputs from the chronic gastric fistula rats.

EFFECTS OF DE-NOL, SUCRALFATE AND METHYLATED PROSTAGLANDIN ANALOGUE ON ACUTE GASTRIC MUCOSAL LESIONS INDUCED BY ABSOLUTE ETHANOL AND ACIDIFIED ASPIRIN
In control experiments without De-Nol or sucralfate, the mean lesions area induced by absolute ethanol averaged $76 \pm 10 \text{ mm}^2$ and $83 \pm 10 \text{ mm}^2$ (Fig. 1). Both De-Nol and sucralfate reduced dose dependently the severity of ethanol induced gastric necrosis, the dose reducing the mean lesion area by 50% (ID_{50}) was

Fig. 3 Effects of De-Nol or sucralfate given po in various doses on mean ulcer area induced by acidified aspirin. For comparison, the effect of po administration of a standard dose of 16,16 dimethyl PGE_2 on aspirin-induced ulcer area is presented.

Table 2 Effects of De-Nol (40 mg/kg) or sucralfate (100 mg/kg) alone or in combination with indomethacin (5 mg/kg) given po 90 min earlier, on mean lesion area and lesion number induced by absolute ethanol

Treatment	Rats (n)	Lesion area mm^2	Lesion number
100% Ethanol alone	12	78 ± 16	15.2 ± 2.6
100% Ethanol+De-Nol	8	$2 \pm 1^*$	$2.1 \pm 0.8^*$
100% Ethanol+sucralfate	12	$8 \pm 2^*$	$3.2 \pm 1.0^*$
100% Ethanol+De-Nol +indomethacin	9	61 ± 12	12.4 ± 1.6
100% Ethanol+sucralfate +indomethacin	9	60 ± 11	11.8 ± 2.5
100% Ethanol+indomethacin	8	82 ± 24	18.6 ± 2.2
Indomethacin alone	8	2 ± 1	0.6 ± 0.2

*Significant ($p < 0.05$) decrease from the control value obtained with 100% ethanol alone.

about 3 mg/kg and 22 mg/kg, respectively. Methylated PGE_2 at a standard dose of $10 \mu\text{g/kg}$ reduced the lesion area by about 95%.

The duration of gastroprotective action of the drugs used is shown on Figure 2. The strongest protective effects of De-Nol and sucralfate were observed within the first 60 minutes after their administration. Their effects were reduced to about 50% after 120 minutes and disappeared almost completely after 240 minutes. 16,16 dimethyl PGE_2 almost completely prevented ethanol-induced gastric necrosis and this effect disappeared after about 360 minutes.

Figure 3 shows the effects of De-Nol and sucralfate

Fig. 4 Effects of various doses of De-Nol or sucralfate given po on mucosal generation of PGE_2 in intact rats.

Fig. 5 Effects of various doses of De-Nol or sucralfate on luminal release of PGE₂ in intact rats.

on ulcers induced by acidified aspirin. Both agents dose dependently prevented the formation of aspirin-induced lesions, the ID₅₀ for De-Nol was about 24 mg/kg and that for sucralfate 41 mg/kg. PGE₂ analogue at a dose of 10 µg/kg almost completely prevented the production of aspirin induced ulcerations.

EFFECTS OF DE-NOL AND SUCRALFATE ON PROSTAGLANDIN FORMATION IN GASTRIC MUCOSA

In intact rats without administration of De-Nol or sucralfate, the capability of the mucosa to generate PGE₂ averaged 480±60 ng/g wet tissue weight and the release of PGE₂ into the gastric lumen was about 372±35 ng/ml. As shown in Figures 4 and 5, De-Nol given po in increasing doses caused a dose dependent increase in the generation and release of PGE₂ starting with the dose of 20 mg/kg. Similarly, sucralfate caused an increase in PGE₂ generation at doses 100 mg/kg and higher.

The results on the reversal of the protective effects of De-Nol against ethanol-induced lesions are presented in Table 2. Indomethacin given po in a dose of 5 mg/kg failed to affect the integrity of the gastric mucosa. De-Nol in a dose of 40 mg/kg showed usual protection against absolute ethanol and this was almost completely reversed by the pretreatment with indomethacin given ig 90 minutes before the combination of De-Nol and ethanol. In similar studies with sucralfate, used in a dose 100 mg/kg, the reversal of the protection by indomethacin with respect to the area and number of ethanol lesions was not significantly different from that obtained with De-Nol (Table 2).

Discussion

This study shows that De-Nol is effective in the prevention of the formation of acute gastric ulcerations induced by acidified aspirin and in the protection of gastric mucosa against acute necrotic lesions caused by absolute ethanol.

As in previous studies,¹² we found that the anti-ulcer action of De-Nol against various ulcerogens is dose-dependent and similar to that of sucralfate but occurs at lower doses than sucralfate. This indicates that De-Nol is several times more potent on a weight basis than sucralfate, a standard anti-ulcer drug. Because aspirin induced mucosal lesions depend on gastric acid secretion and can be prevented by potent inhibitors of this secretion such as ranitidine¹⁰ additional secretory tests were done to find out what effect, if any, De-Nol and sucralfate had on gastric acid secretion. It was found that both acid and pepsin secretions were similar in the vehicle and De-Nol or sucralfate treated rats. Thus, we exclude the mediation of decreased gastric acid secretion in the anti-ulcer effect of De-Nol or sucralfate.

This study shows for the first time that De-Nol, like sucralfate¹² is highly effective in the protection of gastric mucosa against acute necrosis induced by corrosive substances such as absolute ethanol. Although De-Nol produced a similar onset and duration of reduction of ethanol lesions as sucralfate, the dose of CBS was about 10 times lower. As this protection can be reversed by indomethacin, a potent inhibitor of prostaglandin biosynthesis, it may be assumed that mucosal prostaglandin are implicated. Direct support for this assumption was obtained from the studies with mucosal generation and luminal release of prostaglandin. Our study showed that both De-Nol and sucralfate administered into the intact stomach caused a dose-dependent increase in the ability of the fundic mucosa to generate PGE₂ and in the release of this prostaglandin into the gastric lumen. It is likely that prostaglandin formed in large amounts account for the effects observed after De-Nol or sucralfate including stimulation of mucosal alkaline secretion, tightening the mucosal barrier³ and the increase in the mucosal resistance and mucosal cell renewal.¹²

The mediation by endogenous prostaglandin in the gastroprotective activities of sucralfate was previously proposed.¹² The present study provides evidence that De-Nol also protects the gastric mucosa through increased biosynthesis of endogenous prostaglandin.

Both De-Nol and sucralfate have been shown to exhibit high efficacy not only in healing of gastroduodenal ulcerations but also in reducing the ulcer relapse rate.^{6-9 13 14} Lower relapse rates appear to

confer a therapeutic advantage on this gastroprotective agent when compared with agents acting through antisecretory mechanisms, such as H_2 -blockers. It remains to be established whether increased mucosal prostaglandin biosynthesis contributes to the therapeutic efficacy of these agents.⁴⁻⁹

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