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

S J KONTUREK, T RADECKI, IRENEUSZ PIASTUCKI, TOMASZ BRZOZOWSKI, AND DANUTA DROZDOWICZ

From the Institute of Physiology Medical Academy, Kraków, Poland

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.1,2 De-Nol was reported to protect the gastric mucosa against various ulcerogens in animals3,4 and to be effective in promoting the healing of gastric and duodenal ulcers5 in man. Moreover, ulcer relapse has been shown to be reduced after healing with De-Nol compared with healing with H2-antagonists.6,7 The mechanism of these effects has not been fully elucidated. Because prostaglandins are known to exhibit both anti-ulcer and protective activities9 and sucralfate is considered to be a standard protective agent in experimental animals10,11 and effective drug in the healing and the reduction of the recurrence of peptic ulcer in man,12,13 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 cannuas 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 described14 and expressed as outputs/60 minute period after
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. 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. 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.

All experiments were carried out on Wistar rats 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 secretion of 16,16 dimethyl PGE2 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.
Gastroprotection by De-Nol and sucralfate

Fig. 2  Effects of De-Nol, sucralfate of 16,16 dimethyl PGE₂ 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 lesion area induced by absolute ethanol averaged 76±10 mm² and 83±10 mm² (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% (ID₅₀) was about 3 mg/kg and 22 mg/kg, respectively. Methylated PGE₂ at a standard dose of 10 µg/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 PGE₂ 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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rats (n)</th>
<th>Lesion area (mm²)</th>
<th>Lesion number</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Ethanol alone</td>
<td>12</td>
<td>78±16</td>
<td>15.2±2.6</td>
</tr>
<tr>
<td>100% Ethanol + De-Nol</td>
<td>8</td>
<td>2±1*</td>
<td>2.1±0.8*</td>
</tr>
<tr>
<td>100% Ethanol + sucralfate</td>
<td>12</td>
<td>8±2*</td>
<td>3.2±1.0*</td>
</tr>
<tr>
<td>100% Ethanol + De-Nol + indomethacin</td>
<td>9</td>
<td>6±12</td>
<td>12.4±1.6</td>
</tr>
<tr>
<td>100% Ethanol + sucralfate + indomethacin</td>
<td>9</td>
<td>60±11</td>
<td>11.8±2.5</td>
</tr>
<tr>
<td>100% Ethanol + indomethacin</td>
<td>8</td>
<td>82±24</td>
<td>18.6±2.2</td>
</tr>
<tr>
<td>Indomethacin alone</td>
<td>8</td>
<td>2±1</td>
<td>0±0.2</td>
</tr>
</tbody>
</table>

*Significant (p<0.05) decrease from the control value obtained with 100% ethanol alone.
on ulcers induced by acidified aspirin. Both agents
dose dependently prevented the formation of
aspirin-induced lesions, the ID_{50} for De-Nol was
about 24 mg/kg and that for sucralfate 41 mg/kg.
PGE_{2} analogue at a dose of 10 μg/kg almost com-
pletely 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
PGE_{2} averaged 480±60 ng/g wet tissue weight and
the release of PGE_{2} 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 PGE_{2}
starting with the dose of 20 mg/kg. Similarly, sucr-
alfate caused an increase in PGE_{2} 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 pre-
sented 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 combi-
ation 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 signi-
cantly 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 ulcer-
ations induced by acidified aspirin and in the protec-
tion of gastric mucosa against acute necrotic lesions
caused by absolute ethanol.

As in previous studies, 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 ranitidine\textsuperscript{10} addi-
tional 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 media-
tion 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
sucralfate\textsuperscript{12} 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 PGE_{2} 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 mucus-
alkaline secretion, tightening the mucosal barrier\textsuperscript{1}
and the increase in the mucosal resistance and
mucosal cell renewal.\textsuperscript{12}

The mediation by endogenous prostaglandin in the
gastroprotective activities of sucralfate was pre-
viously proposed.\textsuperscript{12} The present study provides
evidence that De-Nol also protects the gastric
mucosa through increased biosynthesis of endogen-
ous prostaglandin.

Both De-Nol and sucralfate have been shown to
exhibit high efficacy not only in healing of gastro-
duodenal ulcerations but also in reducing the ulcer
relapse rate.\textsuperscript{10 13 14} Lower relapse rates appear to
Gastroprotection by De-Nol and sucralfate

confer a therapeutic advantage on this gastroprotective agent when compared with agents acting through antisecretory mechanisms, such as H₂-blockers. It remains to be established whether increased mucosal prostaglandin biosynthesis contributes to the therapeutic efficacy of these agents.  

References

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

S J Konturek, T Radecki, I Piastucki, T Brzozowski and D Drozdowicz

Gut 1987 28: 201-205
doi: 10.1136/gut.28.2.201

Updated information and services can be found at:
http://gut.bmj.com/content/28/2/201

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Stomach and duodenum (1689)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/