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Aspirin-Induced Gastric Injury in the Rat: Histologic Changes and Sucralfate Cytoprotection (42496)

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Abstract. The effect of pretreatment with intragastric sucralfate on aspirin acid-induced gastric mucosal lesions in the rat was studied. The finding by others that sucralfate is cytoprotective and that this cytoprotective effect probably is mediated, at least in part, by stimulation of endogenous prostaglandin synthesis was confirmed. In addition, a time course study revealed that the maximum cytoprotective effect was present 1 min after sucralfate administration and persisted for at least 6 hr. Microscopic evaluation of histologic sections revealed that sucralfate significantly decreased aspirin-induced deep mucosal erosions (those extending into the parietal cell area) but not superficial mucosal damage. Superficial mucosal damage (surface cell injury and erosions involving the mucous neck cell area) could not be detected grossly. The lesions seen grossly were deeper erosions involving the parietal cell area of the mucosa.

Cytoprotection has been defined as protection against mucosal injury through some mechanism other than inhibition of acid secretion (1). Sucralfate, a nonantisecretory agent, has been shown to protect the rat gastric mucosa against injury by various noxious agents (2–6). There is evidence that sucralfate acts by stimulating endogenous prostaglandin synthesis. Ligumsky *et al.* found sucralfate protected the gastric mucosa against damage by aspirin and stimulated the release of prostaglandins (6). Hollander *et al.* found that sucralfate's cytoprotective ability was inhibited when endogenous prostaglandin synthesis was inhibited (2).

The time of onset and duration of cytoprotection by sucralfate have not been studied. In addition, there are no studies concerning the correlation between gross and histologic damage in aspirin-induced gastric mucosal injury. Lacy and Ito (7) demonstrated that with gastric mucosal damage by 100% ethanol, the grossly visible lesions correlated with hyperemia and deep necrosis (necrosis of tissue deeper than the mucous neck cell area). Histologically there was superficial damage (surface mucous cell damage) in areas that were normal in appearance grossly. Pretreatment with prostaglandin analog protected against the ethanol-induced hyperemia and deep necrosis but not against the superficial mucosal damage. Others have confirmed these findings with ethanol injury (8, 9) but analogous studies with aspirin injury have not been performed.

The aim of the present study was first to confirm the protective effect of sucralfate against aspirin-induced injury in the rat and its mediation by prostaglandin synthesis, and then to determine (i) the time course of sucralfate cytoprotection and (ii) the correlation between histologic and gross injury.

Materials and Methods. *Animal model.* Male Sprague–Dawley rats weighing 150–200 g were used throughout this investigation. They were fasted overnight in individual tubular fasting cages that prevented the animals from ingesting hair or feces. Aspirin, 200 mg/kg in 0.15 *N* HCl (1 ml/100 g body wt), was instilled intragastrically by orogastric intubation. One hour after administration of the aspirin, the animals were killed with carbon dioxide, the abdomens were opened, and the stomachs were removed and opened along the greater curvature. Lesions were graded and scored as follows by an observer who was unaware of the drugs adminstered: petechial lesions = 1, erosions less than 1 mm = 2, erosions between 1 and 2 mm = 3, erosions between 2 and 4 mm = 4, and erosions greater than 4 mm = actual length in mm.

1. Sucralfate dose-response study. This study was designed to determine the lowest dose of sucralfate that would protect the gastric mucosa against aspirin acid injury. Six groups

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of 19 to 24 rats were pretreated with the intragastric instillation of 1 ml of vehicle only (0.15 *M* NaCl solution) or vehicle plus sucralfate in one of these doses: 500, 250, 125, 62.5, or 31.25 mg/kg. The sucralfate was supplied by Marion Laboratories, Inc., as a powder. Thirty minutes later, the aspirin acid injury procedure as described above was performed. The results were analyzed to determine the dosage range of sucralfate's cytoprotection and the lowest dose that provided effective cytoprotection.

2. Prostaglandin inhibition study. The purpose of this study was to investigate the effect of inhibition of endogenous prostaglandin formation on sucralfate protection. Two groups of eight rats each were pretreated with either 5 mg/kg indomethacin subcutaneously to inhibit prostaglandin cyclooxygenase activity or with saline subcutaneously. Ninety minutes later 250 mg/kg sucralfate was given intragastrically, followed 1 hr later by aspirin in HCl. The animals were killed, the stomachs were removed, and lesions were scored as described above.

3. Time course study. This study was designed to determine the time of onset and duration of sucralfate's protection. Seven groups of 10 rats each were given 250 mg/kg sucralfate intragastrically. Aspirin in HCl was instilled intragastrically 1, 10, 30, or 60 min, or 3, 6, or 12 hr later. An eighth (control) group received the vehicle intragastrically, followed 30 min later by aspirin in HCl intragastrically. The animals were killed, the stomachs were removed, and lesions were scored as described above.

TABLE I. SUCRALFATE DOSE–RESPONSE

Pretreatment	*n*	Lesion score
Vehicle	24	16.1 ± 2.3
Sucralfate (mg kg^{-1})		
31.25	23	16.8 ± 3.6
62.5	24	14.4 ± 3.1
125	23	15.8 ± 3.7
250	24	7.2 ± 2.4*
500	19	5.7 ± 2.9*

Note. In this and subsequent tables, *n* = number of rats and results are presented as means ± SE.

* $P < 0.05$ vs vehicle (analysis of variance with contrasts).

TABLE II. EFFECT OF PROSTAGLANDIN INHIBITION ON SUCRALFATE CYTOPROTECTION

First pretreatment	Second pretreatment	*n*	Lesion score
Indomethacin	Sucralfate	8	20.6 ± 6.5
Saline	Sucralfate	8	7.0 ± 2.5*

Note. *n* as in Table I.

* $P < 0.05$ vs indomethacin–sucralfate (*t* test).

4. Histologic study. The purpose of this study was to determine the extent of histologic injury induced by aspirin in HCl and to determine whether sucralfate protects against the histologic as well as the gross injury. Four groups of six rats each were pretreated and then treated 30 min later (both intragastrically) as follows: (i) vehicle, and then 0.15 *N* HCl; (ii) 250 mg/kg sucralfate, and then 0.15 *N* HCl; (iii) vehicle, and then aspirin in HCl; or (iv) 250 mg/kg sucralfate, and then aspirin in HCl. The animals were killed, the stomachs were removed, and the lesions were scored as described above. A strip of tissue across the entire width of the corpus in the area of the maximal gross lesions was then removed, fixed in Bouin's solution, paraffin embedded, sectioned, and stained with hematoxylin and eosin. The sections were then coded (so the microscopist was unaware of the treatment group each slide came from) and evaluated microscopically for the percentage length of gastric mucosa involved with the following abnormalities: surface mucous cell damage; erosions down to, but not deeper than, the mucous neck cell area; and erosions extending into the parietal cell area.

Results. *Sucralfate dose-response study.* Results of the dose–response study are presented in Table I. Doses up to 125 mg/kg of sucralfate did not protect the rat gastric mucosa against aspirin in HCl injury. Doses of both 250 and 500 mg/kg sucralfate, however, did significantly reduce gross lesions scores as compared with vehicle-pretreated rats ($P < 0.05$, analysis of variance with contrasts). Therefore, 250 mg/kg was used for the subsequent studies.

Prostaglandin inhibition study. Results of pretreatment with indomethacin are shown in Table II. Indomethacin significantly blocked

TABLE III. TIME COURSE OF SUCRALFATE CYTOPROTECTION

Time of pretreatment (min before ASA/HCl)	*n*	Lesion score
1 min	10	0*
10 min	10	1.0 ± 0.8*
30 min	10	2.9 ± 2.3*
60 min	10	6.0 ± 5.4*
3 hr	10	3.5 ± 1.4*
6 hr	10	2.7 ± 1.6*
12 hr	10	15.2 ± 5.4
Vehicle – 30 min	10	20.4 ± 3.6

Note. *n* as in Table I.
* $P < 0.05$ vs vehicle (analysis of variance with contrasts).

the cytoprotective effect of sucralfate ($P < 0.05$, *t* test), suggesting that sucralfate cytoprotection is dependent on stimulation of endogenous prostaglandin synthesis.

Time course study. The effect of increasing duration of time between pretreatment with sucralfate and treatment with aspirin in HCl on sucralfate protection is shown in Table III. Significant protection was seen as early as 1 min after sucralfate administration and persisted at this same level for at least 6 hr ($P < 0.05$, analysis of variance with contrasts). At 12 hr, protection declined and the lesion score of animals pretreated with sucralfate approached that of animals pretreated with vehicle only.

Histologic study. In rats receiving vehicle + HCl, there was no gross injury and little histologic injury (only surface cell damage involving 12% of the mucosa; Table IV). Sucralfate + HCl also did not cause gross injury. Histologically, however, there was extensive superficial mucosal damage (significantly greater than vehicle + HCl, $P < 0.05$), over half the mucosa being involved with surface cell damage or superficial erosions (Table IV, Fig. 1). With aspirin in HCl (after vehicle pretreatment) there was both gross and histologic injury. Histologically, in addition to superficial mucosal damage of similar extent to that occurring with sucralfate, there were deep erosions involving 21% of the mucosa (Table IV, Fig. 2). Sucralfate pretreatment significantly reduced both the gross injury and the deep erosions ($P < 0.05$, analysis of variance with contrasts) but not the superficial mucosal damage seen histologically.

Discussion. This study confirms previous findings that sucralfate pretreatment will protect against gastric mucosal injury by topical barrier breakers (2–5), that it causes superficial mucosal damage in a manner similar to that caused by mild irritants (10), and that its cytoprotective action appears to result from stimulation of endogenous prostaglandin synthesis (2, 6). In this study, the dose found to be needed for protection was relatively large, 250 to 500 mg/kg. The protective dose for sucralfate has varied in several studies from 50 to 500 mg/kg (2, 4, 5). Okabe *et al.* (5) found that a dose of 100 to 300 mg/kg was required for protection against aspirin injury. Nagashima *et al.* (4) found that the protective effect

TABLE IV. COMPARISON OF GROSS AND HISTOLOGIC INJURY

		% Mucosa involved with:			
Group	*n*	Gross score	Surface damage[a]	Superficial erosions[b]	Deep erosions[c]
Vehicle + HCl	6	0	12.4 ± 1.9	0	0
Sucralfate + HCl	6	0	38.4 ± 3.2*	29.4 ± 2.0*	0
Vehicle + ASA/HCl	6	25.4 ± 4.8*	26.4 ± 4.2*	36.1 ± 5.7*	21.1 ± 4.4*
Sucralfate + ASA/HCl	6	7.7 ± 2.1**	39.8 ± 7.9*	28.5 ± 6.8*	6.6 ± 3.1**

Note. *n* as in Table I.
[a] Surface mucous cell abnormality.
[b] Erosions involving mucous neck cell area.
[c] Erosions extending into parietal cell area.
*,** $P < 0.05$ vs vehicle–HCl and vehicle–ASA/HCl, respectively (analysis of variance with contrasts).

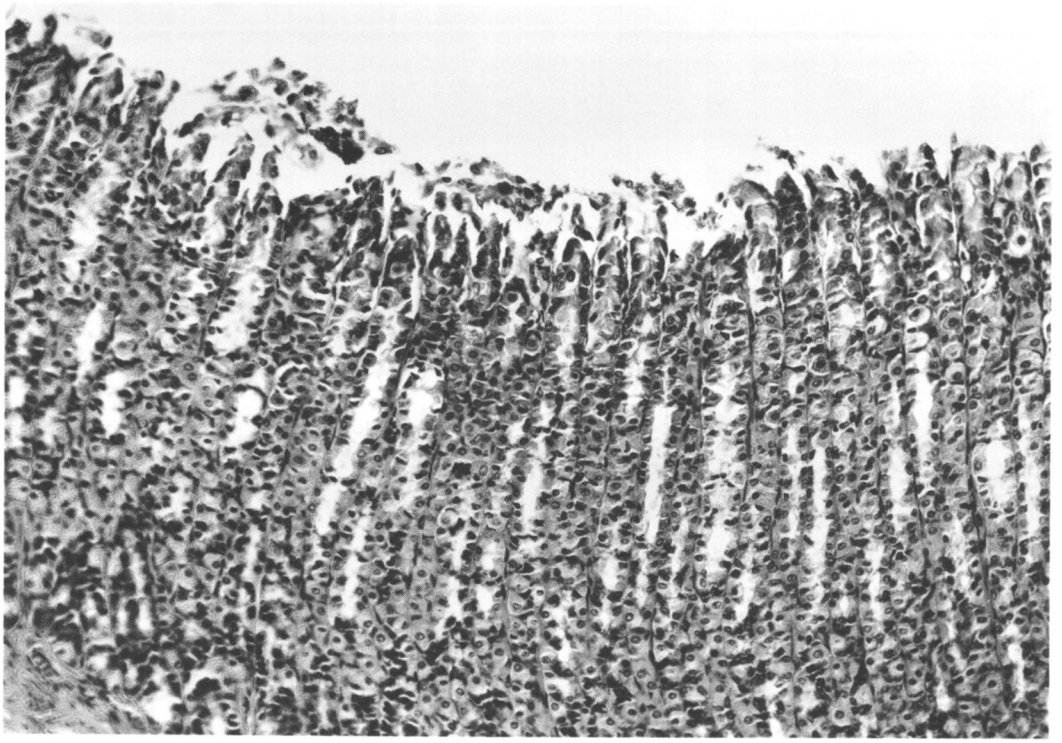

FIG. 1. Photomicrograph of an H and E-stained section from the stomach of a rat pretreated with sucralfate and then HCL (but no aspirin) ig. A very superficial erosion with desquamation of surface epithelial cells is present over the middle and left half of the field. Original magnification ×200.

of sucralfate against ethanol injury was dose dependent: 50 mg/kg caused a significant 61.6% inhibition of lesions and protection increased up to 99.6% inhibition as the dose was progressively increased to 800 mg/kg. In the present study, there appeared to be a threshold for protection, 250 mg/kg or more being required for protection.

The protective effect of the 250 mg/kg dose was blocked by prior administration of indomethacin, an inhibitor of endogenous prostaglandin synthesis. This suggests that sucralfate cytoprotection is dependent on stimulation of endogenous prostaglandin synthesis as was previously shown by Hollander *et al.* (2). A word of caution is in order, however. In the latter study (2), alcohol (which does not inhibit prostaglandin synthesis) was used as the damaging agent. Intragastric aspirin, on the other hand, rapidly inhibits prostaglandin synthesis; e.g., 100 mg kg^{-1} ig produced a 98% inhibition of prostaglandin synthesis in 15 min in the rat (11). Even if there is marked stimulation of prostaglandin synthesis by sucralfate, in the present study aspirin must have induced a profound depletion of mucosal prostaglandin concentrations since the half-life of prostaglandins is short (minutes). Therefore the blocking of sucralfate protection by indomethacin might have been due to other factors.

Robert *et al.* (1) previously showed that prostaglandin pretreatment provides protection of the mucosa for 1 hr against damage by necrotizing agents. In addition, pretreatment with a mild irritant provided protection against the damaging effects of a strong irritant, called adaptive cytoprotection (12, 13). The results of the present time course study show that there is a very prompt onset of protection with sucralfate, marked protection against aspirin acid injury beginning just 1 min after sucralfate administration. Furthermore, this protec-

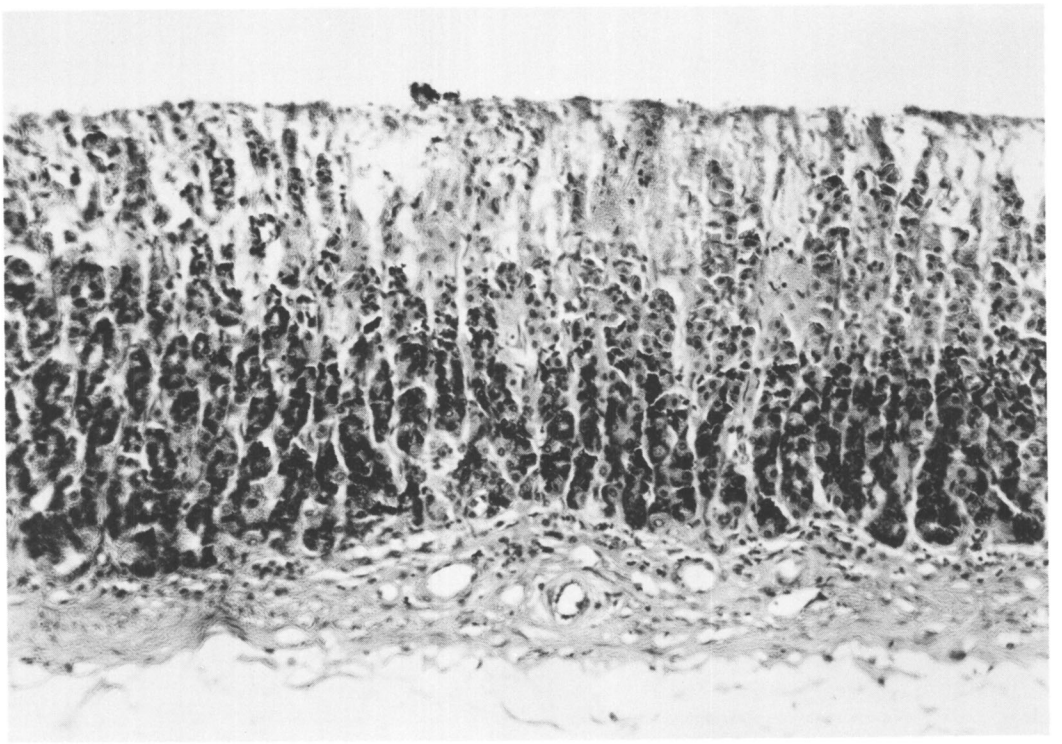

FIG. 2. Photomicrograph of an H and E-stained section from the stomach of a vehicle pretreated rat that received aspirin in HCl ig. There is a deep erosion with necrosis of cells involving 25 to 50% of mucosal depth across the entire field. Original magnification ×200.

tive effect is relatively long lasting, persisting for at least 6 hr.

In the histologic study of Tarnawski *et al.*, the authors found that sucralfate causes superficial mucosal damage, suggesting that its protective effect involves its acting as a mild irritant (10). The finding in the present study that sucralfate + HCl resulted in significantly greater superficial mucosal damage than did vehicle + HCl is consistent with that concept. Sucralfate caused no deep erosions. Although it did not protect against superficial mucosal damage caused by aspirin + HCl, it did significantly protect against deep erosion formation. In this regard, it is similar to prostaglandin's action in protecting against the deep necrotic injury, but not the surface cell injury caused by ethanol (7–9).

The histologic study also confirmed the importance of histologic evaluation in mucosa injury studies. Surface cell injury and superficial erosions involving the mucous neck cell area could not be detected grossly. The lesions seen grossly were deeper erosions involving the parietal cell area of the mucosa. As with prostaglandin protection against ethanol injury (7), the protective effect of sucralfate involved the gross lesions and its histologic counterpart, but it did not protect against superficial mucosal injury.

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