Naftidrofuryl Protects the Rat against Chronic Gastric Ulceration

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Abstract □ Intraperitoneal reserpine (5 mg/kg every day for 5 days) produced solitary chronic ulceration of the rat stomach after 2 weeks. Gavage with 1 mL/day of 1% naftidrofuryl oxalate for 2 weeks protected 30% of rats against ulceration and this protection extended to 70% of cases with a 2% solution. Similar gavage with a 5% solution protected all rats against ulceration without significantly influencing the basal H^+ output (14.8 ± 0.6 versus 15.4 ± 0.5 μmol, mean ± SEM, n = 10); that is, cytoprotection was achieved.

Reduced gastric mucosal blood flow plays a key role in the evolution of gastric ulceration. As early as 1855, Virchow[1] described ischemic necrosis in the base of gastric ulcers. Since then, clinical and laboratory experience have confirmed the damaging nature of mucosal ischemia. Clinically, gastroscopy in trauma patients shortly after injury has identified zones of mucosal pallor that later develop frank epithelial disruption.[2,3] Experimentally, the pathogenicity of ischemia has also been established by several shock models of acute gastritis.[4,5] In dogs, a mixture of acid and bile salts causing no gastric mucosal injury will erode the epithelium of this mucosa if the blood supply is reduced.[6] In the rat, persistence of gastric mucosal ischemia produces solitary chronic ulceration of the stomach.[7,8] Naftidrofuryl oxalate, an acid ester of diethylaminoethanol, is a vasodilator[9,10] that directly enhances tissue oxidative metabolism by activation of succinic dehydrogenase, a tricarboxylic acid cycle enzyme.[11,12] These actions and, in particular, vasodilatation and the observation that naftidrofuryl protects the rat against ischemia-induced acute gastric mucosal injury[13] prompted investigation of the effect of the drug on chronic gastric ulceration.

Experimental Section

Experiments were conducted with groups of 10 Sprague-Dawley rats of either sex (240–290 g). Animals were denied solid food for 24 h before acid collection and were housed in cages with wide mesh wire bottoms to prevent coprophagy. Solutions of naftidrofuryl oxalate (Praxilene, Lipha Ltd., West Drayton, Middlesex, U.K.) and 1 mg/mL of reserpine (Sigma, St. Louis, MO) were freshly prepared each day as detailed elsewhere.[13] Injections were administered ip into the left iliac fossa using a 25-G needle, and gavage was undertaken under light ether anesthesia using a 6 FG Infant's Feeding Tube 400/420 (Portex Ltd, Hythe, U.K.). The surgical procedures in this study were carried out as previously described.[14,15] Results are expressed as mean ± SEM. The statistical significance ($p < 0.05$) of observed differences between the groups was determined using the Mann-Whitney U test for nonparametric data.

To minimize the impact of day-to-day variation in response to treatment, each part of the present study was designed to be conducted over several days and on each of these days rats were allocated to the control and all of the treatment groups.

The accuracy of the scorer of chronic gastric ulceration was determined by assessing his ability to reproduce the scores of 10 rats injected ip with 5 mg/kg of reserpine every day for 5 days and then housed for 2 weeks before being sacrificed. The association between the scores of two runs was determined by measuring the coefficient of correlation (r) using Spearman's rank correlation coefficient test. The r value for the test was 0.98 and its level of significance value was $p < 0.001$. Thus, the scorer had the ability to reproduce these scores accurately.

Preliminary studies were undertaken to determine the effect of naftidrofuryl on the stimulated H^+ output of the pyloric-ligation rat over a period of 2 h. Rats were anesthetized by inhalation of diethyl ether (B.P.) and the pylorus was ligated. The abdomen was closed and 1 mL of 1, 2, or 5% solutions of naftidrofuryl were instilled into the stomach by orogastric intubation. Control animals were similarly treated with 1 mL of saline. Animals were completely fasted and allowed to recover from anesthesia. Two hours later, they were killed by ether overdose, the stomach was removed, the gastric contents were carefully collected and measured, and H^+ output was determined by titration to pH 7.0 with 0.1 M NaOH using an automatic titrator (Radiometer, Copenhagen). The H^+ output was calculated for each animal (μmol), and then the group mean score was determined and expressed as μmol/2 h. The results showed that naftidrofuryl had no significant effect on the H^+ output of this rat model (control output 247 ± 19 μmol).

In controlled pilot studies, 2 weeks after the ip injection of 5 mg/kg of reserpine every day at 10 a.m. for 5 days, all rats developed solitary chronic gastric ulceration, thus demonstrating the validity of this method. These ulcers were oval or round. They developed in the pre-antral region, had a 2–3-mm diameter, and extended into the submucosa with chronic inflammatory cell infiltration and fibroblastic reaction in the tissues surrounding the ulceration.

Following these experiments, secretory and mucosal integrity studies were carried out. One milliliter of 1, 2, or 5% naftidrofuryl oxalate or 1 mL of saline was instilled daily (9 a.m.) into the stomach by orogastric intubation for 2 weeks. Starting after the first day's gavage, animals were injected every day (10 a.m.) for 5 days with reserpine (5 mg/kg) or saline (5 mL/kg). At the end of 2 weeks of gavage, animals were anesthetized with ip pentobarbitone (60 mg/kg; Sagatal, May and Baker, Dagenham, U.K.) and submitted to tracheostomy (to overcome respiratory distress from intubation) and orogastric intubation with a 6 FG tube. The gastric fasting secretion was recovered by slowly instilling 1 mL of double-distilled water and recovering all gastric contents. The basal gastric secretion was then collected every 15 min for 1 h and the H^+ output (μmol/h) was determined by titration to pH 7.0 with 0.1 M NaOH using an automatic titrator (Radiometer, Copenhagen). At the end of this hour, animals were killed by ether overdose and the stomach was removed and opened along the greater curvature. After washing with a direct stream of cold water, the stomachs were pinned out and independently examined for the presence of gastric ulceration by the same scorer, who was blinded as to treatment group, throughout the study. The scorer was instructed to identify the site, shape, and size of any breaches of the gastric mucosa using a hand lens. Sections of ulcerated and apparently nonulcerated stomachs were then cut off each stomach, fixed in formalin, embedded in paraffin, sectioned at right angles to the mucosa, stained (hematoxylin-eosin and periodic acid-Schiff), and examined microscopically.

Results and Discussion

All rats survived without observed distress or changes in activity or weight. During these experiments, the consumption of food and water by the treatment groups was similar to that by control animals. The basal H^+ output for 1 h in rats

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receiving naftidrofuryl solutions for 2 weeks was not significantly different from control values and addition of reserpine did not influence this outcome. The stomach of rats gavaged with naftidrofuryl and injected with saline showed no gastric ulceration and were microscopically similar to control stomachs. All rats in the reserpine-alone group developed solitary oval or round ulceration situated at or immediately adjacent to the lesser curvature in the pre-antral region. These ulcers were always solitary. They were sharply demarcated, punched-out, of vertical edges and a black base depressed below the mucosal surface, and of a diameter ranging from 2 to 3 mm. Naftidrofuryl produced dose-dependent protection against this ulceration; complete protection was noted with the 5% solution. The ulceration that developed with lesser doses of this agent had appearances similar to the ulcers induced by reserpine alone; however, their diameter was always <2 mm.

Microscopically, the reserpine-induced ulceration had a narrow surface layer of fibrinous exudate and consisted of full-depth mucosal necrosis and loss, extending into the submucosa, with chronic inflammatory cell infiltration and fibroblastic reaction, particularly at the base. The gastric mucosa that was protected by naftidrofuryl against ulceration was microscopically similar to that of control animals. It was completely intact and covered by mucus and was not different in thickness or density from that of controls. Furthermore, this mucosa showed no evidence of chronic inflammatory changes. The ulceration occurring with naftidrofuryl plus reserpine was similar microscopically to the reserpine alone ulceration; however, it was less severe and just confined to the mucosa.

The pharmacological actions of naftidrofuryl oxalate remain poorly understood. The clinical effects of the drug are attributed to its actions on cellular metabolism and regional blood flow. Clinical trials and in vitro and in vivo studies of animals have indicated that naftidrofuryl oxalate may directly enhance tissue oxidative metabolism by activation of succinic dehydrogenase.[11,12] The drug was originally proposed to be a spasmolytic substance with obvious vascular tropism that produces central and peripheral vasodilatation by sympathetic blockade.[9,10]

Cytoprotection has been defined as the ability of pharmacological agents—originally prostaglandins—to prevent or reduce gastric, duodenal, or intestinal mucosal injury by mechanisms other than inhibition of gastric acid secretion.[16] Recent studies in the rat[13] demonstrated that naftidrofuryl protects the stomach against ischemia-induced acute gastric mucosal injury by cytoprotective actions.

Reserpine-induced chronic gastric ulceration was reproduced in this study and was associated with normochlorhydria (Table I). Naftidrofuryl had no significant effect on the H^+ output of the rat with or without pyloric ligation (Table I) and protected its stomach against the reserpine-induced ulceration without significantly influencing the basal H^+ output (Table I). Since pyloric ligation produces maximum stimulation of acid secretion in the rat,[14] this action of naftidrofuryl indicates cytoprotection as defined by Robert et al.,[16] the mechanism of which is unknown. The possibility exists, however, that this cytoprotection is achieved by enhancing tissue oxidative metabolism and gastric mucosal blood flow.

Table I—Effect of Naftidrofuryl Oxalate on Reserpine-Induced Chronic Gastric Ulceration[a]

Experimental Group[b]	Incidence of Animals Showing Ulceration, %	H^+ Output (mean ± SEM), μmol/h
Saline, 1 mL ig; saline, 5 mL/kg ip	0	15.4 ± 0.5
1% Naftidrofuryl, 1 mL ig; saline, 5 mL/kg ip	0	14.7 ± 0.3
2% Naftidrofuryl, 1 mL ig; saline, 5 mL/kg ip	0	13.9 ± 0.7
5% Naftidrofuryl, 1 mL ig; saline, 5 mL/kg ip	0	15.6 ± 0.6
Saline, 1 mL ig; reserpine, 5 mg/kg ip	100	13.8 ± 0.4
1% Naftidrofuryl, 1 mL ig; reserpine, 5 mg/kg ip	70	15.1 ± 0.7
2% Naftidrofuryl, 1 mL ig; reserpine, 5 mg/kg ip	30	14.3 ± 0.4
5% Naftidrofuryl, 1 mL ig; reserpine, 5 mg/kg ip	0	14.8 ± 0.6

[a] n = 10. [b] ig: instilled into the stomach every day for 2 weeks; ip: intraperitoneal injection every day for 5 days.

Current treatment for chronic gastric ulceration is largely with H_2-receptor antagonists (e.g., cimetidine, ranitidine), which are used to aid healing. The addition of cytoprotective agents, such as colloidal bismuth or sucralfate, is useful in treating the refractory ulcer. This study suggests that naftidrofuryl may prove to be an added dimension in the treatment of chronic gastric ulceration.

References and Notes

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