DMSO

Stimulation of Healing by Free Radical Scavengers of Ischemia-Induced Acute Gastric Mucosal Injury in the Rat

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Abstract □ Allopurinol and dimethyl sulfoxide (DMSO; 1 mL of 1, 2, or 5% by gavage daily) were used to examine the influence of scavenging oxygen-derived free radicals on the healing of reserpine- (5 mg/kg, intraperitoneal) and 5-hydroxytryptamine- (50 mg/kg, intraperitoneal) induced acute ischemic injury of the rat gastric mucosa. Allopurinol and DMSO demonstrated a time- but not dose-dependent power to stimulate healing of this injury. The magnitude of injury produced by reserpine or 5-hydroxytryptamine (serotonin) followed by gavage with allopurinol or DMSO was significantly ($p < 0.01$) less after day 4 than that after day 3 of this gavage, and the magnitude after day 3 was itself significantly (reserpine, $p < 0.001$; 5-hydroxytryptamine, $p < 0.01$) less than that after day 2 of the same gavage. The actions of allopurinol and DMSO were not associated with any significant influence on H^+ output. These results suggest that oxygen-derived free radicals are detrimental to the integrity of the rat gastric mucosa and that scavenging them stimulates healing of the ischemia-induced injury of the mentioned mucosa.

Oxygen-derived free radicals are cytotoxic and promote tissue injury.[1] These radicals play a direct role in the mechanism of ischemic injury of the gastrointestinal mucosa.[2–4] Recent studies in the rat[4] demonstrated that the scavenging of oxyradicals affords cytoprotection; that is, it protects the stomach against ischemic injury without influencing the H^+ output. The present investigation with rats examined whether this scavenging influences the healing of ischemia-induced acute gastric mucosal injury produced by reserpine[5] and 5-hydroxytryptamine[6] (5-HT; serotonin).

Experimental Section

Animals—Food, but not water, was withheld for 24 h from groups of 10 Sprague–Dawley rats of either sex (220–280 g) before the experiments. Tracheostomy and pyloric ligation were performed as detailed elsewhere.[7]

Source and Preparation of Drugs—All drugs, except allopurinol (Burroughs Wellcome Company, Research Triangle Park, NC), were supplied by Sigma Chemical Company (St. Louis, MO) and prepared as previously described.[4] 5-HT powder was dissolved in double-distilled water to prepare a 10-mg/mL solution. Drugs were freshly prepared for each experimental day. Injections were administered intraperitoneally (ip) into the left iliac fossa, and gavage was performed under light ether anesthesia with a 6 FG infant feeding tube (400/420; Portex Ltd., Hythe, U.K.).

Preliminary Studies—These studies were carried out in groups of 10 rats to determine the effect of the radical scavengers used on the stimulated H^+ output of the pylorus-ligated rat.

Validity and H^+ Output Studies—Rats were injected with double-distilled water (5 mL/kg), the vehicle solution of reserpine (5 mL/kg), reserpine (5 mg/kg), or 5-HT (50 mg/kg) and then completely fasted. After 5 h, they were anesthetized with pentobarbitone and subjected to tracheostomy (to overcome respiratory distress from intubation) and orogastric intubation with a 6 FG feeding tube. The gastric secretion during fasting was recovered by slowly instilling 1 mL of double-distilled water and collecting 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. At the end of this hour, animals were killed by ether overdose, and their stomachs were removed and opened along the greater curvature. After being washed with a direct stream of cold water, the stomachs were pinned out and independently examined for the presence of mucosal injury and determination of surface area (mm^2). Sections of injured and apparently uninjured gastric mucosa were then examined microscopically.

Healing Studies—Rats were injected with reserpine (5 mg/kg), 5-HT (50 mg/kg), or double-distilled water (5 mL/kg). After 6 h, they were started on a daily regimen of gavage under light ether anesthesia with 1 mL of double-distilled water or 1 mL of 1, 2, or 5% allopurinol or dimethyl sulfoxide (DMSO). Animals were killed at 2, 3, or 4 days after the start of gavage. Before each killing, they were denied solid food for 24 h and then anesthetized with pentobarbitone. Tracheostomy and orogastric intubation with a 6 FG feeding tube were performed, and then the basal gastric secretion was collected for 1 hour, and the H^+ output was determined. Rats were killed by ether overdose, and their stomachs were independently examined macroscopically and microscopically.

To minimize the impact of day-to-day variation in response to treatment, the study was conducted over several days, and animals were allocated to the control group and each of the treatment groups on every experimental day.

Scorer Accuracy—The precision of the scorer of the extent of gastric mucosal injury was determined by assessing the ability to reproduce the injury scores of 10 rats injected ip with 5-HT at 50 mg/kg and killed 6 h later. The association between the scores of two runs was determined by measuring the correlation coefficient with Spearman's rank correlation coefficient test. The correlation coefficient for the test was 0.99, and its level of significance value was $p < 0.001$. Therefore, the scorer had the ability to reproduce mucosal injury scores accurately.

Statistical Analysis—The statistical significance ($p < 0.05$) of observed differences between the groups was determined with the Mann–Whitney U test for nonparametric data.

Results

All rats survived their experimental period without any observed distress or changes in activity. During the experiments, food and water consumption by the treatment groups was similar to that by control animals.

Preliminary Studies—Pyloric ligation for 2 h was associated with a stimulated H^+ output of 237 ± 12 μmol, and orogastric instillation of 1 mL of 1, 2, or 5% of either allopurinol or DMSO had no significant influence on this output.

Validity and H^+ Output Studies—The basal H^+ output over 1 h was not significantly influenced by the vehicle solution of reserpine but was significantly ($p < 0.001$) depressed by both reserpine and 5-HT (3.2 ± 0.2 and 3.6 ± 0.3 μmol, respectively, versus 13.9 ± 0.6 μmol).

The stomachs of rats injected with the vehicle solution of reserpine had no mucosal injury and were microscopically similar to control stomachs. All rats injected with reserpine or 5-HT developed oval or round mucosal injury confined to the glandular stomach and of no constant relationship to rugal crests (injury scores, 38.7 ± 3.1 and 28.4 ± 3.1 mm^2, respectively). Microscopically, the reserpine- and 5-HT-

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induced injuries were similar and consisted of partial or full-depth (including the muscularis mucosae) mucosal necrosis and loss. Polymorphonuclear leucocyte infiltration and edema occurred at injury edges and in the submucosa. The mucosal and submucosal blood vessels were severely constricted. The muscularis propria was intact, and no pathological changes were detected in the forestomach or antrum.

Healing Studies—The basal H^+ output of rats injected with reserpine or 5-HT was not significantly different from that of animals injected with distilled water, and gavage with allopurinol or DMSO for up to 4 days did not influence this output.

After 2–4 days of gavage with distilled water, all animals injected with reserpine or 5-HT had gastric mucosal injury similar to that seen during the validity studies. A natural tendency for healing was exhibited by both the reserpine- and 5-HT-induced injuries. Most of the injury seen after day 3 and all of that seen after day 4 of gavage with distilled water consisted of full-thickness mucosal necrosis and loss, which extended into the submucosa. Microscopic examination after days 2, 3, and 4 of the gavage showed mononuclear inflammatory cells infiltrating the tissues surrounding the injury and the submucosa. No submucosal or mucosal vasoconstriction was seen. The gastric epithelium bordering the injured sites had increased mitotic activity of crypt cells and abundant mucous cells, both in crypts and surface epithelium. Vascular granulation tissue was present in the base of the full-thickness mucosal injury as early as day 2 after gavage. By day 4 after the start of gavage, many sections showed an intact mucosa, which was immature and in a regenerating phase, with muscularis mucosae defects bridged by granulation tissue. The injury remaining at this stage extended into the submucosa, was filled with granulation tissue and was particularly noticeable between the edges of the muscularis mucosae. The epithelium showed increased mitotic activity of crypt cells and grew from the edges to cover the surface. Occasional foci of a foreign-body-type giant-cell reaction were found in some of this injury around debris and food material. Healing of partial-thickness mucosal injury occurred as early as day 2 after the start of gavage, when the mucosa was intact but immature and regenerating. At the end of gavage, the mucosa had not completely regained a normal appearance.

Allopurinol and DMSO demonstrated a time- but not dose-dependent power to stimulate the healing of the reserpine- and 5-HT-induced injuries. On days 2, 3, and 4 after the start of gavage with allopurinol or DMSO, the areas of the reserpine- and 5-HT-induced injuries were significantly less than those of animals similarly gavaged with distilled water. The magnitude of the mucosal injury produced by reserpine or 5-HT and gavage with allopurinol or DMSO was significantly ($p < 0.01$) less after day 4 than that after day 3 of this gavage, and the magnitude after day 3 was itself significantly (reserpine, $p < 0.001$; 5-HT, $p < 0.01$) less than that after day 2 of the same gavage. Microscopic examination of the reserpine- and 5-HT-induced injuries remaining after 4 days of gavage with allopurinol or DMSO revealed full-thickness mucosal necrosis and loss, which extended into the submucosa. The histologic features of the injury and its healing characteristics at 2, 3, and 4 days after starting gavage with allopurinol or DMSO were similar to those after gavage for the same periods with distilled water.

Discussion

In addition to inhibiting the enzyme xanthine oxidase, which is responsible for the formation of superoxide radicals,[8,9] allopurinol is a potent scavenger of hydroxyl radicals. DMSO is also a scavenger of hydroxyl radicals.[1] These agents were used in the present investigation to determine whether the scavenging of oxygen-derived free radicals influences healing of ischemia-induced acute gastric mucosal injury.

Parenteral administration of large doses of reserpine (2–10 mg/kg) in experimental animals causes acute gastric mucosal injury.[5,10] This injury is ischemically mediated and is produced by vagal α-adrenoceptor stimulation to gastric submucosal and mucosal blood vessels, which causes vasoconstriction.[5] 5-HT produces acute gastric mucosal injury in rats due to intense vasoconstriction that results in areas of focal ischemia.[6] These injuries were reproduced in the present study, and their relationship to mucosal ischemia was confirmed. The injuries were associated with submucosal and mucosal vasoconstriction and significant depression of H^+ output, which is consistent with the fact that marked inhibition of mucosal blood flow diminishes acid secretion.[11] Two

Table I—Effect of Distilled Water, Allopurinol, or DMSO on the Rate of Healing of Reserpine- and 5-HT-Induced Acute Gastric Mucosal Injury in the Rat[a]

Treatment	Results after 2 Days			Results after 3 Days			Results after 4 Days		
	Incidence, %[b]	Area, mm²[c]	H^+[d]	Incidence, %[b]	Area, mm²[c]	H^+[d]	Incidence, %[b]	Area, mm²[c]	H^+[d]
DW (ip) + DW (ig)	0	0	13.9 ± 0.6	0	0	12.4 ± 0.7	0	0	14.2 ± 0.6
Reserpine									
+ DW (ig)	100	26.1 ± 3.2	12.8 ± 0.5	100	14.6 ± 2.1	13.2 ± 0.3	100	7.1 ± 1.1	13.7 ± 0.5
+ 1% allopurinol	80	15.1 ± 1.1[e]	14.1 ± 0.4	60	5.2 ± 0.9[e]	12.8 ± 0.4	40	0.9 ± 0.3[f]	14.1 ± 0.4
+ 2% allopurinol	80	14.5 ± 0.9[e]	12.8 ± 0.3	60	5.1 ± 0.8[e]	13.2 ± 0.3	40	0.8 ± 0.2[f]	13.9 ± 0.5
+ 5% allopurinol	80	14.1 ± 1.1[e]	13.4 ± 0.4	60	4.8 ± 0.7[e]	13.1 ± 0.6	40	0.9 ± 0.1[f]	12.8 ± 0.4
+ 1% DMSO	80	16.2 ± 1.3[e]	12.7 ± 0.3	60	6.1 ± 1.1[e]	12.9 ± 0.5	40	0.9 ± 0.4[f]	12.9 ± 0.3
+ 2% DMSO	80	15.1 ± 1.2[e]	12.9 ± 0.5	60	5.2 ± 0.8[e]	14.1 ± 0.3	40	0.8 ± 0.3[f]	13.4 ± 0.5
+ 5% DMSO	80	14.7 ± 0.9[e]	12.5 ± 0.6	60	5.1 ± 0.7[e]	14.2 ± 0.4	40	0.8 ± 0.4[f]	14.1 ± 0.2
5-HT									
+ DW (ig)	100	21.2 ± 2.1	14.2 ± 0.3	100	16.1 ± 1.1	13.9 ± 0.5	100	7.2 ± 1.2	12.9 ± 0.4
+ 1% allopurinol	70	13.9 ± 1.9[f]	14.1 ± 0.4	50	6.2 ± 0.9[e]	12.8 ± 0.4	40	0.8 ± 0.3[f]	13.6 ± 0.5
+ 2% allopurinol	70	13.8 ± 1.2[f]	13.8 ± 0.5	50	5.8 ± 0.8[e]	13.6 ± 0.6	40	0.7 ± 0.2[f]	13.2 ± 0.3
+ 5% allopurinol	70	13.6 ± 1.3[f]	12.9 ± 0.6	50	5.7 ± 0.7[e]	13.1 ± 0.5	40	0.7 ± 0.1[f]	12.8 ± 0.4
+ 1% DMSO	70	13.8 ± 1.4[f]	13.4 ± 0.4	50	5.9 ± 0.8[e]	12.6 ± 0.3	40	0.9 ± 0.4[f]	12.9 ± 0.3
+ 2% DMSO	70	13.7 ± 0.9[f]	14.2 ± 0.3	50	5.6 ± 0.8[e]	12.9 ± 0.4	40	0.8 ± 0.3[f]	13.2 ± 0.5
+ 5% DMSO	70	13.8 ± 0.8[f]	12.8 ± 0.4	50	5.5 ± 0.9[e]	13.4 ± 0.5	40	0.7 ± 0.1[f]	13.9 ± 0.3

[a] Distilled water (DW), allopurinol, or DMSO was given by gavage (ig) daily (1 mL); reserpine dose, 5 mg/kg, ip; 5-HT dose, 50 mg/kg, ip; n = 10; all values are expressed as mean ± standard error of the mean. [b] Percent incidence of animals showing injury. [c] Injury area. [d] H^+ output in μmol/h. [e] Significantly different compared with DW (ig); $p < 0.001$. [f] Significantly different compared with DW (ig); $p < 0.01$.

days after these injuries had occurred, the basal H^+ output was similar to that of control animals (Table I), a result suggesting that the ischemic impulse to the mucosal blood vessels had ceased and that mucosal blood flow had returned to normal.[11] Consequently, the reserpine- and 5-HT-induced injuries were allowed to reflect their healing potential under basal conditions. These injuries had a natural tendency for healing; however, gavage with allopurinol and DMSO significantly enhanced this potential (Table I). The partial-thickness mucosal injuries healed by regeneration, whereas the full-thickness mucosal injuries were initially filled with granulation tissue and then the mucosa grew from the edges to cover their surface. The observation that allopurinol and DMSO demonstrated time- but not dose-dependent power to stimulate the healing of reserpine- and 5-HT-induced injuries may be attributed to the possibility that the lower doses of these agents exhibit the maximum ability to enhance healing at any given period, and thus, the higher doses incurred no additional benefits. Prolonging the duration of treatment, however, affords an advantage by extending the therapeutic gain over the period of tissue healing and, thereby, enhancing the process of healing.

The similar efficacies of allopurinol and DMSO and the fact that the only action they share is their ability to scavenge oxygen-derived free radicals suggest that this action is responsible for the results achieved. This study showed that oxyradicals are detrimental to the integrity of the gastric mucosa and mediate its injury. Scavenging these radicals stimulates tissue healing by intensifying the natural tendency of injured tissues to regenerate, an action probably due to removal of deleterious agents that impair this tendency. Inflammatory cells infiltrated the tissues surrounding mucosal injury, and these cells yield free radicals by oxidative bursts. DMSO inhibits the function of such cells,[12] an action that may be involved in its antiradical activity. The earlier comment that the healing potential of the injury was assessed under basal conditions after the ischemic impulse had ceased suggests that the enzyme xanthine was present in the dehydrogenase form when allopurinol was given. It is, therefore, unlikely that the actions of allopurinol were achieved by inhibiting xanthine oxidase.

Gastric mucosal ischemia is an essential prerequisite for stress-induced injury in both humans and animals.[5,13] This injury can be produced pharmacologically by the administration of reserpine,[5,13] and it has been proposed that 5-HT may be a mediating factor in its mechanism.[6] The results of the present study suggest that the scavenging of oxygen-derived free radicals may be an added dimension in the treatment of stress-induced acute gastric mucosal injury in humans.

References and Notes

1. Del Maestro, R. F.; Thaw, H. H.; Bjork, J.; Planker, M.; Arfors, K. E. *Acta Physiol. Scand.* 1980, *492 (Suppl.)*, 43–57.
2. Parks, D. A.; Bulkley, G. B.; Granger, D. N.; Hamilton, S. R.; McCord, J. M. *Gastroenterology* 1982, *82*, 9–15.
3. Itoh, M.; Guth, P. H. *Gastroenterology* 1985, *88*, 1162–1167.
4. Salim, A. S. *Digestion* 1989, *43*, 113–119.
5. Salim, A. S. *J. Physiol.* 1987, *392*, 363–376.
6. Ferguson, W. W.; Edmonds, A. W.; Starling, J. R.; Wangensteen, S. L. *Ann. Surg.* 1973, *177*, 648–654.
7. Salim, A. S. *J. Pharm. Sci.* 1988, *77*, 579–581.
8. Flower, R. J.; Moncada, S.; Vane, J. R. In *The Pharmacological Basis of Therapeutics*; Goodman, L. S.; Gilman, A.; Eds.; Macmillan: New York, 1980; pp 720–722.
9. Moorhouse, P. C.; Grootveld, M.; Halliwell, B.; Quinlan, J. G.; Gutteridge, J. M. C. *FEBS Lett.* 1987, *213*, 23–28.
10. Blackman, J. G.; Campion, D. S.; Fastier, F. N. *Br. J. Pharmacol.* 1959, *14*, 112–116.
11. Jacobson, E. D.; Linford, R. H.; Grossman, M. I. *J. Clin. Invest.* 1966, *45*, 1–13.
12. Beilke, M. A.; Collins-Lech, C.; Solnle, P. G. *J. Lab. Clin. Med.* 1987, *110*, 91–96.
13. Salim, A. S. *J. Psychiatr. Res.* 1988, *22*, 35–42.

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