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PREVENTION OF ETHANOL-INDUCED VASCULAR INJURY AND GASTRIC MUCOSAL LESIONS BY SUCRALFATE AND ITS COMPONENTS: POSSIBLE ROLE OF ENDOGENOUS SULFHYDRYLS

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Abstract: We tested the hypothesis that sucralfate, which contains eight sulfate and aluminum molecules on a sucrose and its other components might decrease ethanol-induced vascular injury and hemorrhagic mucosal lesions through a sulfhydryl (SH)-sensitive process. Experiments performed in rats revealed that the entire sucralfate molecule is not a prerequisite for protection against ethanol-induced mucosal vascular injury and erosions. It appears that sulfate and sucrose octasulfate are potent components of sucralfate, although an equimolar amount of sucralfate is at least twice as effective in gastroprotection than its components. The SH alkylator N-ethylmaleimide abolished the gastroprotection by sucralfate, suggesting SH-sensitive process in the mucosal protection which seems to be associated with the prevention of rapidly developing vascular injury in the stomach of rats given ethanol.

Introduction

Sucralfate is one of the few unusual drugs which exerts acute gastric mucosal protection and accelerates healing of chronic gastric and duodenal ulcers without inhibiting gastric acid secretion or neutralizing intraluminal acid (1). This unexpected wide spectrum of action is due, at least in part, to stimulation of synthesis of endogenous prostaglandins (PG) (2), enhanced bicarbonate secretion (3), protection of mucosal proliferative zone (4) which, in part, may be associated with prevention of vascular injury and maintenance of blood flow (5).

Since endogenous sulfhydryls (SH) were implicated in the mechanism of acute gastric mucosal protection by PG, metals and certain drugs (6-8), we tested the hypothesis that endogenous SH which can be alkylated by N-ethylmaleimide (NEM) might also be involved in gastroprotection by sucralfate. One should recall that the sucralfate molecule contains, in addition to sucrose, eight molecules of sulfate and aluminum which may influence endogenous protein or nonprotein SH groups.

Recent studies also indicate that vascular injury might be a common target of interaction of damaging and protective agents in the stomach (9,10). We thus also examined whether sucralfate and its components might protect against ethanol-induced vascular injury.

Materials and Methods

Sprague-Dawley female rats (150-200 g) initially had unlimited access to Purina laboratory chow and tap water, and every experiment was performed at least twice and the results were pooled (total n = 6-8).

Sucralfate (Marion Laboratories, Inc.) was solubilized in 0.1 N HCl or in the nonionic detergent polysorbate Tween 80 and administered intragastrically (i.g.) by rubber stomach tube to groups of rats in initial dose response experiments at 2, 10 or 50 mg/100 g 30 min before i.g. 100% ethanol (1 ml). NEM (Sigma) was injected subcutaneously (s.c.) at 5 mg/100 g 10 min after sucralfate (20

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min before alcohol). Sucrose octasulfate (Marion), sodium sulfate (Fisher) aluminum hydroxychloride (Marion) or aluminum chloride (Fisher) were administered i.g. in doses equimolar to 50 or 10 mg/100 g of sucralfte 30 min before ethanol.

The animals were killed 1 hr after alcohol. The area of hemorrhagic mucosal lesions was measured by computerized planimetry of the projected stereomicroscopic image of the glandular stomach (11).

For the detection of vascular injury, 3% suspension of monastral blue B (Sigma) was injected at 0.1 ml/100 g into the jugular vein under light ether anesthesia 4 min before autopsy and 1 min after ethanol i.g. (9). At autopsy, the stomach was removed, opened along the greater curvature, pinned flat on cork board and fixed in 10% formaldehyde for 24 hr. The stomach was then cleared in 90% glycerol for 24 hr, mounted in glycerol jelly and examined under stereomicroscope. The area of monastral blue-labelled blood vessels in the glycerol-cleared stomachs was measured by computerized planimetry using the projected stereomicroscopic image (9, 11).

For statistical analysis, the two-tailed student's t-tests and the non-parametric Mann-Whitney U-test were used.

Results

In untreated or solvent pretreated animals, ethanol caused extensive hemorrhagic lesions in the glandular stomach. Most of these lesions were erosions involving about 6% and 20% of the glandular stomach in rats killed at 1 and 60 min respectively after i.g. administration of alcohol.

The initial dose-response studies revealed that unlike 2 mg/100 g, 10 and 50 mg/100 g sucralfate significantly decreased the ethanol-induced hemorrhagic mucosal lesions. Sucralfate was equally effective if solubilized in dilute HCl or Tween 80.

In subsequent studies only 10 and 50 mg/100 g of sucralfate and equimolar doses of its components were used. These results revealed that sucrose octasulfate, sodium sulfate and aluminum chloride, but not aluminum hydroxychloride administered at doses equimolar to sucralfate, 50 mg/100 g

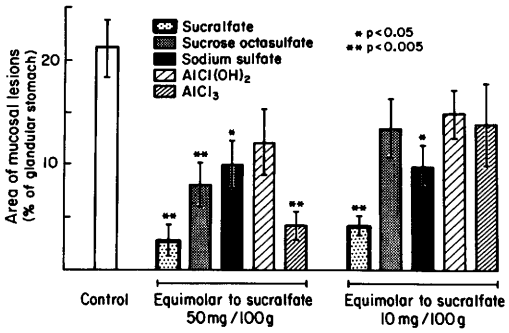

Figure 1. Effect of sucralfate and its components on ethanol-induced gastric erosions. Fasted rats were given sucralfate, 50 or 10 mg/100g, or equimolar amounts of its components i.g. 30 min before ethanol. The animals were killed 1 hr after alcohol.

significantly reduced the alcohol-induced gastric erosions (Fig. 1). At doses equimolar to 10 mg/100 g of sucralfate, only sodium sulfate was able to significantly decrease the hemorrhagic lesions in rats exposed to ethanol. A comparison of the efficacy of sucralfate with its components revealed that sucralfate was at least twice as potent than its constituents.

The effect of the SH alkylator NEM on the gastroprotective effect of sucralfate against ethanol is presented in Table 1. Sucralfate (10 mg/100 g) markedly reduced the area of hemorrhagic lesions, while NEM alone had no influence. When the i.g. administration of sucralfate was followed by s.c. injection of NEM, the gastroprotection was abolished.

In the vascular injury studies, sucralfate and its components administered at doses equimolar to 50 mg/100 g of sucralfate were used. The area of vascular injury as revealed by deposition of monastral blue particles in the damaged capillaries in the gastric pits in rats killed 1 min after 100% ethanol was always larger than the area of early hemorrhagic erosions (Fig. 2). Sucralfate and equimolar amounts of sucrose octasulfate, sodium sulfate and aluminum chloride significantly reduced the extent of vascular injury. At this early time (1 min) after alcohol only sucralfate and equimolar amount of sodium sulfate were able to significantly diminish

Table 1

The effect of the SH alkylator N-ethylmaleimide (NEM) on the gastroprotective effect of sucralfate against ethanol in the rat

Group	Pretreatment	Area of mucosal lesions (% of glandular stomach)
1.	None	25.3 ± 2.2
2.	Sucralfate (i.g.)	4.2 ± 1.0
3.	NEM (s.c.)	28.4 ± 3.6
4.	Sucralfate + NEM	32.2 ± 6.5 *

* = $p < 0.001$, comparison between groups 2 and 4.

Sucralfate (10 mg/100 g) was given i.g. 30 min before 100% ethanol (1 ml, i.g.) which was administered to rats of all groups. NEM (5 mg/100 g) was injected s.c. 10 min after sucralfate (i.e., 20 min before ethanol). Animals were killed 1 hr after alcohol.

the area of hemorrhagic erosions (Fig. 2).

Discussion

Our studies presented here confirm the gastroprotective properties of sucralfate and revealed that the entire molecule is not a prerequisite for protection against acute ethanol-induced gastric erosions. It appears that sodium sulfate and sucrose octasulfate (the moiety containing the sulfate radicals) are potent components of sucralfate although an equimolar amount of sucralfate is at least twice as effective in protecting the gastric mucosa against ethanol than its active constituents. An additive or potentiating interaction among components of sucralfate can thus be postulated.

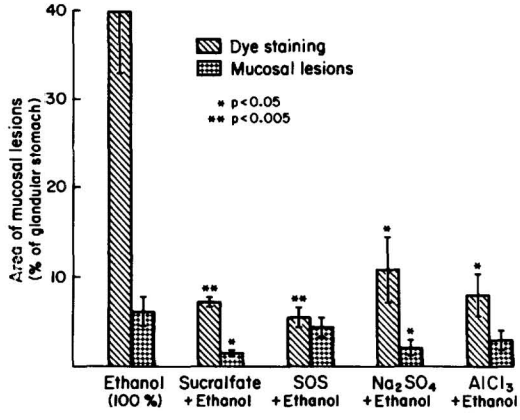

Figure 2. Influence of sucralfate (50 mg/100 g) and equimolar amounts of its components on vascular injury as revealed by monastral blue staining of damaged blood veseels and gastric mucosal lesions 1 min after ethanol in fasted rats. Pretreatment with drugs was given 30 min and the vascular tracer dye was injected i.v. 3 min before alcohol.

Our results on the effectiveness of sodium sulfate against gastric erosions are in agreement with studies demonstrating that sulfate is an active ingredient of sucralfate in protecting the esophageal mucosa against acid-induced injury (12). Sodium thiosulfate protects against aspirin-induced vascular injury and subsequent hemorrhagic erosions in the stomach (13). Thus, although examples exist concerning mucosal protection by sulfate, it is not clear whether sulfate is directly responsible for the beneficial effect or interaction with protein SH or other groups is required. Recent studies with other ions, especially with heavy metals, copper and aluminum, suggest that the interaction is with protein SH (7,8). Our present

data on the complete counteraction by NEM of the gastroprotective action of sucralfate suggest an involvement of protein SH. So far, only endogenous PG were implicated in mediating the action of sucralfate since indomethacin partially counteracted the gastroprotection by sucralfate (2). Gastric SH might thus be another endogenous mediator of sucralfate. Endogenous SH were implicated in the mechanism of action of PG as well since the SH alkylator NEM or iodoacetamide abolished the gastroprotection. NEM does not affect mucus secretion (14) but its effect on PG synthesis and bicarbonate secretion have not been examined.

Aluminum was recently shown to stimulate bicarbonate secretion which might contribute to mucosal protection (3). Our studies, however, demonstrated that only aluminum chloride and not the hydroxychloride, which is apparently the precursor in the synthesis of sucralfate, was active against ethanol-induced mucosal lesions. The role of aluminum and bicarbonate secretion in the mechanism of acute gastroprotection by sucralfate remains to be determined.

A novelty of the present results is that sucralfate and its components protect against vascular injury, which might be the target of an interation of diverse mucosal damaging and protective agents (7-10). The prevention or decrease of ethanol-induced endothelial injury in mucosal capillaries may result in the maintenance of blood flow (Pihan & Szabo, in preparation). Tarnawski et al. demonstrated that the preservation of the proliferative zone in the gastric mucosa is crucial for gastric cytoprotection (4). Cell proliferation and rapid restitution (15) are energy-requiring processes for which a supply of oxygenated blood is essential. Prevention of vascular injury and maintenance of mucosal blood flow may thus be a common link in the proposed mechanisms of action of mucosal protective agents.

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References

1. Marks IN, Samloff IM (eds.). Sucralfate in peptic ulcer disease and gastritis: A worldwide view. Amer J Med 79:(2C)1-64, 1985.
2. Hollander D, Tarnawski A, Gergely H, Zipser RD. Sucralfate protection of the gastric mucosa against ethanol-induced injury: a protaglandin-mediated process? Scand J Gastroenterol 19:(Suppl 101)97-102, 1984.
3. Crampton JR, Gibbons LC, Rees DW. Effect of certain ulcer-healing agents on amphibian gastroduodenal bicarbonate secretion. Scand J Gastroenterol 21:113-116, 1986.
4. Tarnawski A, Hollander D, Stachura J, Krause WJ, Gergely H. Prostaglandin protection of the rat gastric mucosa against alcohol injury - a dynamic time-related process. The role of the mucosal proliferative zone. Gastroenterology 88:334-352, 1985.
5. Szabo S, Majzoubi D, Brown A. Sucralfate and its components protect against ethanol-induced vascular damage and gastric mucosal injury. Gastroenterology 90:1654, 1986.
6. Szabo S, Trier JS, Frankel PW. Sulfhydryl compounds may mediate gastric cytoprotection. Science 214:200-202. 1981.
7. Dupuy D, Szabo S. Protection by metals against ethanol-induced gastric mucosal injury in the rat: comparative biochemical and pharmacologic studies implicate protein sulfhydryls. Gastroenterology 91:966-974, 1986.
8. Szelenyi I, Brune K. Possible role of sulfhydryls in mucosal protection induced by aluminum hydroxide. Dig Dis Sci 31:1207-1210, 1986.
9. Szabo S, Trier JS, Brown A, Schnoor J. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. Gastroenterology 88:228-236, 1985.
10. Leung FW, Robert A, Guth PH. Gastric mucosal blood flow in rats after administration of 16,16-dimethyl prostaglandin E2 at a cytoprotective dose. Gastroenterology 88:1948-1953, 1985.
11. Szabo S, Trier JS, Brown A, Schnoor J, Homan HD, Bradford JC. A

quantitative method for assessing the extent of experimental gastric erosions and ulcers. J Pharmacol Meth 13:59-66, 1985.
12. Tobey NA, Orlando RC, Schreiner VJ, Powers DW. Cytoprotective effect of sulfate ions in acid-exposed rabbit esophagus. Amer J Physiol 251:G866-G869, 1986.
13. Szabo S, Trier JS, Brown A, LaRoque M. Protection against aspirin-induced hemorrhagic gastric erosions and mucosal vascular injury by co-administration of sulfhydryl drugs. Gastroenterology 88:1604, 1985.
14. Lamont JT, Ventola AS, Maull EA, Szabo S. Cysteamine and prostaglandin F2B stimulate rat gastric mucin release. Gastroenterology 84:306-313, 1983.
15. Lacy ER, Ito S. Microscopic analysis of ethanol damage to rat gastric mucosa after treatment with a prostaglandin. Gastroenterology 83:619-625, 1982.

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