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Role of Surface-Active Phospholipids in Gastric Cytoprotection

Abstract. *Intragastric administration of a liposomal surfactant suspension markedly reduced acid-induced gastric ulcerogenesis and bleeding in rats. The concentration of surface-active molecules intrinsically present in the gastric mucosa was increased two to six times by administration of 16,16-dimethyl prostaglandin E₂. Thus, local accumulation of surface-active phospholipids may be an integral component of the cytoprotective mechanism activated by prostaglandin treatment.*

It is accepted that the amphoteric phospholipids secreted by alveolar type 2 cells are particularly active at the cell surface and play major roles in pulmonary mechanics and homeostasis (1). These phospholipids have been studied because of their ability to reduce surface tension at the interface between air and liquid (1, 2), but recently it was reported that the same class of surfactants can adsorb onto inert or biological membranes and enhance the hydrophobic properties of these surfaces (3). It is also recognized in the theory of corrosion inhibition that treatment of surfaces with cationic surfactants provides the underlying material with a hydrophobic coating that protects against hydrochloric acid and other noxious water-soluble agents in the environment (4).

Under normal conditions the mammalian gastric mucosa is protected from acid damage by a physicochemical barrier of unknown composition (5, 6). The integrity of this barrier may be compromised in patients who are predisposed to ulcer disease (7). Since surfactants may play a role in the maintenance of hydrophobic surfaces in the lung, it is possible that these substances have a similar function in the stomach. The gastric epithelium, like the pulmonary epithelium, has a highly hydrophobic surface with biophysical properties similar to those of polyethylene and other nonwetable substances (8). Several research groups including our own have identified surfactant compounds, previously thought to be unique to the lung, in both the gastric juice and mucosal surface of a variety of species including man (9). It is, therefore, of interest to investigate the role of extrinsic and intrinsic surface-active phospholipids in gastric cytoprotection.

Gastric necrosis and bleeding were induced in rats by a modification of the

technique of Robert *et al.* (10). Fasted male rats were anesthetized with pentobarbital, the pylorus was ligated, and a cannula was positioned in the gastric lumen from an esophageal opening. The animals were then treated via the esophageal cannula with saline or a liposomal phospholipid suspension. The composi-

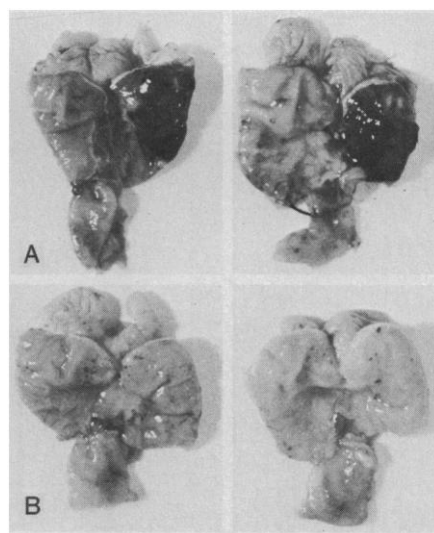


Fig. 1. Gross appearance of excised stomachs of pylorus-ligated rats treated with 1 ml of saline (A) or 1 ml of a surfactant mixture (B) 30 minutes before 1 ml of 0.6M HCl was administered intragastrically. The animals were killed 45 minutes after the acidic challenge. Stomachs were not heparinized when visually examined for ulcer formation. The surfactant mixture contained 135 μ g of DP phosphatidylcholine and 15 μ g of each of the following phospholipids: phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, and sphingomyelin. The organic solvent (ether) was removed by evaporation under N₂ and replaced with saline. A final aqueous liposomal suspension of the phospholipids was prepared by sonication of the mixture at 25°C for 1 minute. The suspension was stored at 4°C under N₂ until needed. In the controls the necrotic lesion formed only along the posterior wall of the stomach, where the acid pooled in the supine position.

tion of phospholipids in the mixture was based on the concentration of these substances in pulmonary surfactant (1). Thirty minutes later an ulcerogenic dose of hydrochloric acid was administered by the same route. After 45 minutes the animals were killed and their stomachs were excised, opened along the greater curvature, and examined.

Acid-induced gastric necrosis was markedly reduced by treatment with the surface-active phospholipids (Fig. 1). Surfactant administration did not provide protection by neutralization or dilution of the exogenous acid, since mean pH and volume of the gastric aspirates were virtually identical in the two groups (pH in control and surfactant-treated rats, 1.43 ± 0.03 and 1.41 ± 0.04 , respectively; volume, 4.6 ± 0.2 and 4.3 ± 0.2 ml, respectively; $N = 5$ per group).

Intragastric bleeding in control and surfactant-treated rats was estimated by measuring the concentration of hemoglobin in heparinized gastric aspirates (11, 12). This technique was validated by demonstrating that the concentration of intragastric hemoglobin paralleled the release of ⁵¹Cr-labeled red blood cells into the gastric juice in response to graded doses of exogenous HCl (0.15 to 0.6M) (Fig. 2A). Intragastric bleeding in surfactant-treated rats was 60 percent less than in controls (Fig. 2B).

Prostaglandins protect against gastric and duodenal ulcerogenesis and bleeding in both laboratory animals and man (10, 13). Evidence has also been obtained that prostaglandins are synthesized locally in the gastric mucosa and play a physiologically important role in protecting the tissue against damaging agents such as acid (13). It is, however, unclear how extrinsic or intrinsic prostaglandins exert their cytoprotective action. We therefore sought to determine (i) whether prostaglandins mediate protection induced by extrinsic phospholipids and (ii) whether the intrinsic phospholipids of the gastric mucosa mediate the cytoprotective mechanism initiated by the prostaglandins. The latter alternative seemed reasonable since prostaglandin treatment stimulates the secretion of surfactants by pulmonary alveolar cells (14).

The first possibility was investigated by treating rats with indomethacin, a selective inhibitor of prostaglandin synthesis (15), before the administration of the test agents. The dose of indomethacin employed was nonulcerogenic when administered alone but elicited marked aggravation of acid-induced bleeding (Fig. 2B). In addition, the phospholipid-induced reduction in gastrointestinal

Table 1. Prostaglandin-induced enhancement of intrinsic gastric mucosal phospholipids. Rats were injected intragastrically with PGE₂ (0.5 µg/kg) or saline and were killed 30 minutes later. The oxyntic mucosa of the stomach was dissected free of the serosa, weighed, and extracted in accordance with the method of Folch *et al.* (20). The extracted sample (30 to 50 µl) was then spotted onto the corner of a silica plate and separated by two-dimensional thin-layer chromatography (16). The phosphorus content of the resolvable spots (visualized by sulfuric acid and formaldehyde and scraped off the plates) was determined by the technique of Rouser *et al.* (17). Values (means ± standard errors) represent micrograms of phosphorus per gram of tissue (wet weight). Abbreviations: LPC, lysophosphatidylcholine; SP, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine. Values in parentheses are number of rats per group.

Treatment	Phospholipid					
	LPC	SP	PC	PI	PS	PE
Saline	3.87 ± 1.32 (5)	7.12 ± 2.10 (5)	6.93 ± 1.63 (5)	5.15 ± 1.60 (5)	2.10 ± 0.70 (4)	3.19 ± 1.54 (4)
Prostaglandin	6.28 ± 1.56 (5)	14.00 ± 3.65 (5)	34.18 ± 12.10 (5)*	12.08 ± 1.58 (5)†	5.90 ± 1.74 (5)	18.72 ± 1.59 (4)‡

**P* < .02 (unpaired Student's *t*-test). †*P* < .01. ‡*P* < .001.

bleeding (60 to 70 percent) was not affected by treatment with indomethacin. Thus it appears that prostaglandin synthesis is not required for extrinsic phospholipid-induced gastric protection.

We then determined the concentration of key surface-active phospholipids in the gastric mucosa of control rats and rats that had been treated for 30 minutes with a cytoprotective dose of 16,16-dimethyl prostaglandin E₂ (PGE₂). Prostaglandin treatment was associated with a

twofold to sixfold increase in the gastric mucosal concentration of the major surfactant species [as separated by thin-layer chromatography (16) and quantitated by phosphorus analysis (17)], with the greatest enhancements found in phosphatidylethanolamine and phosphatidylcholine (Table 1). In a separate experiment, we used ion-exchange chromatography (18, 19) to measure dipalmitoyl phosphatidylcholine. We determined that PGE₂ induced a twofold increase in

the gastric mucosal concentration of this phospholipid [the concentration of dipalmitoyl phosphatidylcholine in rats injected with PGE₂ (*N* = 9) was 199 ± 37 percent of that in control rats (*N* = 11)]. This enhancement of gastric phosphatidylcholine and phosphatidylethanolamine in response to prostaglandin treatment is of particular relevance to the aims of this study, since in a previous study we found that these constituents of pulmonary surfactant were the most efficacious antiwetting agents of all the amphoteric phospholipids tested, with the dipalmitoyl species being the most potent (3).

We have shown that intraluminal administration of surface-active phospholipids effectively protects the gastric mucosa from acid-induced necrosis and bleeding. We believe that these agents protect the tissue by forming an adsorbed hydrophobic layer between the gastric epithelium and the luminal contents. We have also demonstrated that the concentration of surface-active phospholipids in the gastric mucosa is markedly increased by treatment with prostaglandins. It is, therefore, possible that prostaglandin-induced cytoprotection is mediated in part by a localized increase in phospholipid concentration, which in turn enhances the hydrophobicity of the lining of the gastric mucosa.

LENARD M. LICHTENBERGER
LYNNE A. GRAZIANI
ELIZABETH J. DIAL
BRUCE D. BUTLER
BRIAN A. HILLS

Department of Physiology and
Cell Biology and Department of
Anesthesiology, University of Texas
Medical School, Houston 77025

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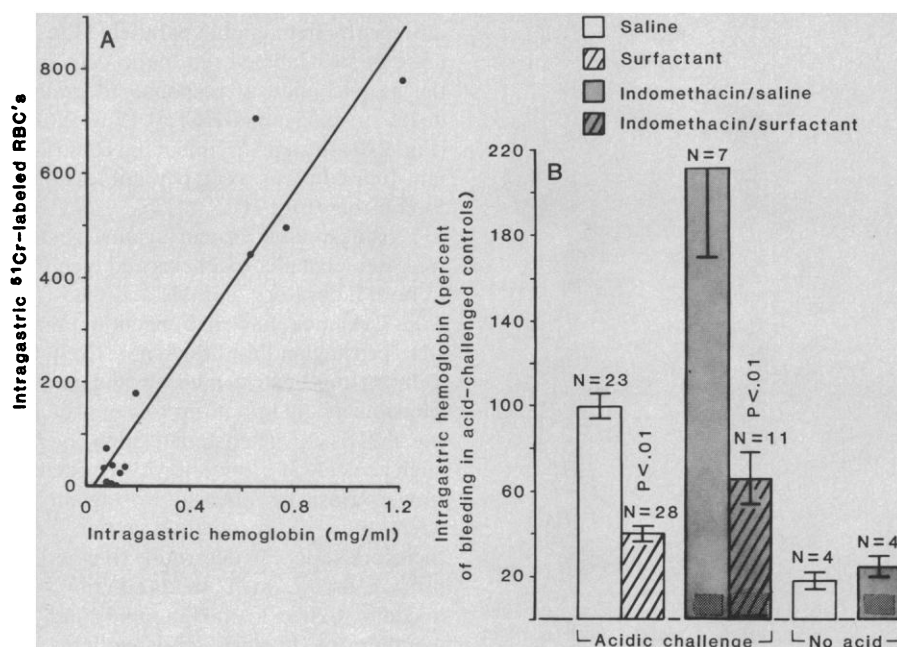


Fig. 2. (A) Linear dependence between the appearance of hemoglobin and ⁵¹Cr-labeled red blood cells in gastric aspirates from rats that were intraluminally challenged with graded doses of HCl (0.15, 0.30, 0.45, and 0.6M). Regression analysis revealed a significant relation (*t* = 10.51, *P* < .001) between these two estimates of intragastric bleeding, with a slope of 14.96 ± 1.42 (± standard error). Heparin (200 U/ml, 0.5 ml) was injected into the excised stomachs 5 minutes before the fluid was collected. Rodent red blood cells were labeled in vitro with ⁵¹Cr (21) and intravenously injected into the rats 10 minutes before they were exposed to the acid. Radioactivity of the gastric aspirates was measured in a Tracor-Analytic gamma counter. Intragastric hemoglobin was quantitated by benzidine analysis (11, 12). (B) Concentration of intragastric hemoglobin in rats treated with 1 ml of saline or the surfactant mixture (same concentrations of phospholipids as detailed in legend to Fig. 1) 30 minutes before being exposed to HCl. A second pair of groups received saline or surfactant plus indomethacin (5 mg/kg, subcutaneously) 2 hours before being killed (75 minutes before the acidic challenge). The concentration of intragastric hemoglobin was also determined in two additional groups of rats who were treated like the first pair described above, except that they received 1 ml of saline instead of the ulcerogenic dose of acid. Rats in one of these groups were subcutaneously injected with indomethacin (5 mg/kg) 2 hours before being killed. The intragastric hemoglobin concentration has been normalized as the percentage of gastrointestinal bleeding measured in rats that were treated with saline and subsequently exposed to acid.

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 12. The benzidine analysis was performed by incubating 25 μ l of the sample (gastric aspirate or hemoglobin standard) in a tube containing 0.5 ml of benzidine reagent (1 percent benzidine dihydrochloride in acetic acid) and 0.5 ml of 1 percent H_2O_2 at 25°C. After 20 minutes 5 ml of 10 percent acetic acid was added to the solution and the incubation was continued for an additional 10 minutes. Absorbance of the colored reaction product was then measured spectrophotometrically at a wavelength of 515 nm.
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Polypeptide Transforming Growth Factors Isolated from Bovine Sources and Used for Wound Healing in vivo

Abstract. *Transforming growth factors, which are polypeptides that induce the transformed phenotype in nonneoplastic cells, have been isolated in bulk amounts from bovine salivary gland and kidney. In experiments in which wound healing chambers were implanted subcutaneously in the backs of rats, these bovine transforming growth factors accelerated the accumulation of total protein, collagen, and DNA in treated chambers. These studies thus show an effect of an isolated transforming growth factor in vivo.*

Although many new peptide growth factors have been isolated and characterized (1), there have been few studies on the activity of these materials in vivo. An important area for potential application of peptide growth factors is the enhancement of wound healing. Despite the need for rapid healing in the treatment of severe burns, trauma, diabetic and decubitus ulcers, and other conditions, there is no practical way at present to accelerate wound healing with pharmacological agents. Although it has been suggested that epidermal growth factor (EGF) might be of benefit (2), it has not yet been extensively used in a practical way for wound healing. The ability of a related and newly discovered set of polypeptides, the transforming growth factors (TGF's), to promote growth of cells under highly restrictive conditions in vitro suggests that TGF's might have useful applications in vivo for wound healing. We now report a large-scale isolation of TGF's from readily available bovine sources and a demonstration of the in vivo activity of an isolated TGF in

an experimental wound healing system.

Transforming growth factors are a heterogeneous set of low molecular weight polypeptides defined by their ability to induce the transformed phenotype—particularly anchorage-independent growth in soft agar—in untransformed indicator cells that ordinarily do not grow in soft agar (3, 4). Transforming growth factors have been found in almost all tissues, both nonneoplastic and neoplastic, from many different species of animals, in-

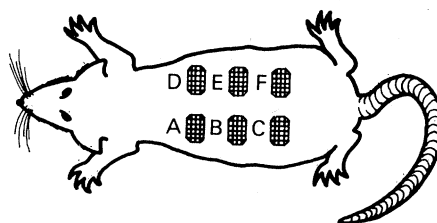


Fig. 1. Diagram of arrangement of wound healing chambers in the back of a rat. The chambers are made of stainless steel wire mesh and are 2 cm long and 1 cm in diameter. Male Buffalo rats, weighing 400 to 500 g and approximately 12 months old, were used in all experiments.

cluding man (4, 5). The finding of TGF's in blood platelets (6) suggests that TGF's may have a role in wound healing and tissue repair. Since TGF's have important functional interactions with EGF and its receptor, we have proposed a new classification of TGF's based on their relationships with EGF (7). Type α TGF's are those that compete with EGF for receptor binding and do not require EGF for induction of colony formation by indicator cells in soft agar, whereas type β TGF's do not compete with EGF for receptor binding, but do require the presence of EGF (or EGF-like polypeptides) for induction of colony formation in soft agar. Both types of TGF activity have been isolated from both neoplastic and nonneoplastic cells and tissues (3–8).

Full details of purification of bovine β -TGF's will be reported elsewhere. Briefly, salivary glands or kidneys, obtained fresh from the slaughterhouse and frozen immediately on dry ice, were extracted in 2-kg portions with acidified ethanol (8). Extracts from 6 to 8 kg of tissue were combined and chromatographed on Bio-Gel P-30 with 1M acetic acid on an 80-liter bed volume column (9). Most of the in vivo studies reported below were done with salivary gland or kidney TGF's purified to this stage; their activity in vitro was enhanced approximately 20-fold by the presence of 2 to 5 ng of EGF per milliliter in the assay. After chromatography on Bio-Gel P-30, the bovine β -TGF's were purified further by high-performance liquid chromatography (HPLC) on μ Bondapak C₁₈ columns for which an acetonitrile gradient in 0.1 percent trifluoroacetic acid was used; this was followed by a second HPLC step on μ Bondapak CN columns with a gradient of *n*-propanol in 0.1 percent trifluoroacetic acid (10).

Activity of isolated salivary gland and kidney β -TGF's in vivo was measured in a standard experimental wound healing model. Six empty wire mesh wound healing chambers (Schilling-Hunt) (11) were surgically inserted subcutaneously in the back of rats in a symmetrically paired fashion (pairs A and D, B and E, and C and F in Fig. 1). The animals respond to these chambers as if they were wounds, and eventually the chambers become filled with fibroblasts and collagen. By the fourth day after insertion, the chambers become encapsulated with connective tissue, but there are few cells within the chambers themselves. There is thus a defined, enclosed space within the chambers where a wound healing response can be quantitatively measured. At this time, daily injections of TGF (0.1 ml in sterile, phosphate-buffered saline) into