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ENHANCEMENT OF THE LIPID CONTENT AND PHYSICAL PROPERTIES OF GASTRIC MUCUS BY GERANYLGERANYLACETONE

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Abstract—The effects of intragastric administration of geranylgeranylacetone (GGA) on the content, composition and physical properties of the mucus component of the gastric mucosal barrier were investigated. One group of rats received twice daily for 3 consecutive days a dose of 100 mg/kg body weight of GGA, while the control group was subjected to daily doses of the vehicle. Sixteen hours following the last dose, the animals were killed, and their stomach was cut open and subjected to measurements of the adherent mucus gel content, analysis of its lipids and molecular forms of elaborated mucin, and evaluation of the viscosity and H^+ retardation capacity. The results revealed that GGA elicited a 62% increase in the adherent mucus gel and caused a marked decrease in the proportion of the lower molecular weight mucin. Furthermore, the mucus of the GGA group exhibited a 67% higher content of covalently bound fatty acids and contained 46% more total lipids which were greatly (143%) enriched in phospholipids. The physical measurements demonstrated that mucus elaborated in the presence of GGA also exhibited 2.3 times higher viscosity and had a 32% greater ability to retard the diffusion of H^+ than the mucus of the control group. The results suggest that GGA exerts a profound effect on the lipid content and the properties of gastric mucus associated with the maintenance of the mucosal integrity.

The layer of mucus covering the epithelial surfaces of gastric mucosa constitutes the first line of mucosal defense against the insults by a variety of exogenous and endogenous agents [1–3]. The extracellular localization and relative ease with which the underlying epithelium can control the quality of elaborated mucus make this layer an ideal renewable protective component of the mucosal barrier. Indeed, the continuous renewal and resilient nature of the gel efficiently counter its erosion by pepsin, assure the viscoelastic and permselective properties, and provide a milieu for neutralization of the diffusing luminal acid by mucosal bicarbonate [3–5]. The constituents of mucus represent a heterogeneous mixture of molecules that find their way to the mucosal surface, either by the process of active secretion or by passive transudation, and include proteins, glycoproteins and lipids [1, 5–7].

Although the gel-forming properties of gastric mucus are intimately associated with mucus glycoprotein [2, 8], evidence is rapidly mounting that the integrity and strength of mucus gel depend also on the content and composition of lipids [3, 5, 9]. Two types of lipids can be distinguished within the mucus gel: one that, through hydrophobic interaction, forms strong heterotypic complexes with mucin, and the other, that exists in a covalent linkage with the glycoprotein [10–12]. These lipids apparently determine the degree of resistance of mucin to peptic degradation and contribute significantly to mucus viscosity, hydrophobicity, and the impedance to hydrogen ion diffusion [5, 9, 13, 14]. Thus, the lipid content of mucus gel, along with its renewable quality, appears to play a major role in the inherent resistance of the mucosa to injury. While this resistance to insults is sufficiently maintained under normal physiological conditions, the situation changes drastically in gastric disease where the integrity of the mucosal defense has been compromised [1, 2, 15]. Among the agents designed to strengthen the gastric mucosal defense is a recently introduced antiulcer drug, geranylgeranylacetone [16–18]. The purpose of this study was to determine the effect of geranylgeranylacetone administration on the lipid content and composition of mucus gel adhering to gastric mucosa and to evaluate its viscoelastic and permselective properties.

MATERIALS AND METHODS

Animal preparation. The study was conducted with male Sprague–Dawley rats weighing 180–200 g. Each animal in the first group was given orally, twice daily for 3 consecutive days, a dose of 100 mg/kg body weight of geranylgeranylacetone (GGA) as an emulsion in 5% gum arabic and 0.6% Tween 80, while animals in the control group were exposed to daily doses of the vehicle. This dose of GGA has been demonstrated to be effective in ulcer treatment with animal models [16–19]. Following the last dose, the rats were fasted for 16 hr and then killed by decapitation. Their stomachs were immediately dis-

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sected, rinsed with cold saline, and subjected to measurements of the adherent mucus gel content, analysis of its mucin and lipid components, and evaluation of the viscosity and hydrogen ion retardation capacity.

Adherent mucus determination. The measurements of the adherent mucus gel content were performed according to the procedure of Corne *et al.* [20]. For this, freshly dissected stomachs were opened along their greater curvatures, and the glandular segments were excised and weighed. Each segment was transferred immediately to 10 ml of 0.1% Alcian blue solution containing 0.16 M sucrose in 0.05 M sodium acetate buffer, pH 5.8. After 2 hr of staining, the excess of dye was removed by soaking the segments in 0.25 M sucrose, first for 10 min and then for 45 min. The Alcian blue complexed with mucus gel adherent to the gastric walls and was extracted from the mucosa with 10 ml of 0.5 M $MgCl_2$ and vigorously shaken with an equal volume of diethyl ether [20, 21]. The resulting emulsion was centrifuged at 4000 rpm for 10 min, and the aqueous layer was used to determine the dye concentration by spectrophotometry at 598 nm. The quantity of Alcian blue extracted per g wet glandular tissue was then calculated from standard curves.

Mucus gel isolation. The gastric mucus gel used for chemical analyses and physical measurements was obtained by instilling the ligated stomachs with 0.10 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0 [3]. Immediately following excision, each stomach was thoroughly rinsed with cold saline to remove its content, and the ligature was placed at the pyloric ring. Using a polyethylene tube introduced through the esophagus, the stomach was instilled for 20 min to capacity with 0.10 M NaCl, 0.05 M phosphate buffer, pH 7.0. The recovered instillate was filtered through a Millopore HA (0.45 μm) filter, subjected to intrinsic pepsin inactivation (pH 9.0 at 37° for 30 min), dialyzed against distilled water, and lyophilized. The mucus obtained was used for physical measurements and chemical characterization.

Viscosity and H^+ retardation capacity measurements. Viscosity determinations were performed with a Brookfield cone/plate digital viscometer, model LVTDCP, equipped with a 1.565° cone and a constant (37°) temperature bath [5]. Shear rates were varied from 1.15 to 230 sec^{-1}, and the sample volumes were 0.5 ml. For the measurements, samples of mucus, dissolved at 30 mg/ml in 0.10 M NaCl, 0.05 M sodium phosphate buffer, pH 6.0, were gently stirred at 4° for 1 hr and then brought to 37°. To calculate the specific viscosity (η_{sp}), measurements were also taken of buffer alone.

The diffusion of H^+ through gastric mucus gel was measured in a specially constructed permeability chamber [9]. Individual samples of mucus, dissolved at 30 mg/ml in 0.15 M NaCl, were placed in the diffusion port separating the two compartments, filled on one side with 0.15 M NaCl and on the other side with 0.15 M HCl, and the change in pH in the NaCl compartment was recorded at 5-min intervals for up to 2 hr with a micro-pH electrode connected to an Accumet recording ionalyzer. The amount of H^+ diffusing through the sample was calculated in mol/sec and the permeability coefficient in cm/sec [3, 5].

Isolation and analysis of lipids. Extraction of lipids from the prepared samples of mucus was performed with chloroform–methanol (2:1 and 1:1, v/v) followed by chloroform–methanol–water (65:35:8, by vol.) [13]. The extracts were filtered through a grade-F sintered glass funnel, and the lipids contained in the filtrates were separated on silic acid columns into neutral lipid, glycolipid, and phospholipid fractions [10]. The neutral lipids were separated into individual components by thin-layer chromatography in hexane–diethyl ether–acetic acid (90:10:1, by vol.), identified by comparison with the chromatographic mobility of authentic standards, and recovered from the gel by elution with chloroform–methanol (4:1, v/v). Following removal of the solvent, the free fatty acids were esterified with diazomethane and analyzed by gas–liquid chromatography [5, 10, 22]. Mono-, di-, and triglycerides were subjected to acid methanolysis [22], and following removal of the fatty acid methyl esters by extraction with cold hexane, the glycerol contained in the lower methanolic phase was derivatized with trimethylsilyl reagent and quantitated by gas–liquid chromatography [22]. The free cholesterol and cholesterol derived from cholesteryl esters after saponification were determined colorimetrically [23].

The phospholipids, eluted from the silic acid column with methanol, were separated into individual compounds by two-dimensional thin-layer chromatography in chloroform–methanol–NH_4OH (65:25:4, by vol.), followed by chloroform–acetone–methanol–acetic acid–water (3:4:1:1:0.5, by vol.), identified by co-chromatography with appropriate standards, and recovered from the gel by elution with chloroform–methanol (1:4, v/v) and methanol [22]. Following removal of the solvents, the individual compounds were quantitated by measuring the phosphorus content [24].

The glycolipids, contained in the acetone–methanol (8:2, v/v) eluates from the silic acid column, were chromatographed on a DEAE-Sephadex A-25 (acetate form) column (0.5 × 10 cm) into neutral and acidic fractions [22], and separated into individual components by thin-layer chromatography in chloroform–methanol–water (65:30:8, by vol.) [10]. Following differentiation of glycosphingolipids and glyceroglucolipids by visualization with benzidine and orcinol reagents [10, 22], the individual compounds were subjected to acid methanolysis and analysis for their carbohydrate and lipid components [10, 22].

For the analysis of covalently bound fatty acids, the delipidated mucus preparations were incubated for 30 min at 37° with 0.3 M methanolic KOH, and the released fatty acid methyl esters were extracted with hexane [13].

Mucin molecular forms distribution. The dry samples of mucus were dissolved at 0.5 mg/ml in 0.10 M NaCl, 0.05 M sodium phosphate buffer, pH 7.0, containing 42% (w/w) CsCl at a loading density of 1.43 g/ml, and centrifuged for 48 hr at 12° and 46,000 rpm in a Beckman 50Ti rotor. The resultant gradient was fractionated into 1-ml fractions using a Beckman fraction recovery system, and

the densities of individual fractions were determined by refractive index measurement [10]. Each fraction was assayed for protein and carbohydrate [11], and the fractions containing mucus glycoprotein were pooled, dialyzed against distilled water, and lyophilized. The powder was dissolved at 10 mg/ml in 6 M urea, 10 mM sodium phosphate buffer, pH 7.0, and chromatographed on a Bio-Gel A-50 column equilibrated in and eluted with buffered 6 M urea, pH 7.0. The eluted fractions were monitored for protein and carbohydrate, pooled accordingly, and subjected to dialysis and lysophilization.

Analytical methods. The protein content of samples was measured by the method of Lowry *et al.* [25]. The phenol-H_2SO_4 method was used for monitoring the carbohydrate in column and density gradient fractions [26]. The content and composition of carbohydrate in various mucus and mucus glycoprotein preparations were determined by gas–liquid chromatography following methanolysis, re-N-acetylation and derivatization with silylating reagent [10, 12]. Gas–liquid chromatographic analyses of methyl glycosides, glycerol and fatty acid methyl esters were performed on 3% SE-30 columns according to previously described procedures [5, 9–12]. For the analysis of trimethylsilyl derivatives of glycerol and methyl glycosides, the temperature was programmed at 2°/min from 100 to 200°. The temperature program for fatty acid methyl esters was 150–290° at 2°/min [22]. All experiments were carried out in duplicate, and the results are expressed as means ± SD. Student's *t*-test was used to determine significance, and P values of 0.01 or less were considered significant.

Antiulcer drug. The geranylgeranylacetone (GGA) sample, Lot. No. K0501100, was donated by the Eisai Co., Tokyo, Japan. The drug was stored at 4° in the dark and was emulsified with 5% gum arabic and 0.6% Tween 80 shortly before each administration. The control animals received freshly made vehicle (aqueous solution of 5% gum arabic and 0.6% Tween 80). The drug or vehicle was given orally in a volume of 0.5 ml/100 g body weight through a dull metal tubing attached to a 2-ml syringe.

RESULTS

Evaluation of the mucus gel adhering to gastric mucosa by the Alcian blue uptake assay revealed that rats treated with GGA showed a 62% increase in the surface mucus as compared to that of the control group (Table 1). The results of chemical analyses, furthermore, showed that adherent gastric mucus of the GGA group exhibited a higher content of lipids and covalently bound fatty acids, but the levels of protein and carbohydrate were quite similar to those found in gastric mucus of the control group. The mucus of the GGA group, in comparison to that of the control, contained 46% more total lipids and 67% more covalently bound fatty acids (Table 1). Of the total lipids identified in the mucus of the GGA group, 43.7% were represented by neutral lipids, 28% by glycolipids and 28.3% by phospholipids. In mucus of the control group, the neutral lipids accounted for 57.9% of the total lipids, glycolipids 25.2%, and phospholipids 16.9% (Table 2).

Lipids derived from the mucus of each group of animals were of a similar composition. The neutral lipids were rich in free fatty acids, triglycerides, cholesterol and cholesteryl esters. The phospholipids exhibited a high content of phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and lysophosphatidylcholine, whereas the glycolipids consisted mainly of glyceroglucolipids. However, quantitative differences were noted. The netural lipids from mucus of the GGA group contained 20% less mono- and diglycerides, 28% more cholesterol, and 23% more cholesteryl esters than those of the control group (Table 2). The phospholipids from mucus of the GGA group showed a higher content of phosphatidylinositol, phosphatidylserine and sphingomyelin, whereas those isolated from mucus of the control group were richer in phosphatidylcholine and phosphatidylethanolamine (Table 3). Less pronounced differences, however, were observed in the content of glycolipids. In both types of samples, glyceroglucolipids were comprised of neutral (59%) and sulfated (41%) compounds, whereas the glycosphingolipids were represented mainly by glucosyl and lactosylceramides. The covalently bound fatty acids of the adherent mucus gel from control and GGA-treated animals exhibited a high content of hexadecanoic and octadecanoic acids. In each case, these two fatty acids accounted for over 75% of the total covalently bound fatty acids.

The effect of GGA administration on the viscosity of the adherent gastric mucus gel is illustrated in Fig. 1, while the data on H^+ retardation capacity are

Table 1. Effect of geranlygeranylacetone (GGA) on the content and composition of the adherent mucus gel in rat stomach

Component	Control	GGA
	(μg Alcian blue/g wet glandular tissue)	
Adherent mucus gel	131.9 ± 9.1	213.4 ± 17.2*
	(mg/100 mg mucus)	
Protein	68.5 ± 7.6	60.4 ± 6.9
Carbohydrate	10.3 ± 1.1	9.6 ± 1.2
Lipids	20.1 ± 1.7	29.3 ± 2.4*
Covalently bound fatty acids	0.3 ± 0.0	0.5 ± 0.1*

Values represent the means ± SD of duplicate analyses performed on the individual samples obtained from twelve animals in each group.
* $P < 0.001$.

Table 2. Effect of geranylgeranylacetone (GGA) on the lipid composition of the adherent mucus gel in rat stomach

Constituent	mg/100 mg mucus	
	Control	GGA
Free fatty acids	5.69 ± 0.71	6.51 ± 0.75
Mono- and diglycerides	0.74 ± 0.08	0.59 ± 0.07*
Triglycerides	2.74 ± 0.31	2.58 ± 0.30
Cholesterol	1.30 ± 0.14	1.66 ± 0.18*
Cholesteryl esters	1.19 ± 0.12	1.47 ± 0.17*
Glycosphingolipids	0.51 ± 0.10	0.37 ± 0.06*
Glucoglycerolipids	4.56 ± 0.50	7.84 ± 0.81*
Phospholipids	3.40 ± 0.23	8.29 ± 0.65*

Values represent the means ± SD of duplicate analyses performed on the individual samples of mucus obtained from twelve animals in each group.
* $P < 0.001$.

summarized in Table 4. Examination of the viscosity of gastric mucus from both control and GGA group samples over the employed range of shear rates showed that the specific viscosity of mucus from the control group ranged from 3.7 at the shear rate of 1.15 sec^{-1} to 1.5 at the shear rate of 230 sec^{-1}, whereas the specific viscosity of mucus from the GGA group at the lowest shear rate was 8.3 and at the highest shear rate 4.1 (Fig. 1). The data on the effect of GGA administration on the permeability of the adherent gastric mucus to H^+ revealed that the H^+ retardation capacity of mucus from GGA-treated rats was about 32% greater than that of gastric mucus of the control group.

The distribution of the molecular forms of mucin in the adherent gastric mucus of rats treated with GGA and that of control is depicted in Fig. 2. Chromatography on the CsCl equilibrium density

Table 3. Effect of geranylgeranylacetone (GGA) on the phospholipid composition of the adherent mucus gel in rat stomach

Phospholipid	% of Total lipid phosphate	
	Control	GGA
Phosphatidylcholine	29.4 ± 3.3	21.3 ± 2.4*
Phosphatidylethanolamine	14.3 ± 1.5	12.2 ± 1.3*
Phosphatidylserine	3.8 ± 0.4	6.0 ± 0.7*
Phosphatidylinositol	4.3 ± 0.5	8.1 ± 0.9*
Sphingomyelin	12.5 ± 1.1	15.3 ± 1.4*
Lysophosphatidylcholine	15.9 ± 1.5	14.5 ± 1.6
Phosphatidic acid	3.6 ± 0.3	3.8 ± 0.4
Phosphatidylglycerol	8.7 ± 0.7	8.0 ± 0.9
Diphosphatidylglycerol	3.5 ± 0.4	4.3 ± 0.4
Unidentified	4.0 ± 0.6	6.5 ± 0.8

Values represent the means ± SD of duplicate analyses performed on the individual samples from each group of animals.
* $P < 0.001$.

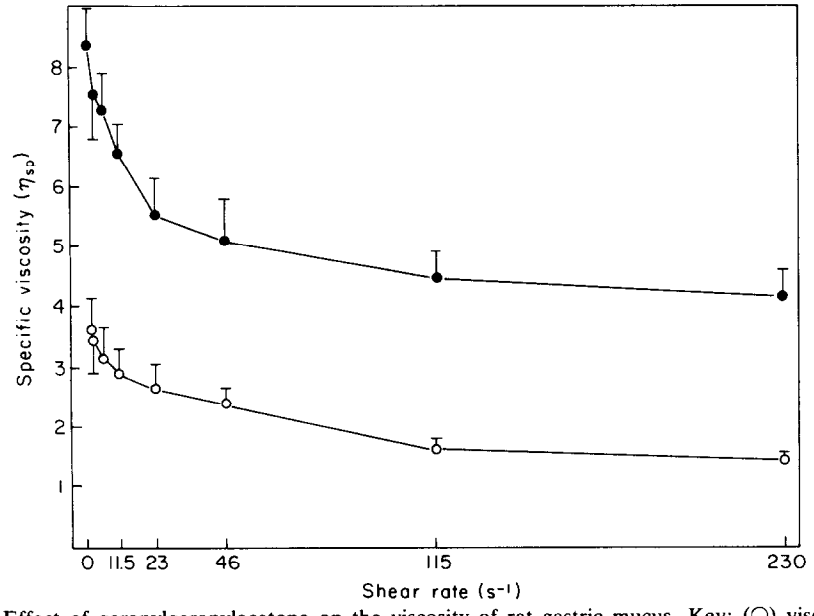

Fig. 1. Effect of geranylgeranylacetone on the viscosity of rat gastric mucus. Key: (○) viscosity of gastric mucus of the control group; and (●) viscosity of gastric mucus elaborated in the presence of geranylgeranylacetone. Values represent means ± SD of duplicate analyses performed on the individual samples obtained from twelve animals in each group. The changes in mucus viscosity evoked by the drug were significant at $P < 0.001$.

Table 4. Effect of geranylgeranylacetone (GGA) on the permeability of the adherent mucus gel to hydrogen ion in rat stomach

Type of sample	Permeability ($mol \cdot 10^{-10} \cdot sec^{-1}$)	Permeability coefficient ($cm \cdot 10^{-6} \cdot sec^{-1}$)
Adherent mucus of control animals	3.51 ± 0.27	5.88 ± 0.48
Adherent mucus of GGA-treated animals	2.63 ± 0.19*	4.45 ± 0.32*

Each value represents the means ± SD of twelve experiments.
* $P < 0.001$.

gradient-purified mucus glycoprotein on Bio-Gel A-50 revealed the presence of a high-molecular-weight ($M_r \approx 2 \times 10^6$) glycoprotein polymer in gastric mucin of the GGA group (Fig. 2A), whereas the mucus glycoprotein of the control group gave two distinct glycoprotein fractions (Fig. 2B). The high-molecular-weight fraction (Fig. 2B, fraction I) corresponded in its elution to mucus glycoprotein polymer ($M_r \approx 2 \times 10^6$), whereas the lower-molecular-weight fraction (Fig. 2B, fraction II) corresponded in its size (≈500 kDa) to that of gastric mucin subunit [2, 11].

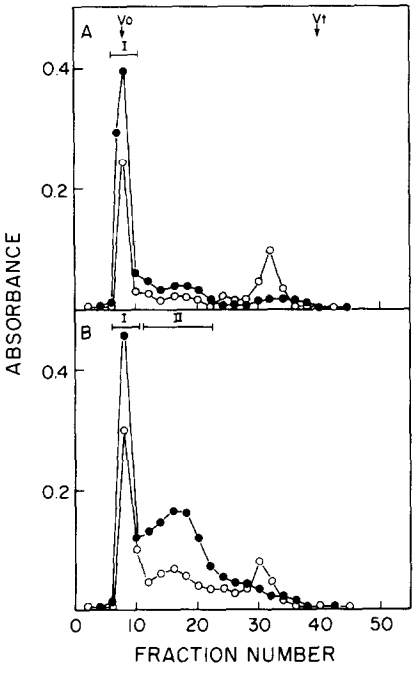

Fig. 2. Bio-Gel A-50 column chromatography in 6 M urea, 10 mM phosphate buffer, pH 7.0, of the CsCl density gradient-purified mucus glycoprotein derived from gastric mucus of rats receiving geranylgeranylacetone (A) and that of the control group (B). Samples of mucus glycoprotein, 7 mg each, recovered from the CsCl gradient were dissolved in 0.7 ml of 6 M urea, 10 mM phosphate buffer, pH 7.0, and applied separately to a column (0.9 × 120 cm). Fractions (2 ml) were collected and monitored for protein (absorbance at 280 nm, ○) and carbohydrate (phenol/H_2SO_4 method, ●). I, mucus glycoprotein polymer ($M_r \approx 2 \times 10^6$); II, mucus glycoprotein subunit ($M_r \approx 500$ kDa). Elution profiles are typical for individual samples representing each group. V_0 = void volume; V_t = total volume.

DISCUSSION

A unique quality of gastric mucosa is its ability to withstand the insults of a variety of endogeneous and exogeneous agents. The integrity of gastric mucosal defense depends upon a delicate dynamic equilibrium, controlled by factors affecting the synthesis, secretion and breakdown of its constituents [1, 2, 27]. While this equilibrium is maintained successfully under normal physiological conditions, injury to gastric mucosa ensues when aggressive forces overcome factors that control mucosal defense. Thus, as an approach to ulcer therapy, mucosal healing can be achieved either by reducing aggressive forces or by strengthening mucosal protective factors. Among the promising agents directed towards strengthening the gastric mucosal defense is a recently introduced drug, geranylgeranylacetone [16–19]. This acyclic polyisoprenoid, also known as teprenone, has been demonstrated to possess a remarkable ability to repair and restore gastric mucosal integrity by stimulating mucus elaboration and by slowing down the rate of its erosion [16, 17, 19].

The results presented in this report demonstrate that intragastric administration of geranylgeranylacetone not only causes a 62% increase in the content of mucus gel adhering to the gastric mucosal surface, but also affects the chemical composition of this protective layer. Our data show that the surface gastric mucus gel of rats receiving the drug, while displaying a protein and carbohydrate content and composition similar to those of the control group, exhibited a 46% higher content of lipids and contained 67% more covalently bound fatty acids. An increase in mucus gel adhering to gastric mucosa also occurs in response to drugs whose action evokes prostaglandin generation [28–30], whereas irritants such as bile salts, aspirin and lysolecithin are known to deplete the mucosa of its mucus coat [31–33]. Furthermore, studies on mucus elaborated along the gastrointestinal tract in response to luminal application of these agents indicated that drugs evoking prostaglandin generation also cause enrichment of the lipids in adherent mucus, whereas the depleting agents exert no effect on the mucus lipid content [12, 34]. Although the mucus lipid enrichment obtained with GGA is comparable to that reported for the prostaglandin precursor, arachidonic acid [12], the evidence for a prostaglandin mechanism of GGA action is not conclusive. Earlier data indicate that GGA is capable of preventing stress-induced ulcer formation by enhancing mucosal prostaglandin generation [35]. More recently, however, in studies with gastric epithelial cells it was found that, although

GGA has the ability to stimulate mucus production, this effect does not appear to be mediated by endogenous prostaglandins [17]. Yet other recent findings suggest that the protective effect of GGA against ethanol-induced damage to gastric mucosa can be diminished significantly with indomethacin pretreatment [18]. Thus, it is possible that the mucus lipid enrichment due to GGA may be independent of prostaglandin generation.

Because lipids present in alimentary tract secretions form strong heterotypic complexes with mucins that protect these glycoproteins from excessive degradation by proteolytic enzymes [13, 36], the elaboration of lipid-rich mucus evoked by GGA may be an important factor in the preservation of the polymeric structure of gastric mucin and, hence, the maintenance of surface mucus gel resilience and integrity. The results reported herein certainly support this contention, as the high-molecular-weight mucus glycoprotein polymer was found to be a major mucin component of gastric mucus of the GGA group, whereas gastric mucus of the control group contained a large proportion of its mucin in the lower-molecular-weight form.

The changes in quality of the adherent mucus elaborated by gastric mucosa in response to GGA were also reflected in the enhancement of such functional properties of mucus as the impedance of hydrogen ion diffusion and viscosity. The lipid-rich gastric mucus of the GGA group exhibited a 32% greater capacity to retard the hydrogen ion diffusion, and its viscosity was 2.3 times higher than that of gastric mucus of the control group. Since, as shown earlier, the viscoelastic and permselective properties of gastric mucus depend strongly upon the content and composition of lipids [3, 5, 9, 37], the GGA-induced increases in the adherent mucus gel lipid contents may be of direct relevance to the ability of gastric mucosa to meet successfully the hostile environment to which it is continuously exposed. The enrichment of mucus in lipids may bring marked changes in the hydrophobicity of the mucus layer and thus affect the perimeters of the first line of mucosal defense not only by increasing its resistance to penetration by a variety of noxious hydrophilic substances including HCl, but also by improving the surface mucus gel capacity to provide a milieu for more efficient utilization of the mucosal bicarbonate. It is thus conceivable that under physiological conditions, the increase in mucus lipids by GGA may have a greater impact on bicarbonate utilization than the retardation of hydrogen ion diffusion. That hydrophobicity of the mucosal surface plays an important role in preservation of gastric mucosal integrity has been demonstrated by studies on the mucosa with fluorescent probes and by goniometric procedures [14, 38].

From the data on lipid composition of the adherent mucus it is apparent that GGA exerted a most striking effect on the content of phospholipids and covalently bound fatty acid, levels of which increased 2.4- and 1.7-fold over the respective control values. These two lipid classes are also known to exert the greatest impact on the physicochemical characteristics of mucus [5, 9, 13, 14]. The covalently bound fatty acids determine the tenacity of heterotypic complexes formed between mucin and lipid components of mucus gel [36, 37], while the phospholipids by interacting with the nonglycosylated regions of mucins help to maintain the extended macromolecular structure of this glycoprotein in the aqueous environment of gastric lumen [5, 9]. The phospholipids have also been suggested to be involved in the maintenance of hydrophobic lining of the stomach [38–40]. These studies, however, attribute the hydrophobic properties of the mucosal surface to a layer of phospholipids underlying the mucus gel. In this concept, the so-called "surface active phospholipids" form a separate entity within the gastric mucosal defense system. As the constituents of gastric mucosal barrier form a dynamic continuum rather than exist as independent domains, assignment of the protective function either to the mucosal lipids [38–40] or only to mucus glycoprotein [2, 8, 28], obviously does not support the multicomponent nature of gastric mucosal protection. It is more likely that the inherent resistance of the mucosa to injury is maintained through the concerted effort of all of its constituents. This does not preclude that some constituents of the mucosal defense bear greater responsibility in the preservation of mucosal integrity.

Although the mechanism by which GGA affects the level of lipids in gastric mucus gel requires further elucidation, the data presented in this report provide strong evidence that this property of the drug may account for its beneficiary effect on ulcer healing.

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