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ENHANCEMENT OF THE PHYSICOCHEMICAL QUALITIES OF GASTRIC MUCUS BY SOFALCONE

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The effect of prolonged administration of an antiulcer drug, sofalcone, on the physicochemical properties of gastric mucus was investigated. The experiments were conducted with groups of rats receiving twice daily for three consecutive days a dose of 100 mg/kg sofalcone, while the control group received daily doses of vehicle. The rats were sacrificed 16 h after the last dose and gastric mucosa subjected to physicochemical measurements. The results revealed that sofalcone evoked a 23% increase in mucus gel dimension, while sulfo- and sialomucins content of the gel increased by 54 and 25%, respectively. These changes were accompanied by a 16% increase in mucus H+ retardation capacity, 2-fold increase in viscosity, and a 39% increase in the gel hydrophobicity. The mucus elaborated in the presence of sofalcone contained 67% more covalently bound fatty acids, exhibited 10% lower content of protein, 30% higher content of carbohydrate, and 18% higher content of lipids. The mucus of the sofalcone group also showed an increase in the proportion of the high molecular weight mucus glycoprotein form, which in the control group accounted for about 30% of gel mucin, while its content in mucus gel of animals receiving sofalcone reached the value of 50%. The results indicate that sofalcone enhances the protective qualities of mucus component of gastric mucosal barrier.

Key words: Sofalcone, gastric mucus, physicochemical properties, mucosal protection

INTRODUCTION

Although the mechanism of gastric mucosal protection is multicomponential in nature, the concensus is that the initial brunt of luminal insult falls on the layer of mucus which constitutes the only barrier between the gastric lumen and the surface epithelial cells of the mucosa (1—3). The resilient nature of this layer efficiently counters peptic erosion of the gel, assures its viscoelastic and permselective properties and provides a milieu for containment of the diffusing luminal acid by mucosal bicar-
bonate (2, 3, 4—6). Disturbances in this delicate balance lead to the impairment of the protective function of mucus layer resulting in gastric disease. The weakening of gastric mucosal defense is intimately associated with the diminished viscoelastic qualities of mucus, decrease in hydrogen ion retardation capacity, and the extensive peptic erosion (2, 7, 8). Hence, agents capable of strengthening the protective qualities of mucus gel are becoming increasingly popular in peptic ulcer therapy. Among the agents in this category is asynthetic analogue of sophoradine, known as sofalcone (9—12).

Evaluation of the mechanism of sofalcone action requires delineation of its direct effect on the physicochemical characteristics of the mucus gel from that brought about in the chemistry of mucus constituents following the drug administration. In the previous studies with sofalcone, we have shown that the direct effect results from its interaction with the surface mucus gel of gastric mucosa leading to the enhancement in mucus viscosity, hydrogen ion retardation capacity, and resistance to peptic degradation (12). We report here the effect of prolonged sofalcone administration on the physicochemical characteristics of gastric mucus gel.

MATERIALS AND METHODS

Animals

The study was conducted with two groups of male Sprague-Dawley rats weighing 180—200 g. Each animal in the first group was given intragastrically, twice daily for three consecutive days, a dose of 100 mg/kg body weight of sofalcone in 1 ml of 5% gum arabic, while animals in the control group were exposed to daily doses of 5% gum arabic. Following the last dose, the rats were fasted in individual wire-bottom cages for 16 h and then killed. Their stomachs were immediately dissected, rinsed with cold saline, opened along the greater curvature, and the surface mucus gel subjected to the measurements of physical and chemical characteristics.

Mucous coat dimension and its mucin content

Sections of gastric mucosa, luminal surface up, were mounted on a Millipore filter base and cut into strips of 1.6 mm in width (13). Except for being sectioned, the tissue was kept immersed in 0.15 M NaCl solution. Cut strips were mounted transversely in Petri dishes and their positioning checked stereoscopically. Throughout the procedure, care was taken not to distort the mucosa by stretching or compression. Such prepared sections of gastric mucosa were subjected to mucus coat thickness measurement by means of an inverted microscope (100 magnification). The distance between the bathing solution-mucus layer interface and mucus-glandular mucosa epithelial surface interface was recorded with the aid of eyepiece graticule (8).

For the measurements of mucus gel mucin content, the glandular segments of freshly dissected stomachs were excised, weighed, and placed in 10 ml of 0.1% Alcian blue
solution containing 0.16 M sucrose in 0.05 M sodium acetate buffer, pH 5.8, for sulfoand sialomucins or in 0.05 M citrate, pH 2.0, for sulfomucin measurements. After 2h of staining, the excess of dye was removed by soaking the segments in 0.25 M sucrose (14). The dye complexed with mucin adherent to the gastric wall was extracted from the mucosa with 10 ml of 0.5 M MgCl₂ and vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged, and the separated aqueous layer was used to determine the dye concentration by spectrometry (14).

**Mucus gel isolation**

The gastric mucus gel used for chemical analyses was obtained by blotting the individual opened stomachs with Whatman No. 3 filter paper (15). The mucus, transferred on to the filter paper, was then recovered by washing the paper with 1.0 M NaCl in 0.05 M sodium phosphate buffer, pH 7.0. Such isolated mucus was filtered through a Millipore HA (0.45 μm) filter, subjected to intrinsic pepsin inactivation (pH 9.0 at 37°C for 30 min), uialyzed against distilled water, and then lyophilized.

**Viscosity and hydrogen retardation capacity**

Viscosity determinations were performed with a Brookfield cone/plate digital viscometer, model LVTDCP, equipped with a 1.565° C cone and a constant (37°C) temperature bath (6). Shear rates were varied from 1.15 to 230 s⁻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°C for 1 h and then brought to 37°C. To calculate the specific viscosity (nsp), measurements were also taken of buffer alone.

The diffusion of H⁺ through gastric mucus gel was measured in a permeability chamber (14). 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 2h with a micro-pH electrode connected to an Accumet recording ionalyzer (15).

**Mucus hydrophobicity**

The hydrophobicity of the isolated gastric mucus gel was evaluated using a fluorescent hydrophobic probe, bis (8-anilino-1-napthalenesulfonate) (bis-ANS) (16). The measurements were conducted with Perkin-Elmer model LS-5 fluorescent spectrophotometer. The excitation wavelength for the probe was 365 nm and the maximum emission was observed at 530 nm. The samples, dissolved at 2 mg/ml in 0.10 M NaCl/0.05 M sodium phosphate buffer, pH 7.0, were reacted at 27°C with increasing amounts of bis-ANS and the induced alterations in the emission spectra of the probe were evaluated (16).

**Mucin molecular form distribution**

The 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 h at 12°C and 46,000 rpm in a Beckman 50 Ti rotor. The resultant gradient was fractionated into 1 ml fractions using a Beckman fraction recovery system (11, 14). Each fraction was assayed for protein and carbohydrate, and the fractions
containing mucus glycoprotein were pooled, dialyzed against distilled water, and lyophilized (14). 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 lyophilization.

**Analytical methods**

For the analysis of lipids, individual samples of mucus powder were extracted with chloroform-methanol. The extracts were filtered through sintered glass funnel, and the lipids contained in the filtrates were separated on silicic acid columns into neutral lipid, glycolipid and phospholipid fractions (5). The neutral lipids were separated into individual components by thin-layer chromatography, identified by comparison with chromatograms of authentic standards, and quantitated (5, 14). The glycolipids were quantitated by measuring their carbohydrate and lipid constituents (5) and the phospholipid were analyzed by measuring their phosphorus content (17). The content and composition of carbohydrate in the prepared mucus samples were determined by gas-liquid chromatography (16), and the protein was measured by the method of Lowry et al. (18). All experiments were carried out in duplicate, and the results were expressed as means±SD. Student’s t test was used to determine significance, and P values of 0.05 or less were considered significant.

**Antiulcer drug**

The sofalcone powder, lot No. 01WV-07, was kindly donated by Taisho Pharmaceutical Co., Ltd., Tokyo, Japan. The drug was stored at 4°C in the dark and was emulsified with 5% gum arabic shortly before experimentation. The drug or vehicle (5% gum arabic) was given orally in a volume of 1 ml through a dull metal tubing attached to a 2 ml syringe.

**RESULTS**

Examination of gastric mucosal surface by means of an inverted microscope technique revealed three distinct regions under light- and dark-field illumination or phase contrast. These were identified as bathing solution, layer of mucus gel, and the glandular mucosa. By examining 10 sections taken from glandular mucosa of each animal used in the study, it was found that in the control group, the dimension of the adherent mucus gel averaged 207 μm, while its thickness in the animals receiving sofalcone was 255 μm, which represents a 23% increase over the control value (Table 1).

The Alcian blue uptake assays, conducted at pH 2.0 for sulfomucin and at pH 5.8 for sulfo- and sialomucins, indicated that the animals treated with sofalcone showed a 54% increase in sulfomucin and 25% increase in sialomucin content of the gel (Table 1).

The data on the effect of sofalcone administration on the permeability
Table 1. Effect of prolonged sofalcone administration on gastric mucus dimension and its mucin content.

<table>
<thead>
<tr>
<th>Type of measurement</th>
<th>Control</th>
<th>Sofalcone</th>
</tr>
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<tbody>
<tr>
<td>Gel thickness (μm)</td>
<td>207 ± 19</td>
<td>255 ± 26*</td>
</tr>
<tr>
<td>Mucin of adherent mucus gel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μg Alcian blue/g wet tissue (pH 5.8)</td>
<td>162 ± 13</td>
<td>204 ± 25*</td>
</tr>
<tr>
<td>μg Alcian blue/g wet tissue (pH 2.0)</td>
<td>96 ± 13</td>
<td>148 ± 25*</td>
</tr>
</tbody>
</table>

Values represent the means ± SD of duplicate analyses performed on 12 animals in each group. *P < 0.05 change from the control value.

Fig. 1. Effect of prolonged sofalcone administration in the permeability of gastric mucus to H+. Values are plotted as mean ± SD (n = 12) of the amount of H+ diffusing through the sample. Asterisk indicates a significant (p < 0.05) difference as compared to that of the control.

of gastric mucus to H+ revealed that the H+ retardation capacity of mucus gel from sofalcone-treated animals was 16% higher than that of gastric mucus of the control group (Fig. 1). The specific viscosity of mucus...
from the control group ranged from 4.4 at the shear rate of 1.15 s\(^{-1}\) to 1.9 at the shear rate of 115 s\(^{-1}\), whereas the specific viscosity of gastric mucus gel from sofalcone group over the employed range of shear rates was about two times greater (Fig. 2).

![Graph showing specific viscosity vs shear rate](image)

**Fig. 2.** Effect of prolonged sofalcone administration on the viscosity of gastric mucus. Values represent mean ± SD of duplicate analyses performed on the individual samples of mucus obtained from 12 animals in each group. The changes in mucus viscosity evoked by the drug were significant at \(p < 0.05\).

The results obtained with the fluorescent hydrophobic probe indicated that in the absence of mucus, its maximum emission was recorded at 530 nm, while in the presence of mucus, the maximum shifted to 490 nm. The shift induced by gastric mucus in the emission maximum of the bis-ANS probe was accompanied by 6.2-fold increase in fluorescent intensity of probe in the presence of gastric mucus from animals treated with sofalcone increased by 8.6-fold (Fig. 3). This represents a 39% increase in mucus gel hydrophobicity over the control value.

Chromatography of the CsCl density gradient-purified mucin preparations on Bio-Gel A-50 m gave two major glycoprotein fractions for each group. The excluded peak represented high molecular-weight mucus
Relative fluorescence

Control | Control | Experiment
Sofalcone+bis-ANS | Mucus+bis-ANS | Mucus+bis-ANS

Fig. 3. Effect of prolonged sofalcone administration on the hydrophobicity of gastric mucus. Values are plotted as means±SD (n = 12) of the increase in relative fluorescence of hydrophobic probe (bis-ANS) due to mucus binding. Asterisk indicates a significant (p < 0.05) increase in hydrophobicity over the control value.

glycoprotein polymer (Mr ± 2 x 10^2 kDa), while the glycoprotein emerging in the included volume (Mr 500 kDa) was molecular-weight mucin. The mucus glycoprotein polymer constituted 30% of the mucin fraction in the control group, while in the sofalcone-treated animals, the polymeric form of mucus glycoprotein accounted for 50% of mucin (Fig. 4).

The chemical composition of gastric mucus elaborated in the presence and absence of sofalcone administration is shown in Table 2. The data indicate that mucus of the sofalcone-treated animals exhibited 10% lower content of protein, 30% higher content of carbohydrate, and 18% higher content of total lipids and 67% higher content of covalently bound fatty acids. Lipids derived from the mucus of each group of rats were of similar composition and consisted of neutral lipids, glycolipids and phospholipids. However, compared to the controls, the mucus elaborated in the presence of sofalcone showed a 20% increase in phospholipids. The
Fig. 4. Effect of prolonged sofalcone administration on the distribution of molecular forms of mucin in gastric mucus. Values are plotted as means±SD (n = 12) of the percent of total. Asterisks indicate significant (p < 0.05) difference as compared to that of the controls.

Table 2. Effect of prolonged sofalcone administration on the chemical composition of mucus gel in rat stomach

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Control</th>
<th>Sofalcone</th>
</tr>
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<tbody>
<tr>
<td>Protein</td>
<td>67.4±7.1</td>
<td>60.5±6.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>11.0±1.3</td>
<td>14.2±1.5*</td>
</tr>
<tr>
<td>Lipids (total)</td>
<td>20.0±2.3</td>
<td>24.3±2.6*</td>
</tr>
<tr>
<td>Neutral lipids</td>
<td>12.0±1.3</td>
<td>13.9±1.6</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>5.1±0.7</td>
<td>6.3±0.9</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>3.4±0.4</td>
<td>4.1±0.4*</td>
</tr>
<tr>
<td>Covalently bound fatty acids</td>
<td>0.3±0.1</td>
<td>0.5±0.2*</td>
</tr>
</tbody>
</table>

Values represent the means±SD of duplicate analyses performed on 12 animals in each group. *P < 0.05 change from the control value.

Neutral lipids in both types of samples were rich in free fatty acids, triglycerides and cholesterol. The phospholipids exhibited a high content of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin, and the glycolipids consisted mainly of glyceroglucolipids.

DISCUSSION

Sofalcone, a synthetic analogue of sophoradine, an ancient Chinese remedy for dyspepsia, is a potent antiulcer agent mode of action of which includes inhibition of prostaglandin-inactivating enzyme, stimulation of the gastric blood flow, acceleration of the gastric mucosal repair and
the biosynthesis of mucin, enhancement of mucus resistance to peptic degradation, and the improvement of its viscoelastic and acid retardation properties (9—12, 19). Furthermore, there are indications that sofalcone is also capable of inhibition of the protease and lipase enzymes associated with H. pylori proliferation (3, 20). While these data clearly point towards the value of sofalcone in peptic ulcer treatment, most of the information on the mechanism of this drug action is derived from acute animal models (21), whereas patients undergoing ulcer therapy require prolonged administration of the drug.

The results obtained herein demonstrate prolonged intragastric administration of sofalcone causes alteration in the chemical composition and physical properties of gastric mucus gel indicative of the mucosal strengthening action of the drug through its enhancement of the protective qualities of the adherent mucus coat. Our data show that mucus gel of animals receiving sofalcone displayed a marked increase in its dimension and exhibited enhanced viscoelastic, hydrophobic and H⁺ retardation qualities. This improvement in physical characteristics of the mucus coat apparently resulted from the increased mucus content of lipids, and sulfo- and sialomucins, the sialic acid and sulfate ester groups of which have been shown to play a major role in the maintenance of viscoelastic and permselective properties of gastric mucin (22).

The details of chemical analyses revealed that gastric mucus from sofalcone-treated animals exhibited a 10% lower content of protein, 30% higher content of carbohydrate, 18% higher content of total lipids, and 67% higher content of covalently bound fatty acids than that of the controls. Furthermore, lipids derived from mucus of sofalcone-treated animals showed a significant (20%) enrichment in phospholipids. The alterations in gastric mucus gel evoked by sofalcone, thus differ from those elicited by other mucosal strengthening agents, such as geranylgeranylacetone or sucralfate (14, 23). In the case of geranylgeranylacetone, prolonged intragastric administration elicits significant increase in the lipid content of mucus gel, but has no effect on the content of protein and carbohydrate, while prolonged administration of sucralfate leads to changes in mucus gel neutral lipid and mucin content, but only marginal changes are observed in the gel dimension and its H⁺ retardation capacity. Since, as shown earlier, the permselective and hydrophobic properties of gastric mucus depend mainly upon its content of mucin and phospholipids (3, 5, 14, 16), the sofalcone-induced increase in the adherent mucus gel mucin and phospholipid content could be of direct relevance to the ability of gastric mucosa to successfully meet the hostile luminal environment by enhancing its resistance to penetration by a variety of noxious hydrophilic substances, including HCl.
While gastric mucosal lipids have been recognized for some time to play an important role in mucosal defense (2, 5, 22, 24, 25), there are differences of opinion as to the way by which they contribute to this function. While some investigators assign the lipid protective function to the so-called “surface active phospholipids” supposedly forming an entity separate from other components of the mucus gel (25—27), our data indicate that lipids are integral part of mucus gel where, together with mucins, form a dynamic continuum and that this complex is responsible for the maintenance of gastric mucosal integrity (2, 3, 5, 14). Thus, the integrity of gastric mucosal defense system depends upon a delicate balance, controlled by factors affecting the elaboration and breakdown of all mucus gel constituents, and not just that of “surface active phospholipids” (25—27), or for that matter, by mucus glycoproteins alone, as suggested by others (1, 7).

As phospholipids form strong heterotypic complexes with mucins that protect these glycoproteins from excessive degradation by pepsin (2, 22), the elaboration of phospholipidrich mucus evoked by sofalcone may be an important factor in the preservation of the polymeric structure of gastric mucin and, hence, the maintenance of surface mucus gel integrity and resilience. Our findings herein on mucin molecular forms distribution certainly attest to the drug’s ability to enhance the high molecular weight mucin content of the gel. As a decrease in the proportion of the high molecular weight form of mucin is a prominent feature of the mucus gel of patients with gastric disease (8, 28), the ability of sofalcone to enhance the gel content of this mucin form could be viewed as another important aspect of the drug’s mode of action.

With the increasing understanding of the mechanism of mucosal strengthening drugs action, it is becoming apparent that the beneficial effects of these agents can manifest through many different venues. Since the mechanism of gastric mucosal protection is multicomponential in nature, each of these agents has value in ulcer therapy. Our data, presented herein, demonstrate that the evaluation of physicochemical parameters of gastric mucus gel provides an important information in the assessment of efficacy of antulcer drugs, such as sofalcone, which are tailored towards strengthening the mucosal defense.

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REFERENCES


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