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FATTY ACID ACYLATION OF MUCIN BY GASTRIC MUCOSA: EFFECTS OF SOFALCONE AND SUCRALFATE

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Abstract—The effects of antiulcer drugs, sofalcone and sucralfate, on the activity of gastric mucosal mucus glycoprotein fatty acyltransferase were investigated. The acyltransferase enzyme, contained in the detergent extracts of the microsomal fraction of rat gastric mucosa, was incubated with the deacylated gastric mucin and palmitoyl-CoA substrates in the presence and absence of drugs, and the formed fatty acid acylated glycoprotein product was quantitated. In the absence of drugs, the enzymatic activity increased proportionally with increased concentrations of both substrates and of enzyme, and gave an apparent K_m value of 5.6×10^{-7} M. Introduction of sofalcone to the reaction mixtures led to an enhancement in the rate of mucus glycoprotein acylation. The rate of enhancement was proportional to sofalcone concentration up to 1.0×10^{-5} M, with an apparant K_m value of 3.7×10^{-7} M. In contrast to sofalcone, the acyltransferase activity was inhibited by sucralfate. The rate of inhibition of mucus glycoprotein acylation by sucralfate was of the competitive type and at 1.0×10^{-4} M reached a value of 25%. The apparent K_I value calculated from the double-reciprocal plots for sucralfate was 9.1×10^{-7} M. As the acylation of mucin with fatty acids plays an important role in the maintenance of gastric mucosal integrity, the results suggest that stimulation of the fatty acyltransferase enzyme by sofalcone may be one of the beneficial effects of this drug towards ulcer healing.

A unique property of gastric mucosa is its ability to withstand the multitude of insults by a variety of endogenous and exogenous agents. Among the components to which the defense of gastric mucosal integrity is most often assigned are the viscous layer of mucus and the cell membranes of gastric epithelium [1–3]. While a number of processes occurring in the mucosal elements offer an array of mechanisms of gastric mucosal protection, the initial brunt of luminal insult falls on the mucus layer which constitutes the only identifiable physical barrier between gastric lumen and the surface epithelial cells of the mucosa [1, 3, 4].

The constituent responsible for the viscoelastic properties of mucus, its strength, and resilience is mucus glycoprotein (mucin) [5–7]. Structural data on gastric mucin indicate that this high molecular weight, extensively glycosylated glycoprotein, in addition to protein and carbohydrate, also contains covalently bound fatty acids [8–10]. These ester-bound fatty acids determine the degree of mucin resistance to peptic degradation and contribute significantly to the glycoprotein viscosity, hydrophobicity, and the impedance of hydrogen ion diffusion [9, 11, 12]. Thus, the extent of gastric mucin acylation with fatty acids along with the renewable quality of the mucus coat appear to play a major role in the inherent resistance of the mucosa to injury. While this resistance to insults is maintained at its best under normal physiological conditions, the situation changes drastically in gastric disease where the integrity of gastric mucosal defense is compromised [1, 2, 5]. Among the agents designed to strengthen the gastric mucosal defense are the recently developed antiulcer drugs, sucralfate and sofalcone [13, 14]. The mechanism of the action of sucralfate includes provision of a protective physical coating impeding the diffusion of acid and pepsin, and the inhibition of mucin degradation [15–18], whereas the sofalcone is known to stimulate the gastric blood flow and to accelerate the gastric mucosal repair and biosynthesis of mucin [19, 20]. Our aim in this study was to evaluate the effects of these two drugs on the activity of gastric mucosal acyltransferase enzyme responsible for the transfer of fatty acids from fatty acyl-CoA to gastric mucin.

MATERIALS AND METHODS

Preparation of enzyme. Male rats (180–200 g), fasted overnight prior to experiment, were killed by cervical dislocation, and their stomachs were removed, opened along the lesser curvature, and washed with cold 0.15 M NaCl. The mucosa was scraped with a glass slide, suspended in 5 vol. of 3 mM phosphate buffer, pH 7.0, containing 0.25 M sucrose and 1 mM EDTA, and disintegrated with a glass–Teflon homogenizer (900 rpm, 3 strokes). The homogenate was centrifuged at 800 *g* for 10 min, the pellet was discarded, and the supernatant fraction was centrifuged at 10,000 *g* for 20 min to sediment the crude mitochondrial fraction [21]. The post-

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mitochondrial supernatant fraction was then separated into microsomal (pellet) and cytosol (supernatant) fractions by centrifugation at 100,000 *g* for 1 hr. The microsomal pellet was suspended in 0.25 M buffered sucrose, pH 7.0, containing 0.5% Triton X-100, stirred at 4° for 30 min, and centrifuged at 100,000 *g* for 1 hr, and the supernatant was used as an enzyme source. Protein content was determined by the method of Lowry *et al.* [22].

Mucin acceptor preparation. The undegraded mucus glycoprotein was prepared from gastric mucus of rat stomachs [21, 23]. The mucus, dissolved in 6 M urea, was chromatographed on a Bio-Gel A-50 column, and the excluded mucus glycoprotein peak was collected. Following rechromatography, the glycoprotein was subjected to equilibrium density gradient centrifugation in CsCl, dialyzed against distilled water, and lyophilized [23]. Results of chemical analyses indicated that purified mucin contained 13.7% protein, 66.5% carbohydrate, 15.6% associated lipids, and 0.4% covalently bound fatty acids. Removal of the covalently bound fatty acids from the isolated mucin was accomplished with hydroxylamine [21].

Antiulcer drugs. The sucralfate powder, Lot No. AMD 1206, was obtained from Marion Laboratories, Inc., Kansas City, MO, while the sofalcone powder, Lot No. 01WV-07, was donated by Taisho Pharmaceutical Co., Ltd., Tokyo, Japan. For the experiments, various concentrations of the drugs were prepared shortly before use in 0.1% aqueous solution of Triton X-100.

Incubation system. The reaction mixtures for fatty acyltransferase assays, incubated at 37° in a total volume of 100 μl, consisted of 200 μg deacylated mucus glycoprotein, 100 μM [1-^{14}C]palmitoyl-CoA (300,000 cpm), 0.5% Triton X-100, 2 mM dithiothreitol, 25 mM NaF, 0–1.0×10^{-3} M sofalcone or 0–1.0×10^{-2} M sucralfate, 100 mM imidazole–HCl buffer, pH 7.4, and enzyme solution containing 50 μg of protein [21]. The tubes containing the complete incubation mixtures were briefly sonicated, and the reaction was initiated by the addition of enzyme preparation. In the initial experiments the reaction mixtures were incubated in a shaking water bath at 37° for various periods of time up to 2 hr. In the standard assays, the reaction was terminated after 30 min by immersing the tubes for 5 min in a boiling water bath. The mixtures were then centrifuged, sediments were washed twice with 0.1 ml incubation buffer, and the combined supernatant and wash from each assay tube was chromatographed on Whatman 3MM paper strips [21]. The zones containing ^{14}C-labeled glycoprotein were cut, washed with trichloroacetic acid, ethanol and diethyl ether, dried, placed in vials containing liquid scintillation solution and counted [21, 23]. The enzyme activity was expressed as nmol of ^{14}C-fatty acid transferred to glycoprotein/mg of enzyme protein·30 min. The experiments were performed in the presence of controls that contained the incubation mixtures minus the glycoprotein acceptor or boiled enzyme. Assays were carried out in duplicate, and the results are expressed as means ± SD. Student's *t*-test was used to test significance, and P values of 0.05 or less were considered as significant.

RESULTS

Analysis of the distribution of fatty acyltransferase activity for the synthesis of acylated mucin in the various subcellular fractions of rat gastric mucosa revealed that the enzyme was located mainly in the microsomal fraction. This fraction contained 45–50% of the total fatty acyltransferase activity of gastric mucosal homogenate and showed a 12-fold enrichment in specific activity. The fatty acyltransferase activity of the microsomal fraction was readily extractable with Triton X-100, and after centrifugation the solubilized enzyme contained 90–95% of the total activity. Under optimal assay conditions [21, 23], the fatty acyltransferase activity of such preparation was proportional to enzyme protein concentration up to 150 μg, remained constant with time of incubation for up to 1.5 hr, and showed proportional increment, over a given range with increasing concentrations of the substrates, deacylated mucin and palmitoyl-CoA. The apparent K_m value for gastric mucin, based on the molecular weight of 1.0×10^6 [21], calculated from Lineweaver–Burk plots was 5.6×10^{-7} M (Fig. 1).

Figure 2 shows the effect of sucralfate concentration on the activity of gastric mucosal fatty acyltransferase. The results indicated that introduction of this antiulcer agent to the reaction mixtures resulted in reduction of the rate of mucin acylation. The rate of inhibition by sucralfate stayed constant with time of incubation for at least 30 min and was proportional to the drug concentration up to 1.0×10^{-4} M, at which concentration a 25% reduction in transferase activity was obtained. The inhibition of mucin acylation caused by sucralfate was of the competitive type with an apparent K_I value of 9.1×10^{-7} M (Fig. 1). Preincubation of the enzymes (1 hr) with sucralfate prior to addition of

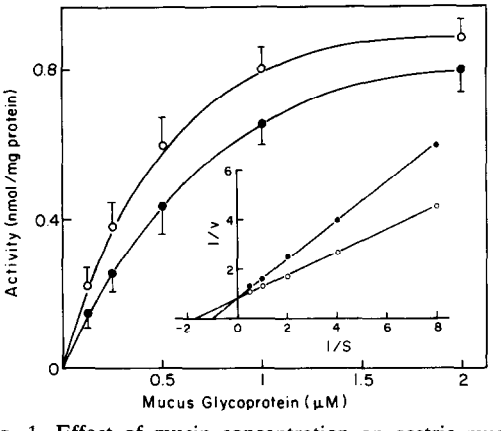

Fig. 1. Effect of mucin concentration on gastric mucus glycoprotein fatty acyltransferase activity in the absence (○) and in the presence (●) of 0.5×10^{-6} M sucralfate. The composition of the incubation mixtures was the same as described in the text, except that varied concentrations (10–300 μg) of mucin were used. The data shows the mean ± SD of five separate experiments performed in duplicate. In the double-reciprocal plots, $1/S = (\mu M)^{-1}$ and $1/V$ = (nmol of [^{14}C]palmitate transferred/mg protein·30 min)$^{-1}$. The change in K_m value due to sucralfate was significant at $P < 0.001$.

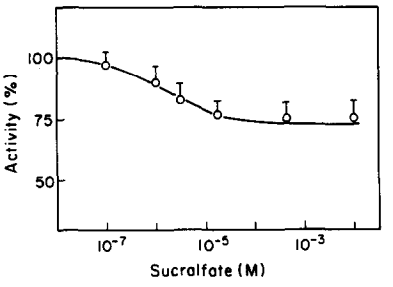

Fig. 2. Effect of sucralfate concentration on the activity of gastric mucosal mucus glycoprotein fatty acyltransferase. The assay conditions were the same as described in the text, except that varied concentrations (0–1.0 × 10^{-2} M) of sucralfate were used. The data shows the means ± SD of four separate experiments performed in duplicate. The inhibition in the rate of mucin acylation by 1.0 × 10^{-4} M sucralfate was significant at $P < 0.001$.

acceptor glycoprotein did not exert any deterrent effect on its activity. When the glycoprotein was preincubated with sucralfate before addition of the acyltransferase enzyme, however, a noticeable decrease in the transferase activity occurred.

The effect of sofalcone on the activity of gastric mucosal mucus glycoprotein fatty acyltransferase is depicted in Fig. 3. The data revealed that addition of sofalcone to the reaction mixtures caused an increase in the rate of mucin acylation with fatty acid. The rate of enhancement remained constant with time of incubation for up to 1 hr and was proportional to sofalcone concentration up to 1.0 × 10^{-5} M, at which point about a 40% increase in mucin acylation was attained. The apparent K_m value for acylation of gastric mucin in the presence of sofalcone was 3.7 × 10^{-7} M (Fig. 4). Results of experiments in which the mucin was preincubated (1 hr) with sofalcone before addition of the acyltransferase enzyme indicated no stimulatory effect

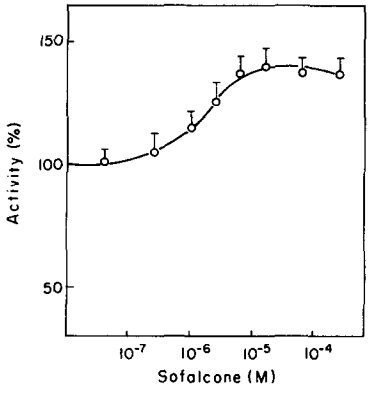

Fig. 3. Effect of sofalcone concentration on the activity of gastric mucosal mucus glycoprotein fatty acyltransferase. The assay conditions were the same as described in the text, except that varied concentrations (0–1.0 × 10^{-3} M) of sofalcone were used. The data show the means ± SD of four separate experiments performed in duplicate. The increase in the rate of mucin acylation by 1.0 × 10^{-4} M sofalcone was significant at $P < 0.001$.

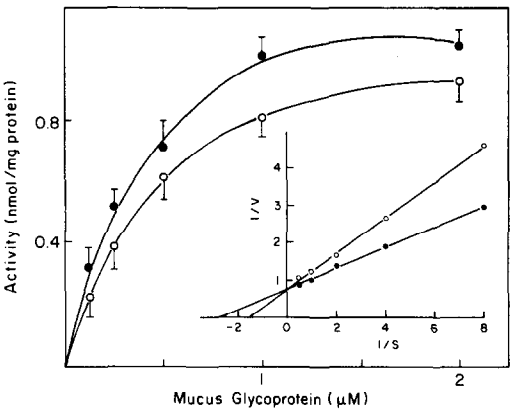

Fig. 4. Effect of mucin concentration on gastric mucus glycoprotein fatty acyltransferase activity in the absence (○) and in the presence (●) of 4.0 × 10^{-6} M sofalcone. The composition of the incubation mixtures was the same as described in the text, except that varied concentrations (10–300 μg) of mucin were used. The data show the means ± SD of five separate experiments performed in duplicate. In the double-reciprocal plots, $1/S = (\mu M)^{-1}$ and $1/V$ = (nmol of [^{14}C]palmitate transferred/mg protein · 30 min). The change in K_m value due to sofalcone was significant at $P < 0.001$.

on the transferase activity. However, when the sofalcone was preincubated with the enzyme before the addition of mucin, a marked increase in the acyltransferase activity was observed.

DISCUSSION

Although the exact sequence of events involved in pathogenesis of peptic ulcer remains elusive, the consensus is that the injury to gastric and duodenal mucosa occurs when the aggressive factors overcome those responsible for the mucosal defense [2, 5, 24]. Thus, in approach to ulcer therapy, the mucosal healing can be achieved either by reducing aggressive factors or by strengthening the mucosal protective factors. Despite relatively early realization that many drugs which promote ulcer healing also stimulate mucus production, the preferred target in designing the antiulcer agents for a long time was the control of acid and pepsin secretion by a variety of antisecretory drugs (i.e. antagonists of histamine H_2 or muscarinic receptors) and antacids [24–26]. This situation has changed considerably in recent years as a direct result of renaissance in research on the mechanisms of gastric mucosal protection and an increase in understanding of the function of mucus gel. Through the concerted efforts of various investigators, it became apparent that the layer of mucus covering the epithelial surfaces of gastrointestinal mucosa constitutes the first line of mucosal defense against a variety of insults and that the integrity of this layer so essential for the protective function remains in a delicate balance controlled by the factors affecting the biosynthesis, secretion, and breakdown of its mucin constituent [1, 4–6, 9, 10, 27–29].

Whilst under normal physiological conditions, the continuous renewal and resilient nature of the mucin

efficiently counter mucus erosion by pepsin, assure the viscoelastic and permselective properties, and provide a milieu for neutralization of the diffusing luminal acid by mucosal bicarbonate [4–6, 11, 12, 28], the mucus integrity is drastically impaired in gastric disease [6, 30]. Hence, the agents capable of strengthening and restoration of the mucus layer integrity are gaining popularity in ulcer therapy. Among the agents belonging to this category are sucralfate and sofalcone [13–15, 20, 31–33]. Studies indicate that, although both drugs are known to enhance directly the mucus viscosity, H^+ retardation capacity and the resistance to pepsin to a similar extent [16, 18, 34], they affect differently other factors contributing to gastric mucosal defense, such as arachidonic acid metabolism [19, 20, 31–33].

The results presented in this report demonstrate that sucralfate and sofalcone also affect differently the process of gastric mucin acylation with fatty acids. Our data indicate that sucralfate exerts a distinctly detrimental effect on mucin acylation which at 1.0×10^{-4} M sucralfate showed a 25% decrease. Apparently, the observed *in vitro* inhibitory effect of sucralfate results from its ability to interact with the naked nonglycosylated regions of mucin molecule that are known to serve as attachment points for fatty acids [17, 23]. Hence, the activity of acyltransferase enzyme responsible for gastric mucin acylation with fatty acids may be also impaired in patients undergoing the sucralfate treatment. This effect of sucralfate is in contrast to the results obtained with sofalcone which showed a marked stimulatory effect on gastric mucin acylation. The rate of enhancement of mucin acylation was proportional to this drug concentration up to 1.0×10^{-5} M, at which point a 40% increase in fatty acid transfer occurred. The results of preincubation experiments suggest that this effect is due to the interaction of the drug with enzyme protein rather than with mucin. Interestingly, the observed effect of sucralfate and sofalcone occurred at doses well below those used in peptic ulcer therapy [20, 35].

Since the extent of gastric mucin acylation with fatty acids affects the viscoelastic properties of the mucus gel and its ability to resist the corrosive action of acid and pepsin [9–12], the factors capable of enhancement of the activity of acyltransferase enzyme may play an important role in the repair and maintenance of gastric mucus integrity. Thus, the stimulation of fatty acid acylation of gastric mucin by sofalcone may be one of the beneficial effects of this drug towards ulcer healing.

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