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SUPPRESSION OF *HELICOBACTER PYLORI* PROTEASE ACTIVITY
TOWARDS GROWTH FACTORS BY SULGLYCOTIDE

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Infection with *H. pylori* is now recognized as a major factor in the pathogenesis of gastric disease. Here, we examined the susceptibility of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor- β (TGF β) and platelet derived growth factor (PDGF) to degradation by *H. pylori* protease, and assessed the effect of a cytoprotective agent, sulglycotide, on this process. The ^{125}I -labeled EGF, bFGF, TGF β and PDGF were incubated with *H. pylori* protease, obtained from the filtrates of saline washes of the bacterium culture, in the presence of 0—100 μg sulglycotide. The results showed that, under the assay conditions, *H. pylori* protease caused only 5% degradation of EGF and 7% degradation of bFGF. However, the protease evoked a 61.7% degradation of PDGF and a 62.3% degradation of TGF β . Introduction of sulglycotide to the reaction assay system caused a dose-dependent inhibition in PDGF and TGF β proteolysis by the *H. pylori* enzyme. The maximal inhibitory effect was obtained with sulglycotide at 100 $\mu\text{g}/\text{ml}$, at which dose an 84.4% decrease in PDGF and 88.3% decrease in TGF β degradation was achieved. The results provide a strong evidence for the effectiveness of sulglycotide in the protection of gastric mucosal growth factors against degradation by *H. pylori*.

Key words: *Helicobacter pylori*, degradation, growth factors, sulglycotide.

INTRODUCTION

Sulglycotide is a potent cytoprotective sulfated glycopeptide derived from pig duodenal mucin by the process of extensive enzymatic proteolysis and the chemical esterification of its carbohydrate chains with sulfate groups (1—3). These modifications endow the agent with gastroprotective and antiulcer properties, including the stimulation of bicarbonate secretion, inhibition of peptic erosion of mucus gel, and the endogenous prostaglandin generation (3—5). Moreover, sulglycotide has the ability to interfere with *H. pylori* mucosal attachment, counteracts the disruptive effects of its lipopolysaccharide on the epithelial cells receptors interaction with the proteins of extracellular

matrix and the glycoproteins of mucus layer, and is a potent inhibitor of the protease, lipase and phospholipase enzymes elaborated by the bacterium (6—8).

The anti-*H. pylori* activities of sulglycotide and its structural resemblance to indigenous gastric sulfomucins, which are known to interfere with *H. pylori* colonization of mucosa (9), strongly suggest that the agent may be of value in augmenting the inherent anti-*H. pylori* defense potential of gastric mucins. Yet, the use of sulglycotide in the therapy of *H. pylori* associated gastric disease remains poorly explored.

Earlier, we provided evidence that sulglycotide, like other antiulcer agents that exhibit gastroprotective properties (10, 11), has the ability to enhance gastric mucosal expression of growth factors and their receptors (12, 13). In this study, we assessed the effect of sulglycotide on the susceptibility of EGF, bFGF, TGF β and PDGF to degradation by *H. pylori* protease.

MATERIALS AND METHODS

H. pylori enzyme preparation

The experiments were conducted with *H. pylori* strain MCTC 11673, a well characterized American Type Culture Collection No. 43504 clinical isolate (14). The bacterium was cultured on Brucella broth supplemented with 10% horse serum and 5% tryptone soya in a microaerophilic atmosphere. The organisms were maintained at 37°C, yielding after 72 h a viable count of 5×10^7 CFU/ml (9). The plates with grown colonies of bacteria were gently washed with 0.15 M NaCl, and the solution was filtered through a Nalgene sterilization filter (0.2 μ m) to retain the bacteria. The filtrate was dialyzed at 4°C against distilled water, and lyophilized (7). Such prepared powder was used as an enzyme source for the assays of protease activity. The protein content of samples was measured by the method of Lowry *et al.* (15).

Preparation of ^{125}I -labeled growth factors

Growth factors, EGF, bFGF, TGF β and PDGF (Sigma, St. Louis, MO), were labeled with ^{125}I using Iodo-Gen method (16). Aliquots of growth factors (50—100 μ g) in 0.1 M Tris — HCl buffer, pH 7.2, were reacted with 1 mCi of Na[^{125}I] at room temperature for 15 min, and radiolabeled product was separated from free [^{125}I] by gel filtration on a Bio-Gel P-30 column (16).

Antiulcer drug

Sulglycotide, batch No. 1448, was kindly donated by Crinos Industria Farmacobiologica, Villa Guardia, Italy. The drug was stored at 4°C in the dark and was suspended in 0.05 M sodium phosphate buffer — 0.10 M NaCl, pH 7.0, shortly before each experiment.

Protease activity assay

The incubation mixtures for *H. pylori* protease activity assays consisted of the following components: ^{125}I -labeled EGF, bFGF, TGF β , or PDGF as substrates, *H. pylori* filtrate containing 50—100 g protein; sulglycotide, 0—100 μ g; and 50 mM phosphate buffer, pH 7.0, in a final volume

of 0.22 ml. Incubation was carried out at 37°C for up to 1 h. The reaction mixtures were applied to a Bio-Gel P-2 column (0.5 × 8 cm), and eluted with 0.5 M NaCl. Collected 1 ml fractions were monitored for the degraded ¹²⁵I-peptide fragments by counting in a gamma counter, and the proteolytic activity of *H. pylori* towards the growth factors was assayed by following the ¹²⁵I-fragments production (17).

Statistical analysis

All experiments were carried out in triplicate, 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 significant.

RESULTS

The data on the susceptibility of EGF, bFGF, TGFβ, and PDGF to degradation by *H. pylori* are summarized in Fig. 1. The results of assays revealed that under the conditions employed, the *H. pylori* protease produced about a 5% degradation of EGF, while the extent of bFGF degradation reached a maximum value of 7%. In contrast, the *H. pylori* protease caused an extensive degradation of PDGF and TGFβ. With both growth factors, the rate of proteolytic degradation was proportional to *H. pylori* protease concentration up to 100 μg and remained constant with time of incubation for at least 30 min, at which point a 62.3% degradation was registered with TGFβ and a 61.7% degradation occurred with PDGF (Fig. 1).

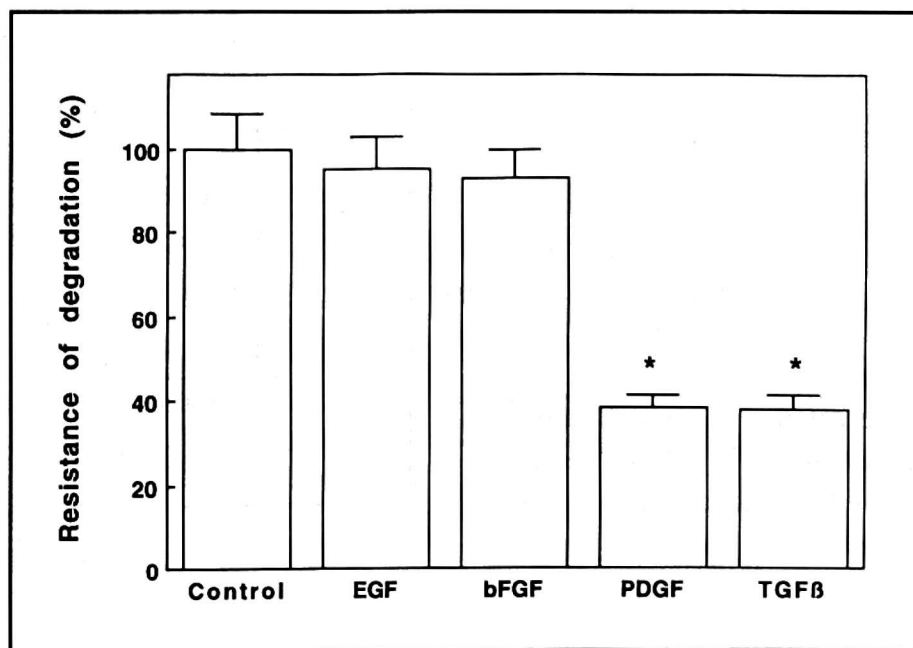


Fig. 1. Effect of *H. pylori* protease on the degradation of EGF, bFGF, TGFβ and PDGF. Values represent the means ± SD of five experiments performed in triplicate. *P < 0.05 compared with that of the control.

Introduction of sulglycotide to the reaction mixture containing TGFβ or PDGF led, in both cases, to a reduction in the rate of proteolysis by *H. pylori* enzyme. The effect of sulglycotide on the activity of *H. pylori* protease towards TGFβ is presented in Fig. 2, while Fig. 3 shows the effect of sulglycotide on the degradation of PDGF by *H. pylori* protease. With both growth factors, the

maximal inhibitory effect on *H. pylori* protease was attained at 100 $\mu\text{g/ml}$. This dose of sulglycotide produced an 88.3% decrease in the *H. pylori* protease degradation of TGF β and an 84.4% decrease in the degradation of PDGF.

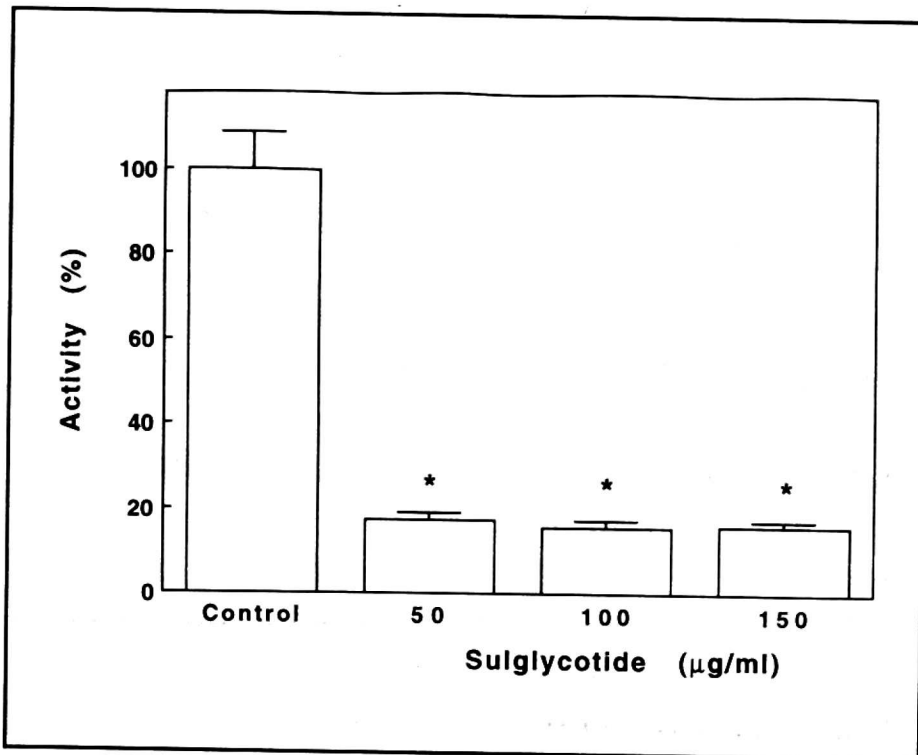


Fig. 2. Effect of sulglycotide on the degradation of TGF β by *H. pylori* protease. Values represent the means \pm SD of five experiments performed in triplicate. *P < 0.05 compared with that of the control.

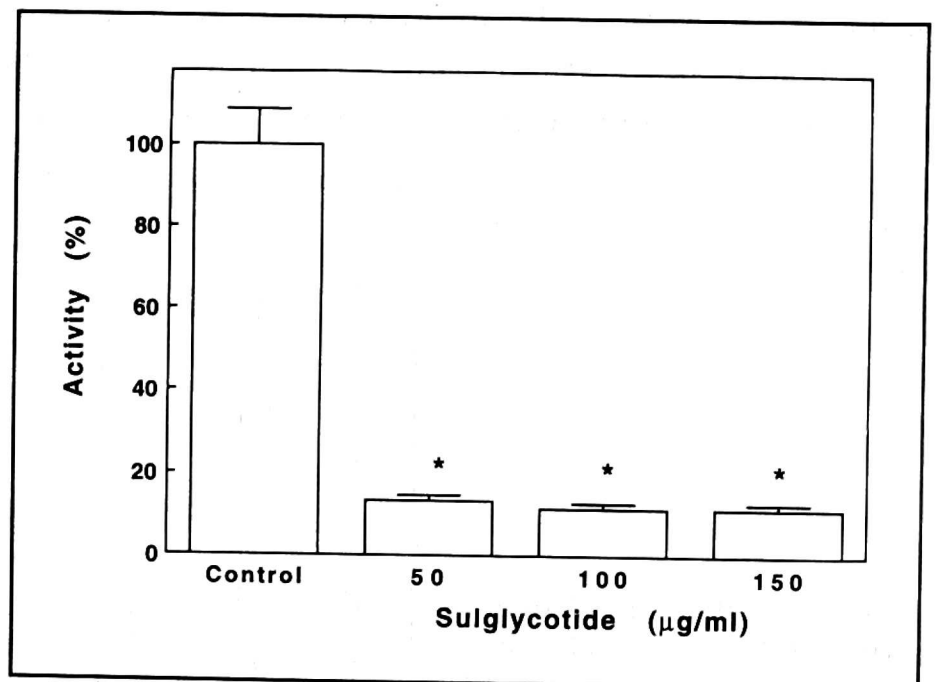


Fig. 3. Effect of sulglycotide on the degradation of PDGF by *H. pylori* protease. Values represent the means \pm SD of five experiments performed in triplicate. *P < 0.05 compared with that of the control.

DISCUSSION

Helicobacter pylori, a pathogen implicated as a causative factor in the etiology of gastric disease, is recognized for its ability to elaborate a number of toxins, proinflammatory mediators, and enzymes capable to rapid compromising the processes associated with maintenance of gastric mucosal homeostasis and the epithelial repair (8, 18—21). Among the enzymes directed

towards disruption of the protective mucus layer is *H. pylori* protease, a 50 kDa metalloproteinase exhibiting an optimum activity at pH 7.0 (22, 23). While the most apparent consequences of *H. pylori* protease action accompanying the bacterial proliferation is the erosion of mucus perimeter of gastric mucosal defense, the role of this protease, in deterring the intrinsic repair processes associated with the availability at the site of injury of growth factors is less obvious (17).

Indeed, in addition to its role as a barrier to acid, gastric mucus is recognized as the important repository of a variety of growth factors and cytokines, including those which are considered the key regulators of cellular proliferation and differentiation at the ulcer crater edge. The cues from these bioactive polypeptides conserved within the mucus coat may be of significant help to coordinate and guide the mucosal cells proliferative and migratory activities at the site of injury. Moreover, the capacity of mucus coat for storage of growth factors and to make them available in the need may be even a part of programmed set of events that aim to restore normal gastric mucosal histology. Therefore, alterations in the structure of mucus gel evoked by excessive peptic activity or brought about by *H. pylori* mucolytic activities could lead to severe disturbances in the orderly mucosal repair processes due to growth factors degradation or their release from the gel matrix.

Earlier, we have shown that peptic degradation of gastric mucus leads also to an extensive proteolysis of EGF (24). The findings reported herein revealed that in contrast to pepsin, *H. pylori* protease causes only marginal proteolysis of EGF and bFGF. The *H. pylori* protease, however, produced extensive fragmentation of PDGF (61.7%) and TGF β (62.3%). Hence, *H. pylori* through the action of its protease may indeed interfere with the time frame of activation of the mucosal repair processes at the side of injury, and affect not only the efficiency of healing process, but also the quality of mucosal repair.

Therefore, agents capable of counteracting the detrimental effects of *H. pylori* protease offer a promising venue in ulcer treatment. Among the antiulcer agents exhibiting this property is sulglycotide (2, 7). Indeed, the results obtained from this study demonstrate that sulglycotide exerts a potent inhibitory effect on the degradation of PDGF and TGF β by *H. pylori* protease. This inhibitory action of sulglycotide was dose-dependent and effective well below the therapeutic doses (500—1200 mg/day) used in gastric and duodenal ulcer treatment (25, 26). The maximal inhibitory effect on *H. pylori* protease was achieved with sulglycotide at 100 μ g/ml, at which dose an 88.3% decrease in TGF β and 84.4% decrease in PDGF degradation was attained.

Taken together, our findings demonstrate that sulglycotide by exerting a strong inhibitory activity on the *H. pylori* protease towards PDGF and TGF β is capable of promoting the mitogenic action of these cytokines in the

mucosal repair process. This property of sulglycotide may be of importance in the ability of the agent to efficiently repair, restore, and maintain the gastric mucosal integrity.

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