THE STUDIES ON THE ROLE OF GASTRIC GLYCOPROTEINS WITH REFERENCE TO CYTOPROTECTION: PROTECTIVE EFFECT OF PROSTAGLANDIN E$_2$ AND SOFALCONE ON ETHANOL-INDUCED NECROSIS

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(Received May 25, 1984)

The gastric cytoprotective action of prostaglandin E$_2$ (PGE$_2$) and sofalcone was studied in rats. The in vitro incorporating activity of $^3$H-glucosamine into the gastric macromolecular glycopolypeptides was examined when PGE$_2$ (0.1 mg/kg, p.o.) or sofalcone (100 mg/kg, i.p.) was administered 5, 15 or 30 min before the oral administration of absolute ethanol. The cytoprotective effect of PGE$_2$ against gastric mucosal damage was demonstrated 5 min after PGE$_2$ was given orally. The cytoprotective effect by sofalcone was seen after 15 min. However, during this period, the decrease in gastric macromolecular glycopolypeptide synthesis induced by the ethanol damage could not be restored by pretreatment with PGE$_2$ or sofalcone. On the other hand, the reduction in the content of the gastric macromolecular glycopolypeptides by the ethanol damage was found to be prevented to a significant extent by pretreatment with PGE$_2$. The same phenomenon was also observed in the administration of sofalcone. Accordingly, PGE$_2$ has stimulating effect on the gastric glycopolypeptide biosynthesis, but this effect can not be considered as the mechanism responsible for cytoprotection, if indeed a single mechanism exists. Thus, it is suggested that the adhesion or maintenance of secreted macromolecular glycopolypeptides to the gastric tissue is closely related to cytoprotection.

Keywords—prostaglandin E$_2$; cytoprotection; gastric; glycopolypeptide; ethanol damage; sofalcone

INTRODUCTION

Prostaglandins (PGs) have been shown to prevent ulceration even at doses much lower than the gastric antisecretory and to protect gastric mucosa against the lesions induced by necrotizing agents such as absolute ethanol. The property to protect the cells of gastrointestinal epithelium against a variety of potentially necrotizing agents has been termed "cytoprotection," but the exact mechanism of this new property has not been defined.

On the other hand, a previous study indicated that 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy)chalcone (sofalcone; SU-88), which had an antiulcer potency against experimental ulcers, had a cytoprotective effect and that this effect was mediated by the increase in endogenous PGs.

It is well known that gastric macromolecular glycopolypeptides play an important role in the defence against ulceration. Therefore, in the present study, we tried to further clarify the mechanism of cytoprotection using PGE$_2$ and sofalcone from the viewpoint of gastric glycoprotein synthesis and the adhesion capacity of the gastric macromolecular glycopolypeptides.

MATERIALS and METHODS

Induction of Gastric Lesions —— Gastric necrosis was produced according to the method of Robert

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et al. Rats (180–200 g male Donryu and Wistar rats were used) were made to fast and deprived of water for 24 h prior to the experiments. One ml of absolute ethanol was administered orally, and 1 h later the animals were sacrificed by decapitation. The stomach was removed, cut along the greater curvature and washed with a cold Krebs-Ringer bicarbonate buffer. The gastric lesions that occurred in the glandular portion were determined—0: no damage; 1: small erosion (<2 mm); 2: 2–5 small erosions (<2 mm); 3: >5 small erosions (<2 mm) or large erosion (>2 mm) and 4: >2 large erosions (>2 mm).

**Drug Administration** — Sofalcone suspended in 0.4% carboxymethyl cellulose (CMC) and PGE$_2$ (sigma) dissolved in 5% ethanol were used in these experiments. Each drug was given 5, 15 or 30 min before the oral administration of absolute ethanol. Positive control rats damaged by absolute ethanol were given 0.5 ml/kg of 0.4% CMC intraperitoneally or 0.5 ml/kg of 5% ethanol orally. Since the same values were obtained in the both cases, the same controls were used in the following experiments.

**Histochemical Study of the Stomach** — The rat stomach opened along the greater curvature was fixed in a 10% buffered formalin containing 1% cetyl pyridinium chloride, and embedded in paraffin and then the sections cut at 4 μm were stained with periodic-acid-Schiff (PAS) and Hematoxylin-Eosin (HE).

**Incubation of the Gastric Tissue and Isolation of the Gastric Macromolecular Glycoproteins**

— The wet weight of the glandular portion of each stomach was determined and the tissue was preincubated for 30 min at 37°C in a 95% O$_2$-5% CO$_2$ atmosphere with 20 ml of the Krebs-Ringer bicarbonate buffer containing antibiotics (penicillin G 65 U/ml, streptomycin 65 U/ml), pH 7.4, and then incubated for another 6 h at 37°C in the presence of 50 μCi of $^3$H-6-galactosamine (34.6 Ci/mmol; Amersham). After the incubation, the tissue and medium were separated by centrifuging at 10000 × g for 10 min at 4°C. The tissue obtained was placed in a boiling-water bath for 3 min to inactivate the enzymes, and 2 ml of a 50 mM Tris-HCl buffer containing 2% Triton X-100, pH 7.2, was added to the tissue. The tissue was then homogenized in the same buffer using a motor-driven glass homogenizer. The homogenate was centrifuged at 10000 × g for 30 min at 4°C. Then the supernatant and the medium were dialyzed exhaustively against running tap water for 2 d and then distilled water for 3 d at 4°C. The medium was lyophilized and resolved with 2 ml of a 50 ml Tris-HCl buffer containing 2% Triton X-100, pH 7.2, for gel filtration. These dialysates were applied to a Bio-Gel A 1.5 m column (100–200 mesh, 1.4 × 55 cm, Bio Rad Laboratories) equilibrated with a 50 mM Tris-HCl buffer containing 2% Triton X-100, pH 7.2, and the column was eluted with the same buffer. The macromolecular glycoproteins fraction eluted in the void volume was designated as Peak I. The fractions of volume 2.3 ml were collected.

**Determination of Labeled Glycoprotein and Glycoprotein Contents** — The radioactivity of glycoproteins was measured in a Aloka 903 liquid scintillation counter. Hexose was determined by the phenol-H$_2$SO$_4$ method with galactose as the standard. The results were expressed as the means ± S.E. Statistical analyses were made using the Student's t-test.

**RESULTS**

**Effect of PGE$_2$ and Sofalcone on Ethanol-Induced Gastric Lesion in Rats**

When absolute ethanol was orally administered, the hemorrhagic lesions occurring mostly in the corpus were observed. The negative control was administrated orally with 1 ml of distilled water in place of absolute ethanol. The positive control was administered orally with 1 ml of absolute ethanol. As shown in Table I, inhibition of the ethanol-induced gastric lesions by PGE$_2$ was clearly recognized each time prior to the oral administration of absolute ethanol. On the other hand, sofalcone reduced the severity of the ethanol-induced lesions 15 min prior to the administration of absolute ethanol.

**Changes in PAS Staining Positive Substances in the**
Stomach

As shown in Fig.1(b), PAS staining positive substances were distributed in the superficial mucus layer and in the neck of the gland of the gastric mucosa in the intact rats. However, a complete loss of surface epithelium and, in some cases, the loss of the upper part of the gastric gland, accompanied by intramucosal hemorrhage and necrosis, were observed in the gastric tissue by the oral administration of absolute ethanol (c), and PAS staining positive substances showed a remarkable decrease (d). By pretreatment with PGE$_2$ and sofalcone the PAS staining positive substances were maintained over the normal level (f,h). The damage was, in the most part, limited only to the luminal epithelium cells and not extended to the gland (e,g).

Effect of PGE$_2$ and Sofalcone on the Synthesis of the Gastric Glycoproteins

As shown in Fig.2, the synthetic activity of the macromolecular glycoproteins in the ethanol-damaged gastric tissue was decreased to about half of the negative control tissue. Pretreatment of the rats with PGE$_2$ (0.1 mg/kg, p.o.) or sofalcone (100 mg/kg, i.p.) prevented the significant decrease in the synthetic activity of the macromolecular glycoproteins in comparison with the positive control 30 min prior to absolute ethanol, but this value was lower than that of the negative control. Therefore, when PGE$_2$ or sofalcone was administered 5 or 15 min prior to absolute ethanol, the recovery of the gastric macromolecular glycoprotein synthesis which was lowered by alcohol damage was not observed. Effect of PGE$_2$ or Sofalcone on the Content of the Macromolecular Glycoproteins in the Gastric Tissue

Figure 3 shows the hexose content of the macromolecular glycoprotein fraction (Peak I) in the gastric tissue after incubation. The hexose content of the positive control tissue decreased significantly, and more than that of the negative control tissue. The decrease in the hexose content of the macromolecular glycoproteins induced by alcohol damage was recovered due to the administration of PGE$_2$ or sofalcone. However, this recovery was not observed when sofalcone was administered 15 min prior to absolute ethanol.

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<th>TABLE 1. Effect of Prostaglandin E$_2$ and Sofalcone on Ethanol-Induced Gastric Lesions in Rats</th>
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<td>Dose (mg/kg)</td>
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$^a)$ Significant difference from control: $p<0.05$.

$^b)$ Significant difference from control: $p<0.01$.

$^c)$ Significant difference from control: $p<0.001$.

$PGE_2$ (0.1 mg/kg, p.o.) and sofalcone (100 mg/kg, i.p.) were given 5, 15 and 30 min before the administration of absolute ethanol. One ml of absolute ethanol was administered orally and 1 h later the animals were sacrificed by decapitation. Significantly different from positive control: $a)$ $p<0.05$, $b)$ $p<0.01$, $c)$ $p<0.001$. All values are present as the means ± S.E.
FIG. 1. Histochemical Improvement by the Administration of PGE$_2$ and Sofalcone on Gastric Mucous Membrane in Ethanol-Induced Necrotic Rats

PGE$_2$ (0.1mg/kg, p.o.) and sofalcone (100mg/kg, i.p.) were given 30 min before the administration of absolute ethanol. (a) intact (HE staining; × 150), (b) intact (PAS staining; × 150), (c) ethanol-induced necrosis (HE staining; × 150), (d) ethanol-induced necrosis (PAS staining; × 150), (e) ethanol-induced necrosis + sofalcone (HE staining; × 150), (f) ethanol-induced necrosis + sofalcone (PAS staining; × 150), (g) ethanol-induced necrosis + PGE$_2$ (HE staining; × 150), (h) ethanol-induced necrosis + PGE$_2$ (PAS staining; × 150).
was administered 5 min prior to absolute ethanol. Release of the Macromolecular Glycoproteins into the Medium from the Gastric Tissue

As shown in Fig. 4, the $^3$H-labeled macromolecular glycoprotein released into the medium from the tissue in the ethanol-damaged tissue increased more than that in the negative control tissue. Pretreatment of rats with PGE$_2$ or sofacline had a tendency to decrease the release of the labeled macromolecular glycoprotein in comparison with the positive control. On the other hand, as shown in Fig. 5, the hexose content of the macromolecular glycoproteins released into the medium in the ethanol-damaged tissue decreased more than that of the negative control tissue. The amount of the macromolecular glycoproteins released in the positive control was almost the same in comparison with the group administered

**FIG. 2.** Incorporation of $^3$H-Glucosamine into the Macromolecular Glycoprotein Fraction (Peak I) in the Rat Gastric Tissue

PGE$_2$ (0.1mg/kg, p.o.) and sofacline (100mg/kg, i.p.) were given (A)5, (B)15 and (C)30 min before the administration of absolute ethanol. Significantly different from negative control: b) $p<0.01$, c) $p<0.001$. Significantly different from positive control: d) $p<0.05$. All values are present as the means ± S.E. NC; negative control, PC; positive control, PG; PGE$_2$ treated, SU; sofacline (SU-88) treated.

**FIG. 3.** The Hexose Content of the Macromolecular Glycoprotein Fraction (Peak I) in the Rat Gastric Tissue

PGE$_2$ (0.1mg/kg, p.o.) and sofacline (100mg/kg, i.p.) were given (A)5, (B)15 and (C)30 min before the administration of absolute ethanol. Significantly different from negative control: b) $p<0.01$, c) $p<0.001$. Significantly different from positive control: e) $p<0.01$, f) $p<0.001$. All values are present as the means ± S.E. NC; negative control, PC; positive control, PG; PGE$_2$ treated, SU; sofacline (SU-88) treated.
with PGE$_2$ or sofalcone.

DISCUSSION

It is well known that gastric mucin glycoproteins form an adherent gel over the surface epithelium and protect the stomach against chemical damage.\textsuperscript{9,17}

Recently, Robert and his associates demonstrated that PGs had a cytoprotective effect on the stomach when administered orally as soon as 1 min prior to exposing the gastric epithelium to absolute ethanol.\textsuperscript{20} Accordingly, changes in the gastric glycoproteins observed after PGs are given orally should occur equally rapidly if such a change is responsible for cytoprotection. In the present study, the incorporating activity of $^3$H-glucosamine into the gastric macromolecular glycoproteins in the isolated gastric tissue of rats,

FIG. 4. The Release of the $^3$H-Labeled macromolecular Glycoprotein Fraction (Peak I) into the Medium from the Gastric Tissue

PGE$_2$ (0.1mg/kg, p.o.) and sofalcone (100mg/kg, i.p.) were given (A)5, (B)15 and (C)30 min before the administration of absolute ethanol. Significantly different from negative control: a) \( p < 0.05 \), b) \( p < 0.01 \). Significantly different from positive control: c) \( p < 0.01 \). All values are present as the means \( \pm S.E. \) NC; negative control, PC; positive control, PG; PGE$_2$ treated, SU; sofalcone (SU-88) treated.

FIG. 5. The Hexose Content of the Gastric Macromolecular Glycoprotein Fraction (Peak I) in the Medium

PGE$_2$ (0.1mg/kg, p.o.) and sofalcone (100mg/kg, i.p.) were given (A)5, (B)15 and (C)30 min before the administration of absolute ethanol. Significantly different from positive control: a) \( p < 0.05 \), b) \( p < 0.01 \). All values are present as the means \( \pm S.E. \) NC; negative control, PC; positive control, PG; PGE$_2$ treated, SU; sofalcone (SU-88) treated.
when PGE₂ (0.1 mg/kg, p.o.) or sofalcone (100 mg/kg, i.p.) was administered, 5, 15 or 30 min before the oral administration of absolute ethanol, was used as an index of the mucous synthesis.

The cytoprotective effect of PGE₂ against gastric mucosal damage was demonstrated 5 min after PGE₂ was given orally. The effect of PGE₂ has also been histologically indicated as described by Lacy et al. 18) In the present experiment, however, the decreased gastric glycoprotein synthesis caused by ethanol damage could not be restored by pretreatment with PGE₂. The prevention of the decreased incorporating activity was not observed until 30 min after PGE₂ or sofalcone was administered.

The macromolecular glycoproteins synthesized in the gastric mucosal gland were gradually released into the medium through the incubation period, while the release of the labeled glycoprotein from the gastric tissue into the medium was markedly increased by the oral administration of absolute ethanol. However, pretreatment with PGE₂ or sofalcone was able to significantly prevent the increase in the labeled glycoprotein released into the medium. The mechanism of cytoprotection is unknown yet, but the stimulation of the macromolecular glycoprotein secretion can be considered as one of the proposed mechanisms for PGs cytoprotection.19-23) PG has been reported to stimulate the biosynthesis of glycoproteins and glycosaminoglycans in chondrocyte and fibroblast cell culture systems.24,25) From the present study, PGE₂ has stimulating effect on the gastric glycoproteins biosynthesis, but this effect can not be considered the mechanism responsible for cytoprotection, if indeed a single mechanism exists.

On the other hand, a large part of the gastric macromolecular glycoproteins that already exist in the gastric mucosa were readily degraded to smaller molecules containing hexose and released into the gastric juice by the oral administration of absolute ethanol (unpublished data). Consequently, the total amount of the gastric macromolecular glycoproteins coating the surface of the gastric mucosa before incubation significantly decreased compared with the negative control tissue and treated tissues. About half of these glycoproteins were gradually released into the medium by incubation, but the maintenance of the glycoproteins to the tissue was significantly enhanced by pretreatment with PGE₂ or sofalcone. These effects were observed 5 min after administration of PGE₂ and 15 min after administration of sofalcone. The time lag seen by sofalcone may be explained by the previous observations that the mechanism of cytoprotective effect by this drug is depending on the increase in the endogenous PG content through its inhibitory effects on the PG inactivating enzyme, 15-hydroxy-PG-dehydrogenase.26,27)

Thus, the increase in such adhesion capacity of the glycoproteins coating the surface of the gastric mucosa may be very important as a defensive mechanism by PGE₂ and sofalcone against ethanol damage. However, it is still not known whether the enhancement of the adhesion or maintenance of macromolecular glycoproteins by PGE₂ or sofalcone is the cause of the cytoprotective effect or the way of preventing gastric lesions. Further experimentation is necessary to more fully explain this interesting observation.

Acknowledgement  The authors wish to thank Mr. Y. Kohno for their histochemical examinations.

REFERENCE
Gastric Glycoprotein and Cytoprotection


