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## Abstract

UDP-galactosyltransferase (UDP-Gal-T) is a key enzyme in the synthesis of mucus glycoprotein which plays an important role in gastric mucosal defensive mechanisms. Analysis of gastric UDP-Gal-T activity should clarify the mechanisms of the action of antiulcer drugs regarding gastric defensive factors. Here, we examined UDP-Gal-T activity in rat gastric mucosa treated with the antiulcer drugs geranylgeranylacetone (GGA) and cetraxate hydrochloride (CET). The effects of coadministration of indomethacin and exogenous administration of prostaglandins (PGs) were also studied. GGA and CET significantly increased UDP-Gal-T activity, and coadministration of indomethacin inhibited the increase of enzyme activity. UDP-Gal-T activity level with GGA was significantly higher than the control level, even in the presence of indomethacin. With CET, however, this was not the case. Among PGs, PGE1 significantly increased enzyme activity. Concomitant administration of PGE1 and GGA or CET increased UDP-Gal-T activity even with indomethacin to the levels achieved when these antiulcer drugs were administered without indomethacin. Our findings suggest that GGA and CET exert antiulcer effects by increasing mucus glycoprotein synthesis and that endogenous PG synthesis may be involved in this process. However, mechanisms not mediated by endogenous PGs may also exist in the stimulatory action of GGA on UDP-Gal-T activity.

**KEYWORDS:** antiulcer drug, galactosyltransferase, prostaglandin, mucin

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## Geranylgeranylacetone and Cetraxate Hydrochloride Increase UDP-Galactosyltransferase Activity in Rat Gastric Mucosa

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UDP-galactosyltransferase (UDP-Gal-T) is a key enzyme in the synthesis of mucus glycoprotein which plays an important role in gastric mucosal defensive mechanisms. Analysis of gastric UDP-Gal-T activity should clarify the mechanisms of the action of antiulcer drugs regarding gastric defensive factors. Here, we examined UDP-Gal-T activity in rat gastric mucosa treated with the antiulcer drugs geranylgeranylacetone (GGA) and cetraxate hydrochloride (CET). The effects of coadministration of indomethacin and exogenous administration of prostaglandins (PGs) were also studied. GGA and CET significantly increased UDP-Gal-T activity, and coadministration of indomethacin inhibited the increase of enzyme activity. UDP-Gal-T activity level with GGA was significantly higher than the control level, even in the presence of indomethacin. With CET, however, this was not the case. Among PGs, PGE<sub>1</sub> significantly increased enzyme activity. Concomitant administration of PGE<sub>1</sub> and GGA or CET increased UDP-Gal-T activity even with indomethacin to the levels achieved when these antiulcer drugs were administered without indomethacin. Our findings suggest that GGA and CET exert antiulcer effects by increasing mucus glycoprotein synthesis and that endogenous PG synthesis may be involved in this process. However, mechanisms not mediated by endogenous PGs may also exist in the stimulatory action of GGA on UDP-Gal-T activity.

**Key words:** antiulcer drug, galactosyltransferase, prostaglandin, mucin

Gastric mucosal injury results from a disrupted balance between aggressive and defensive factors. Gastric mucus is a major component of the defensive factors and protects gastric mucosa from irritants such as HCl through its viscous and gel-forming properties (1, 2). Gastric mucus consists of glycoproteins called mucin which are highly glycosylated in *O*-glycosidic linkages to a peptide backbone. UDP-galactosyltransferase (UDP-Gal-T) is a key enzyme in the synthesis of the mucus glycoprotein, playing a key role in the sequence of events creating carbohydrate chains linked to its peptide backbone (3). Analysis of gastric UDP-Gal-T activity should help clarify mucus glycoprotein synthesis, the physiology of gastric ulcer formation, and the mechanisms of the effect of antiulcer drugs regarding gastric defensive mechanisms.

We previously demonstrated that UDP-Gal-T activity decreased in gastric mucosa of patients with liver cirrhosis, and that this enzyme activity returned to normal levels in patients who were taking an antiulcer drug, geranylgeranylacetone (GGA) (4), an acyclic polyisoprenoid developed in Japan (5). This antiulcer drug, which is thought to enhance mucosal defensive factors, is widely used in Japan for treating gastric mucosal damage. However, the mechanisms by which the drug exerts its antiulcer effects is not yet fully understood. In this study, we examined UDP-Gal-T activity in the gastric mucosa of rats treated with GGA or another antiulcer drug, cetraxate hydrochloride (CET), a compound possessing antiplasmin, anti-casein and anti-trypsin actions (6). To clarify the role of prostaglandins (PGs) in the action of these antiulcer drugs, the effects of coadministration of indomethacin on UDP-Gal-T activity as well as effects of exogenous administration of PGs were also studied.

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## Materials and Methods

GGA and CET were obtained from Eisai Co., Ltd., and Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan, respectively. PGE<sub>1</sub>, PGE<sub>2</sub> and PGD<sub>2</sub> were gifts from Ono Pharmaceutical Co., Ltd., Tokyo, Japan. Gastric specimens were prepared from male Sprague-Dawley rats weighing 180-250 g. Rats were starved for 24 h before use. GGA (100 mg/kg), CET (500 mg/kg), or saline as a control was given orally with or without coadministration of indomethacin (6 mg/kg). The effects of oral administration of PGE<sub>1</sub> (50 μg/kg), PGE<sub>2</sub> (50 μg/kg), and PGD<sub>2</sub> (50 μg/kg) were also examined. The doses of these drugs were arbitrarily determined based on the findings of previous reports; GGA (7), CET (6), indomethacin (7), and PGs (8, 9).

Rats were anesthetized 6 h after drug administration. Stomachs were then rapidly isolated, cut along the greater curvature, and rinsed with cold saline. Gastric mucosa were scraped off and homogenized in 10 volumes (v/w) of 0.25 mol/L sucrose solution. Then, the homogenates were centrifuged at 500 *g* for 10 min, and the supernatants were used as enzyme preparations for determining UDP-Gal-T activity. Protein concentrations of these enzyme preparations were determined using the method of Lowry *et al.* (10).

The assay procedure for UDP-Gal-T using radio-labeled galactose has been described previously (11). Briefly, mucins free of terminal *N*-acetylneuraminic acid (asialomucin) were prepared from bovine submaxillary mucin (Sigma Chemical Co., St. Louis, MO, USA) by acidic cleavage of *N*-acetylneuraminic acid. The enzyme preparation was mixed with asialomucin, UDP-galactose, and UDP-(<sup>3</sup>H)-galactose (New England Nuclear Co., Boston, MA, USA) in 50 mmol/L Tris-HCl buffer, pH 7.5, containing 2-trichloroacetic acid, spotted on Whatman GF/B glass filter, and counted in a liquid scintillation counter. Enzyme activity was expressed as pmoles/mg of protein/min.

Six rats were examined for each experimental group, and the result of UDP-Gal-T activity in each group was expressed as mean ± SD. Comparisons among groups were made with one-way analyses of variance.

## Results

Oral administration of GGA and CET significantly

increased UDP-Gal-T activity in the rat gastric mucosa when compared with control rats ( $P < 0.05$ ) (Fig. 1). Indomethacin slightly decreased enzyme activity, but the decrease did not reach statistical significance. Coadministration of indomethacin inhibited the increase of enzyme activity induced by these antiulcer drugs (Fig. 1). However, the level of UDP-Gal-T activity with GGA was still

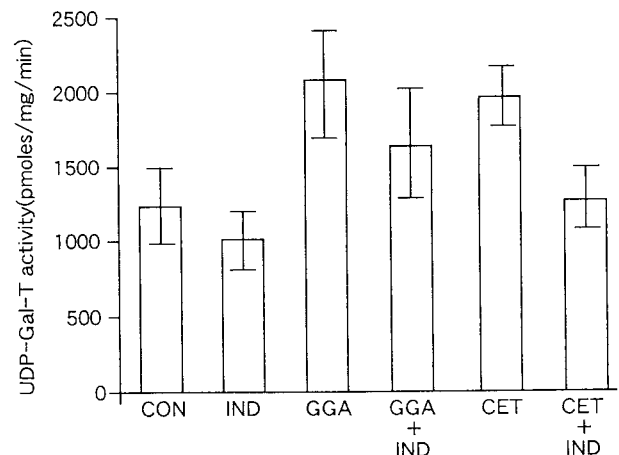


Fig. 1 Effects of geranylgeranylacetone (GGA) and cetraxate hydrochloride (CET) on UDP-galactosyltransferase (UDP-Gal-T) activity in rat gastric mucosa with or without coadministration of indomethacin (IND). Bars = means ± SD. Significant differences ( $P < 0.05$ ) included: Control (CON) vs. GGA, CET; IND vs. GGA, GGA + IND, CET; GGA vs. GGA + IND; CET vs. CET + IND.

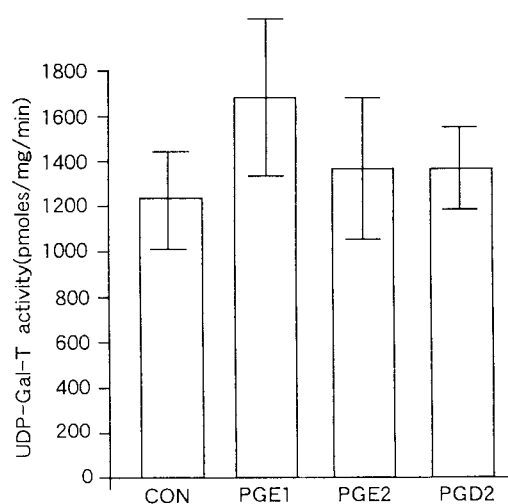
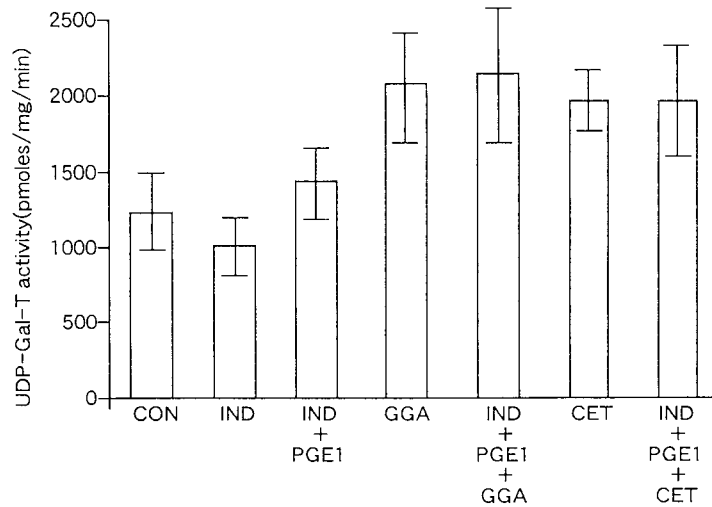


Fig. 2 Effects of prostaglandins (PGs) on UDP-galactosyltransferase (UDP-Gal-T) activity in rat gastric mucosa.

Bars = means ± SD. Significant differences ( $P < 0.05$ ) included: Control (CON) vs. PGE<sub>1</sub>.



**Fig. 3** Effects of concomitant administration of prostaglandin (PG) E<sub>1</sub> and geranylgeranylacetone (GGA) or cetraxate hydrochloride (CET) on UDP-galactosyltransferase (UDP-Gal-T) activity in rat gastric mucosa in the presence of indomethacin (IND). Bars = means  $\pm$  SD. Significant differences ( $P < 0.05$ ) included: Control (CON), IND, IND + PGE<sub>1</sub> vs. GGA, IND + PGE<sub>1</sub> + GGA, CET, IND + PGE<sub>1</sub> + CET.

significantly higher than the control level, even in the presence of indomethacin ( $P < 0.05$ ). With CET, though, this was not the case.

Then, we examined the effects of exogenous administration of PGs on UDP-Gal-T activity (Fig. 2). PGE<sub>1</sub> significantly ( $P < 0.05$ ), and PGE<sub>2</sub> and PGD<sub>2</sub> slightly increased the enzyme activity. Among these PGs, a PGE<sub>1</sub> analogue, ornoprostil, is clinically available as an antiulcer drug in Japan (12). Thus, we examined the effects of PGE<sub>1</sub> on UDP-Gal-T activity in the presence of indomethacin (Fig. 3). PGE<sub>1</sub> was given orally with or without GGA and CET together with indomethacin. PGE<sub>1</sub> slightly increased the enzyme activity in the presence of indomethacin, but this increase did not reach statistical significance. Concomitant administration of PGE<sub>1</sub> and GGA or CET increased UDP-Gal-T activity even in the presence of indomethacin to the levels achieved when these antiulcer drugs were administered without indomethacin (Fig. 3).

## Discussion

In the present study, we studied the effects of the antiulcer drugs GGA and CET on the biosynthesis of mucus glycoproteins in the rat gastric mucosa by examining UDP-Gal-T activity and demonstrated that these two drugs increased this enzyme activity. We have previously shown that UDP-Gal-T activities positively correlate with

the amount of the mucin in gastric surface epithelial cells (4), indicating that GGA and CET exert protective effects on the gastric mucosa by increasing mucus glycoprotein synthesis leading to the increase of gastric surface mucin.

Indomethacin, a potent inhibitor of PG synthesis (13), inhibited the increase of UDP-Gal-T activities by these antiulcer drugs. PGs have several important effects on gastric defensive factors (14). Among them, PGs are known to stimulate mucus production in cultured rat gastric mucosa (15). *In vivo* administration of GGA has been shown to promote PGE<sub>2</sub> synthesis in a dose-dependent manner in the rat gastric mucosa (16), but this drug did not enhance PGE<sub>2</sub> and I<sub>2</sub> production in a cell culture of rat gastric fundic mucosa (5). This inconsistency may arise from the difference between *in vivo* and *in vitro* conditions. The findings of the present study suggest that endogenous PG synthesis in gastric mucosa may play a role, at least to some extent, in the increase of UDP-Gal-T activity induced by GGA and CET. GGA, however, increased enzyme activity even in the presence of indomethacin, although the degree of the increase was significantly inhibited. Thus, mechanisms not mediated by endogenous PGs may also exist in the stimulatory action of this drug on UDP-Gal-T activity. GGA has been shown to induce heat shock proteins in cultured guinea pig mucosal cells and rat gastric mucosa (17), and the relevance of this action to the increase of gastric mucosal defense by this drug has been suggested.

Among PGs, both PGE<sub>1</sub> and PGE<sub>2</sub> and their derivatives have been shown to increase gastric blood flow in the canine stomach (18) and to stimulate mucus glycoprotein synthesis in rat gastric mucous cells (9, 19). In our study, PGE<sub>2</sub> slightly increased UDP-Gal-T activity, while the increase induced by PGE<sub>1</sub> was more prominent and significant. A PGE<sub>1</sub> analogue is clinically available as an antiulcer drug in Japan. Our findings suggest that the drug may be clinically useful in stimulating gastric mucus synthesis for treating gastric mucosal injury.

In treating various disease conditions, non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used. Adverse effects such as hemorrhagic gastric mucosal injury are often encountered (20), and their prevention is an important clinical issue. Gastric mucosal lesions induced by NSAIDs result from a decrease in defensive factors due to their effects on cyclooxygenase (COX), a key enzyme in the synthesis of PGs, leading to the inhibition of PG synthesis (21). This disrupted cytoprotective function is believed to be caused by the inhibition of COX1 isoenzyme, the constitutive form of the enzyme, by NSAIDs. Accumulating evidence suggests that the development of drugs which selectively inhibit COX2 isoenzyme may decrease the occurrence rate of gastrointestinal side effects of NSAIDs (22, 23). Indomethacin, which is a potent widely used NSAID, has a relatively low selectivity to COX2 isoenzyme (24) and the use of it, therefore, poses a higher risk for developing gastrointestinal damage requiring hospitalization (20). In this study, concomitant administration of PGE<sub>1</sub> and GGA or CET increased UDP-Gal-T activity even in the presence of indomethacin to the levels achieved when these antiulcer drugs were administered alone. Thus, using antiulcer drugs in combination, as shown in this study, may be an alternative choice to prevent gastric damage induced by NSAIDs until a selective inhibitor of COX2 is clinically available.

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