Gastroprotective effect of ranitidine bismuth citrate is associated with increased mucus bismuth concentration in rats

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Abstract

Background—Antisecretory and bismuth compounds protect the gastric mucosa from injury resulting from non-steroidal anti-inflammatory drugs.

Aim—To study the mechanism underlying the gastroprotective effects of ranitidine bismuth citrate (GG311) in rats.

Methods—Indomethacin rat injury model and in vivo microscopy in which acid output, surface cell intracellular pH (pH_i), gastric mucus gel thickness, and mucosal blood flow were measured simultaneously.

Results—In injury studies, GG311 dose dependently protected against severe injury induced by indomethacin (60 mg/kg subcutaneously). In in vivo microscopic studies, indomethacin significantly decreased mucus gel thickness and increased the initial rate of acidification of gastric surface cells when the superfusate pH was lowered from 7.4 to 1.0, and impaired pH_i during acid exposure. Indomethacin had no effect on mucosal blood flow or acid output. GG311 alone had no effect on gel thickness, blood flow, or pH_i homeostasis during acid exposure, but improved the initial acidification rate and pH_i during superfusion with pH 1.0 solutions in the presence of indomethacin. In separate experiments, indomethacin pretreatment considerably increased gastric mucus bismuth concentrations in rats given GG311.

Conclusions—The gastroprotective effect of GG311 against indomethacin induced gastric injury is associated with high and prolonged gastric mucus bismuth concentrations, which may impair proton permeation across the mucus gel.

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Keywords: stomach, intracellular pH, ranitidine bismuth citrate, gastric mucus, proton diffusion, mucosal blood flow.

Bismuth salts protect the gastric mucosa from damage caused by non-steroidal anti-inflammatory drugs (NSAIDs) by a yet undefined mechanism.^{1,2} The most usual explanations advanced have suggested that the primary mode of action of bismuth on the gastric mucosal barrier is at the pre-epithelial level.³⁻⁵ Antisecretory compounds, such as H_2 receptor

antagonists, have also been shown to prevent duodenal ulcers in patients taking NSAIDs.^{6,7}

Ranitidine bismuth citrate (GG311) is a novel drug that combines two compounds known to protect the gastric mucosa: an H_2 receptor antagonist and a bismuth salt. This compound has been shown to prevent NSAID induced gastric ulcers in an animal model⁸ and in a clinical study.⁹ However, the mechanism by which GG311 protects gastric mucosa has not been clearly explained. The aim of this study is therefore to ascertain the effect of this novel compound on gastric defensive mechanisms, as determined with an established gastric corpus injury model induced by indomethacin,^{10,11} and with a novel system developed in our laboratory that enables the gastric barrier function to be measured in vivo.

Methods

ANIMALS

Male Sprague-Dawley rats weighing 200-250 g were fasted overnight, but had free access to water. All studies were approved by the Animal Use Committee of the West Los Angeles Veterans Administration Medical Center.

ASSESSMENT OF GASTRIC MUCOSAL LESIONS

Rats were killed in a carbon dioxide chamber, the abdomens opened, and the stomachs removed and opened along the greater curvature. Severe lesions were assessed according to previously described methods.¹¹ The lesions were graded and scored by an observer who was unaware of the drugs administered as follows: petechial lesions=1, erosions less than 1 mm=2, erosions between 1 and 2 mm=3, erosions between 2 and 4 mm=4, and erosions greater than 4 mm=actual length in mm. The individual lesion scores in each rat were summed to provide a lesion score for each animal.

For histological evaluation, three strips of tissue were taken from across the entire posterior wall (parallel to the limiting ridge): (a) just below the limiting ridge, (b) the mid-corpus, and (c) the distal-corpus. The severity of gastric mucosal injury was evaluated on the sections stained with haematoxylin and eosin using a modification of established criteria.¹² Damage was graded as follows: surface mucous cell damage (surface damage); erosions down to, but not deeper than, the

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mucous neck cell area (superficial erosions); and erosions extending down into the parietal cell area (deep erosions). At least 100 glands were assessed in each specimen. The lesion score was calculated by multiplying the percentage of glands with no injury by 0, the percentage with surface damage by 1, the percentage with superficial erosions by 2, and the percentage with deep lesions by 3. The scores were then added and averaged among the three specimens.

IN VIVO MICROSCOPIC MEASUREMENTS

An *in vivo* microfluorometric technique, described in detail elsewhere,¹³⁻¹⁷ was used to measure intracellular pH (pH_i) and mucous gel thickness. The initial acidification rate was calculated from the fall of pH_i during the first two minutes of acid exposure as described previously.¹⁴ Gastric mucosal blood flow was measured by laser-Doppler flowmetry, as a percentage of baseline, and acid secretion was measured by back titration.¹⁸ In brief, the technique entails microscopic observation of the gastric mucosa of an urethane anaesthetised rat under epifluorescent illumination. The mucosa is continuously superfused by means of a perfusion chamber. Carbon particles, placed on top of the mucus gel, delineate the mucus-luminal interface,¹⁹ enabling measurement of mucus gel thickness by up and down focusing. Intracellular pH is measured by a ratiometric technique using the trapped intracellular dye 5,6 carboxyfluorescein diacetate (CF). Image analysis is used to limit the area of interest for pH_i measurements to two to three surface cells.

MEASUREMENT OF MUCUS AND SERUM BISMUTH CONCENTRATION

The bismuth concentration in gastroduodenal mucus was measured using a modification of the method of Slikerveer *et al.*,²⁰ with mucus collected according to the method of Muñoz *et al.*²¹ Gastric and duodenal mucus were gently scraped with a glass slide and weighed. Blood was collected by cardiac puncture. Mucus and serum were solubilised in 5 M HNO_3 for 12 hours at 70°C. Water was then added for a final concentration of 0.5 M HNO_3 . Bismuth concentration was measured by inductive coupled plasma atomic emission spectroscopy (ICP) by the UCLA Environmental Analysis Laboratory, and expressed as $\mu\text{g/g}$ wet weight for mucus bismuth concentrations or $\mu\text{g/l}$ for serum bismuth concentrations.

EXPERIMENTAL DESIGN – INJURY STUDIES

After an overnight fast, rats received GG311 (1 ml volume) or vehicle (water) by gavage. One half hour later, the animals were treated with 60 mg/kg indomethacin (1 ml volume) or vehicle (PEG 400) by subcutaneous injection. Six hours after receiving indomethacin, the animals were killed and gastric lesions were quantified.

EXPERIMENTAL DESIGN – IN VIVO MICROSCOPY STUDIES

Rats were divided into six groups, control, indomethacin, GG311, GG311+indomethacin, bismuth citrate+indomethacin, and ranitidine+indomethacin. GG311 (100 mg/kg in 1 ml volume), ranitidine (45 mg/kg), bismuth citrate (57 mg/kg) or vehicle (water) were given by gavage. One half hour later, the animals were treated with 60 mg/kg indomethacin (1 ml volume) or vehicle (PEG 400) by subcutaneous injection. Two hours after the injection of indomethacin or vehicle, *in vivo* microscopic observation was started, with measurement of acid output, mucosal blood flow, surface cell pH_i , and mucus gel thickness. The chamber was superfused with pH 7.4 Krebs' solution for the first 30 minutes, then the superfusate was changed to pH 1.0 solution.

EXPERIMENTAL DESIGN – BISMUTH MEASUREMENTS

Rats were treated with indomethacin, 60 mg/kg or vehicle (PEG 400) subcutaneously, and GG311, 100 mg/kg, orally as described above. Two groups were studied: GG311+vehicle, GG311+indomethacin. Rats were killed at one, three, six, and 12 hours after GG311 treatment.

SOLUTIONS

The dye solution consisted of CF, which was first dissolved in dimethylsulphoxide and then diluted with Krebs' solution. The final concentration of the solvent was less than 1%. Krebs' solution contains (in mM) 136 NaCl, 2.6 KCl, 1.8 CaCl_2 , N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) for pH 7.4. For acid superfusion, Krebs' solution was (in mM) 36 NaCl, 2.6 KCl, 1.8 CaCl_2 , HEPES, and titrated to pH 1.0 with 5 N HCl. Indomethacin was dissolved in PEG 400. GG311, ranitidine, and bismuth citrate were dissolved in distilled water.

CHEMICALS

CF was purchased from Molecular Probes (Eugene, OR, USA). Indomethacin, bismuth citrate, ranitidine, and PEG400 were purchased from Sigma Chemicals (St Louis, MO, USA). Ranitidine bismuth citrate (GG311) was a gift from Glaxo Research and Development (Greenford, UK).

STATISTICAL ANALYSIS

Results were expressed as means (SEM). Comparisons between the two groups were calculated by the Student's *t* test. Multiple group comparisons were performed by analysis of variance (ANOVA, factorial or repeated measures) followed by Fisher's contrast. A probability level <0.05 was considered significant.

Figure 1: Inhibitory effect of GG311 on gastric damage induced by subcutaneous injection of 60 mg/kg indomethacin. Rats received GG311 or vehicle by gavage 30 minutes before indomethacin (60 mg/kg subcutaneous) treatment. Six hours after receiving indomethacin, gastric lesions were assessed as described in Methods. (A) Effect of GG311 on gross gastric damage. (B) Effect of GG311 on histological gastric damage. Results are the mean of rats per group. In this and subsequent figures, vertical error bars represent SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ v vehicle treated rats by ANOVA.

Results

INJURY MODEL

Severe lesions were present in indomethacin injected, vehicle pre-treated rats (lesion score = 33.4 (3.3), $n = 24$). GG311 decreased the lesion scores in a dose dependent fashion. Lesion scores were 23.5 (7.8) ($n = 8$), 8.0 (4.4) ($n = 8$), and 1.6 (1.1) ($n = 8$) in rats treated with 10, 30, and 100 mg/kg GG311, respectively (Fig 1A). Lesion scores in rats treated with the two highest doses were significantly decreased from the lesion scores in untreated rats. The histological lesion score in rats treated with indomethacin alone was 134 (10) ($n = 8$). Pretreatment with 10 mg/kg GG311 significantly increased the lesion score to 160 (5) ($n = 8$), although pre-treatment with GG311, 30 and 100 mg/kg decreased the scores to 106 (8) ($n = 8$) and 77 (11) ($n = 8$), respectively (Fig 1B). Deep lesions, defined as injury extending into the glandular portion of the gastric pits were significantly prevented by the highest dose (100 mg/kg) of GG311. Deep lesions were

present in 4.7 (1.1)%, 5.3 (1.4)%, 3.0 (1.7)%, and 0.5 (0.3)%, of glands in rats pretreated with 0, 10, 30, and 100 mg/kg GG311, respectively.

IN VIVO MICROSCOPIC STUDY

Acid output

Acid output was significantly suppressed, as expected, by GG311 or ranitidine. Acid output was 0.45 (0.05) $\mu\text{mol/min/cm}^2$ in controls ($n = 10$), 0.11 (0.03) $\mu\text{mol/min/cm}^2$ in GG311 treated rats ($n = 9$), 0.31 (0.06) $\mu\text{mol/min/cm}^2$ in indomethacin treated rats ($n = 9$), 0.09 (0.03) $\mu\text{mol/min/cm}^2$ in GG311+indomethacin treated rats ($n = 8$), 0.39 (0.06) $\mu\text{mol/min/cm}^2$ in bismuth citrate+indomethacin treated rats ($n = 6$), and 0.13 (0.03) $\mu\text{mol/min/cm}^2$ in ranitidine+indomethacin treated rats ($n = 6$). Acid output was thus significantly decreased in groups treated with GG311 or ranitidine ($p < 0.05$ v controls, by ANOVA).

Mucosal blood flow

Figure 2 depicts relative gastric mucosal blood flow. Relative gastric mucosal blood flow was not affected by any treatment during in vivo microscopic experiments. Blood flow gradually declined in all groups to a level of 83.8 (1.4)% ($n = 10$), 78.1 (4.9)% ($n = 9$), 79.1 (2.5)% ($n = 8$), 90.8 (5.4)% ($n = 8$), 81.7 (4.1)% ($n = 6$), and 88.8 (3.3)% ($n = 6$) of baseline at 60 minutes. Consistent with our prior studies,²² acid superfusion did not affect mucosal blood flow.

Figure 2: Effect of GG311 and its components on relative gastric mucosal blood flow. Rats were pretreated with GG311, bismuth citrate (Bis), ranitidine (Ranit) or vehicle by gavage 30 minutes before subcutaneous injection of 60 mg/kg indomethacin (Indo) or vehicle. Two hours after indomethacin or vehicle injection, gastric mucosal blood flow measurement by laser-Doppler flowmetry was started.

Mucus gel thickness

Figure 3 depicts mucus gel thickness, as measured by the fluorometric technique. Indomethacin significantly decreased mucus

Figure 3: Effect of GG311 and its components on mucus gel thickness. Rats were pretreated with GG311, bismuth citrate (Bis), ranitidine (Ranit) or vehicle by gavage 30 minutes before subcutaneous injection of 60 mg/kg indomethacin (Indo) or vehicle. Two hours after indomethacin or vehicle injection, measurement of mucus gel thickness was started. * $p < 0.05$ v controls by repeated ANOVA.

gel thickness over the 50 minute measurement period ($p < 0.05$ v control, by repeated ANOVA). At time=0 min, gel thickness was 119.8 (5.7) μm ($n=10$), 112.0 (4.8) μm ($n=9$), 90.0 (5.0) μm ($n=9$), 88.2 (5.6) μm ($n=8$), 78.4 (7.3) μm ($n=6$), and 83.5 (6.1) μm ($n=6$) in the control, GG311, indomethacin, GG311+indomethacin, bismuth citrate+indomethacin, and ranitidine+indomethacin treated groups, respectively. GG311 pretreatment did not affect gel thickness of the control or indomethacin pre-treated groups. In accord with our prior studies,²² acid superfusion had no effect on gel thickness.

Intracellular pH and initial acidification rate

During superfusion with pH 7.4 Krebs' buffer, pH_i remained stable in the range of 6.98 (0.06) to 7.11 (0.04), with no statistical differences between any of the groups. During superfusion with pH 1.0 buffer, pH_i was significantly lower in the indomethacin and ranitidine+indomethacin groups than in the control group

Figure 4: Effect of GG311 and its components on intracellular pH (pH_i) of gastric surface cells. Rats were pretreated with GG311, bismuth citrate (Bis), ranitidine (Ranit) or vehicle by gavage 30 minutes before subcutaneous injection of 60 mg/kg indomethacin (Indo) or vehicle. Two hours after indomethacin or vehicle injection, gastric pH_i measurement was started. Intracellular pH was compared among six groups both during superfusion with pH 7.4 and pH 1.0 solutions by repeated ANOVA. * $p < 0.05$ v controls, † $p < 0.05$ v indomethacin treated group by repeated ANOVA for the period of acid superfusion (20–50 min, inclusive).

($p < 0.05$, by repeated ANOVA during acid superfusion), and was significantly higher in the GG311+indomethacin, and bismuth citrate+indomethacin groups compared with the indomethacin group ($p < 0.05$, by repeated ANOVA during acid superfusion). GG311 itself had no significant effect on pH_i during acid superfusion in the absence of indomethacin (Fig 4).

The initial acidification rate was calculated from the initial drop of pH_i after the superfusion of pH 1.0 buffer. Initial acidification rate was 7.53 (0.53) mM/min ($n=20$), 6.63 (0.54) mM/min ($n=20$), 11.75 (0.55) mM/min ($n=18$), 8.08 (0.54) mM/min ($n=16$), 9.32 (0.98) mM/min ($n=12$), and 10.34 (0.94) mM/min ($n=12$) in the control, GG311, indomethacin, GG311+indomethacin, bismuth citrate+indomethacin, and ranitidine+indomethacin treated groups, respectively. The acidification rates in the GG311, GG311+indomethacin, and bismuth citrate+indomethacin groups were statistically similar to that of the controls, and was significantly faster in the indomethacin, and ranitidine+indomethacin groups ($p < 0.05$ v control, by ANOVA).

MUCUS BISMUTH CONCENTRATIONS

Figure 5 shows mucus bismuth concentration in rats treated with either GG311 alone or GG311 plus indomethacin. Bismuth concentrations in the gastric mucus was 7.3 (2.8) $\mu\text{g/g}$ wet weight ($n=5$) at baseline. In rats treated with GG311 alone, gastric mucus bismuth concentrations were 4903 (2519) ($n=4$), 149.6 (53.5) ($n=5$), 40.8 (12.2) ($n=6$), and 31.9 (14.3) $\mu\text{g/g}$ ($n=3$) at one, three, six, and 12 hours, respectively. Indomethacin increased the bismuth concentration in GG311 treated rats to 6944 (1596) ($n=4$, NS), 2017 (456) ($n=5$, 13.5-fold increase, $p < 0.01$), 1788 (669) ($n=6$, 49.4-fold increase, $p < 0.05$), and 207.3 (20.2) ($n=3$, 6.5-fold increase, $p < 0.01$) $\mu\text{g/g}$ at one, three, six, and 12 hours, respectively (Fig 5A). Bismuth concentrations in the duodenal mucus was 12.8 (8.5) $\mu\text{g/g}$ wet weight ($n=5$) at baseline. In rats treated with GG311 alone, duodenal mucus bismuth concentrations were 1133 (608) ($n=4$), 16.6 (12.1) ($n=5$), 15.6 (10.5) ($n=6$), and 15.0 (5.4) $\mu\text{g/g}$ ($n=3$) at one, three, six, and 12 hours, respectively. Indomethacin also increased the duodenal mucus bismuth concentration to a lesser extent. Bismuth concentrations in indomethacin+GG311 treated rats were 1296 (687) ($n=4$, NS), 208.9 (66.9) ($n=5$, 12.5-fold increase, $p < 0.05$), 51.2 (7.6) ($n=6$, 3.2-fold increase, $p < 0.05$), and 29.7 (12.9) ($n=3$, NS) $\mu\text{g/g}$ at one, three, six, and 12 hours, respectively (Fig 5B).

Administration of GG311 resulted in low levels of bismuth absorption. Basal serum bismuth concentration was 14.0 (7.0) $\mu\text{g/l}$ ($n=4$). In rats treated with GG311 alone, serum bismuth concentrations were 68.0 (32.3) ($n=3$), 173.3 (115.7) ($n=3$), and 112.9 (44.8) ($n=3$) $\mu\text{g/l}$ at three, six, and 12 hours, respectively. In indomethacin+GG311 treated

Figure 5: Bismuth concentration in gastroduodenal mucus gel. Rats were pretreated with GG311 by gavage 30 minutes before subcutaneous injection of 60 mg/kg indomethacin (Indo) or vehicle. Rats were killed at one, three, six, and 12 hours after GG311 treatment. Bismuth concentration was measured by inductive coupled plasma atomic emission spectroscopy as described in Methods. (A) Bismuth concentration in gastric mucus gel. (B) Bismuth concentration in duodenal mucus gel. * $p < 0.05$, ** $p < 0.01$ v rats treated with GG311 alone by the Student's t test.

rats, serum bismuth concentrations were 97.8 (35.3) ($n=3$), 84.9 (25.1) ($n=3$), and 37.3 (7.1) $\mu\text{g/l}$ ($n=3$) at three, six, and 12 hours, respectively. There was no statistically significant difference between the two groups.

Discussion

GG311 dose dependently prevented severe mucosal lesions in indomethacin treated rats and prevented microscopic injury at the 30 and 100 mg/kg doses, and prevented deep injury to the gastric glands at the 100 mg/kg dose. GG311 (100 mg/kg) significantly suppressed acid output. No treatment affected relative mucosal blood flow. Indomethacin significantly reduced mucus gel thickness, increased the initial acidification rate, and impaired pH_i homeostasis during luminal acid superfusion. In indomethacin treated rats, GG311 (100 mg/kg) normalised the initial acidification rate to control values without affecting gel thickness, and improved pH_i homeostasis during acid superfusion. The most striking and novel finding was that indomethacin substantially increased at times >one hour the gastric and duodenal mucus gel bismuth content of GG311 treated rats.

Although bismuth compounds have been used for several centuries to treat gastro-intestinal problems, their mechanism of action has remained incompletely understood. Bismuth salts including GG311 have been shown to protect the gastric mucosa against a variety of injurious stimuli, including NSAIDs.^{1 2 8 9 23} This latter effect suggests that bismuth might exert at least some of its protective effect in a prostaglandin independent fashion. Mechanisms that have been proposed to explain the gastroprotective effects of bismuth include inhibition of pepsin activity,⁴ binding of epidermal growth factor,²⁴ binding to ulcers,²⁵ stimulation of prostaglandin synthesis and bicarbonate secretion,^{26 27} antibacterial activity,²⁸ and binding to mucus.²⁹ Antisecretory compounds such as the H_2 receptor antagonist ranitidine have also been shown to protect the stomach against injury.³⁰ The primary protective action of H_2 receptor

antagonists presumably stems from their anti-secretory effect, and not from an enhancement of gastroprotective factors.

In this study, indomethacin reduced mucus gel thickness and impaired pH_i homeostasis during acid exposure. The mucus gel layer covering the epithelial surfaces of gastric mucosa constitutes the first line of mucosal defence against luminal acid. The thinned mucus gel in indomethacin treated rats is either caused by diminished mucus synthesis or increased degradation. In previous studies, indomethacin thinned the adherent mucus gel, and decreased the synthetic rate of gastric mucus^{31 32} rendering the first possibility plausible. On the other hand, it has been shown that gastric mucus content is increased after fasting, which is assumed to be due to the decreased mechanical abrasion of the gastric mucosa.³³ Therefore, it is also possible that gastric hypercontraction, which is a known effect of ulcerogenic doses of indomethacin,^{34 35} may increase the degradation of the gastric mucus resulting in the thinned mucus gel. Gastric mucosal blood flow, which has been shown to affect pH_i homeostasis during superfusion with acidic solutions²² is known to be decreased by indomethacin.^{36 37} Laser-Doppler flowmetry used in this study is a good technique to measure relative blood flow changes, but is not suitable for the measurement of absolute blood flow because its accuracy is dependent on the pressure and direction of the laser probe.³⁸ Although relative gastric mucosal blood flow was not different among all of the groups during the experiments, absolute mucosal blood flow may be decreased in indomethacin treated rats. The decrease in absolute blood flow would reduce delivery of bicarbonate needed for the preservation of pH_i during acid exposure.

The explanation most consistent with the finding that bismuth compounds protected the stomach from indomethacin associated injury is that bismuth, present in high concentrations in the gastric mucus gel, decreased its acid permeability. This impaired permeability was manifest as a slowed acidification rate and improved pH_i homeostasis during acid

superfusion, compared with rats treated with indomethacin alone. We assume that the rate of rapid, initial drop of pH_i (initial acidification rate) is primarily dependent on the rate of acid permeation through the mucus gel. We have previously shown that the initial acidification rate is inversely correlated to mucus gel thickness – that is, the thicker the gel, the slower the diffusion.³⁹ Other homeostatic mechanisms that change pH_i such as increased Na^+/H^+ exchange are induced relatively slowly, and thus would have little effect on the initial drop in pH_i .^{40–41} Although GG311 decreased acid secretion, luminal pH was held constant by the high flow rate of the superfusate. Furthermore, the small change in acid secretory state of the mucosa – that is, from basal to fully inhibited – was unlikely to change pH_i regulation, as we have shown previously in cimetidine treated rats,²² and also in this study in the ranitidine only group. On the basis of the foregoing considerations, the most plausible explanation for the normal acidification rate despite a thinned mucus gel in the indomethacin + GG311 group is decreased proton permeability of the adherent mucus gel.

The mechanism for the decreased permeability of adherent gastric mucus in the presence of NSAIDs, and high concentration of bismuth, however, is unclear. Indomethacin, if anything, increased mucus proton permeability in and of itself, as the increase in initial acidification rate (56%) in indomethacin treated rats was greater than would have been predicted from the 19% fall in gel thickness.* The initial acidification rate in the GG311 group was unchanged from control rats, suggesting that GG311 alone had no measurable effect on mucus proton permeability. Thus, high mucus bismuth concentrations, found only in the indomethacin + GG311 group at three hour time point, correlated with decreased proton permeability of adherent gastric mucus. This suggests a direct, probably physical interaction between bismuth and the mucus gel. The nature of this interaction is not clear, although there is experimental evidence suggesting that divalent cations such as Mg^{2+} , Ca^{2+} , and Fe^{2+} can alter the physical properties of adherent mucus.^{42–44} Moreover, Lee found that precipitated gastric mucus glycoprotein complexed with colloidal bismuth subcitrate in vitro produced a mucus-bismuth complex that impeded proton permeability.⁴⁵ These in vitro experiments thus provide a plausible basis for hypothesis that bismuth, in high concentrations, changes the physical structure of adherent gastric mucus so as to decrease its permeability to protons.

The reason why indomethacin increased gastric mucus bismuth concentrations by the substantial extent observed is also not obvious. One possibility is that bismuth might be adhering to damaged areas or an inflammatory exudate in the gastric mucosa. Indeed, it

has been shown that bismuth binds to injured gastric mucosa in a human study⁴⁵ and experimental ulcer models.²⁵ In this study, however, severe and histological injury were nearly absent under the conditions in which mucus bismuth concentrations were studied. Recently, several reports have suggested that the pathogenesis of indomethacin induced gastric injury involves gastric hypercontraction and microvascular disturbance in addition to a prostaglandin deficiency.^{34–35–46–47} Takeuchi *et al*^{46–47} reported that an ulcerogenic dose of indomethacin induced a pronounced increase in frequency and amplitude of gastric contractions, which was associated with oscillatory fluctuations of mucosal blood flow, and increased extravasation of Evans blue dye. These findings occurred prior to the appearance of gastric lesions. Therefore, it is plausible that bismuth was bound to an exudate derived from microvessels before the appearance of gastric lesions. The second prospect is that indomethacin slowed gastric emptying, allowing more contact time between GG311 and the gastric mucosa. This latter hypothesis is not supported by experimental motility studies, which suggest that gastric emptying, if changed at all, is hastened by indomethacin.^{48–49} Furthermore, gastric hypercontraction induced by indomethacin may enhance the mechanical contact between GG311 and the gastric mucosa, increasing the diffusion of bismuth particles into the gastric mucus gel. Another explanation is that indomethacin changed the chemical composition of the gastric mucus gel in such a manner as to increase binding to cationic metals. For example, indomethacin decreased the amount of lipids in rat gastric mucus.⁵⁰ The NSAID aspirin also decreased the density of phospholipids and the hydrophobicity of canine gastric mucus.^{51–52} Indomethacin, by decreasing the hydrophobicity and lipid content of the surface of the adherent mucus, may have increased the accessibility of the mucus to exogenous bismuth. The studies with bismuth citrate and ranitidine alone strongly suggest that the bismuth, and not the ranitidine component of GG311 decreases the permeability of gastric mucus.

The potentiation of bismuth concentrations in the gastric mucus by indomethacin has significance beyond the observed alterations of acid permeability and protection against indomethacin induced gastric mucosal injury. *Helicobacter pylori*, the organism associated with chronic gastritis and recurrent peptic ulcer disease, resides in the juxtamucosal region of the gastric mucus. This organism is sensitive to bismuth in vitro,⁵³ although clinical trials in which bismuth compounds were used as monotherapy to eradicate *H. pylori* have been disappointing.^{54–55} Furthermore, *H. pylori* infection is associated with decreased gastric mucus bismuth concentrations in human postmortem specimens treated in vitro with colloidal bismuth subcitrate.²¹ This study suggests that the combination of a bismuth containing compound such as GG311 combined with an NSAID such as indomethacin,

*Our previous data demonstrated that the per cent change in initial acidification rate was 68% of the per cent change in mucus gel thickness. We would thus predict that for a 19% decrease in mucus gel thickness, initial acidification rate would increase by only 13%.

by virtue of its high and prolonged concentration of bismuth in mucus, might be a logical combination for the large scale eradication of *H. pylori* in selected clinical populations.

In conclusion, this study indicates that bismuth concentrations in the gastric mucus are increased during the inapparent gastric injury induced by indomethacin, and are associated with impaired acid permeability of the mucus gel. Thus, GG311 protects gastric mucosa from indomethacin induced injury by strengthening pre-epithelial defence mechanisms in addition to inhibiting acid secretion. As gastroprotective properties of bismuth compounds may also result from inhibition of pepsin activity, binding of bile acids and epidermal growth factor, and induction of PG synthesis, and because bismuth compounds also protect the gastric mucosa from other noxious stimuli such as ethanol and cold restraint stress,^{2,23} it is possible that other mechanisms may also be involved in the gastroprotective effect of GG311.

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- 1 Konturek SJ, Kwiecien N, Obtulowicz W, Hebzda Z, Oleksy J. Effects of colloidal bismuth subcitrate on aspirin-induced gastric microbleeding, DNA loss, and prostaglandin formation in humans. *Scand J Gastroenterol* 1988; 23: 861-6.
- 2 Goldenberg MM, Honkomp LJ, Burrous SE, Castellon AW. Protective effect of Pepto-Bismol liquid on the gastric mucosa of rats. *Gastroenterology* 1975; 69: 636-40.
- 3 Slomiany BL, Nishikawa H, Bilski J, Slomiany A. Colloidal bismuth subcitrate inhibits peptic degradation of gastric mucus and epidermal growth factor in vitro. *Am J Gastroenterol* 1990; 85: 390-3.
- 4 Tay HP, Chaparala RC, Harmon JW, Huesken J, Saini N, Hakki FZ, et al. Bismuth subsalicylate reduces peptic injury of the oesophagus in rabbits. *Gut* 1990; 31: 11-6.
- 5 Shorrock CJ, Crampton JR, Gibbons LC, Rees WDW. Effect of bismuth subcitrate on amphibian gastroduodenal bicarbonate secretion. *Gut* 1989; 30: 917-21.
- 6 Ehsanullah R, Page M, Tildesley G, Wood J. Prevention of gastroduodenal damage induced by non-steroidal anti-inflammatory drugs: controlled trial of ranitidine. *BMJ* 1988; 297: 1017-21.
- 7 Robinson MG, Griffin JWJ, Bowers J, Kogan FJ, Kogut DG, Lanza FL, et al. Effect of ranitidine on gastroduodenal mucosal damage induced by nonsteroidal anti-inflammatory drugs. *Dig Dis Sci* 1989; 34: 424-8.
- 8 Stables R, Campbell CJ, Clayton NM, Clitherow JW, Grinham CJ, McColl AA, et al. Gastric anti-secretory, mucosal protective, anti-pepsin, and anti-helicobacter properties of ranitidine bismuth citrate. *Aliment Pharmacol Ther* 1993; 7: 237-46.
- 9 Hudson N, Murray FE, Cole AT, Turnbull GM, Lettis GM, Hawkey CJ. Ranitidine bismuth citrate and aspirin-induced gastric mucosal injury. *Aliment Pharmacol Ther* 1993; 7: 515-21.
- 10 Djahanguiri B. The production of acute gastric ulceration by indomethacin in the rat. *Scand J Gastroenterol* 1969; 4: 265-7.
- 11 Satoh H, Guth PH, Grossman MI. Role of food in gastrointestinal ulceration produced by indomethacin in the rat. *Gastroenterology* 1982; 83: 210-5.
- 12 Itoh M, Paulsen G, Guth PH. Hemorrhagic shock and acid gastric injury in the rat. *Gastroenterology* 1986; 90: 1103-10.
- 13 Kaneko K, Guth PH, Kaunitz JD. In vivo measurement of rat gastric surface cell intracellular pH. *Am J Physiol* 1991; 261: G548-52.
- 14 Kaunitz JD, Nishizaki Y, Kaneko K, Guth PH. Effect of orogastric nicotine on rat gastric mucosal gel thickness, surface cell viability, and intracellular pH. *J Pharmacol Exp Ther* 1993; 265: 948-54.
- 15 Nishizaki Y, Guth PH, Kaunitz JD. Isoproterenol enhances rat gastric mucosal barrier function in vivo. *Gastroenterology* 1993; 105: 340-6.
- 16 Nishizaki Y, Guth PH, Quintero E, Del Rivero M, Bover J, Kaunitz JD. Prostaglandin E₂ enhances gastric defense mechanisms against acid injury in uremic rats. *Gastroenterology* 1994; 107: 1382-9.
- 17 Tanaka S, Kaunitz JD. Indomethacin does not alter the effect of pentagastrin on rat gastric defense mechanisms. *Peptides* 1996; 17: 155-9.
- 18 Garner A, Heylings JR. Stimulation of alkaline secretion in amphibian-isolated gastric mucosa by 16,16-dimethyl PGE₂ and PGF_{2a}. *Gastroenterology* 1979; 76: 497-503.
- 19 Kerss S, Allen A, Garner A. A simple method for measuring thickness of the mucus gel layer adherent to rat, frog, and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. *Clin Sci* 1982; 63: 187-95.
- 20 Slikkerveer A, Helmich RB, de Wolff FA. Analysis for bismuth in tissue by electrothermal atomic absorption spectrometry. *Clin Chem* 1993; 39: 800-3.
- 21 Muñoz DJB, Tasman-Jones C, Pybus J. Effect of *Helicobacter pylori* on colloidal bismuth subcitrate concentration in gastric mucus. *Gut* 1994; 33: 592-6.
- 22 Nishizaki Y, Guth PH, Kim G, Wayland H, Kaunitz JD. Pentagastrin enhances gastric mucosal defenses in vivo: luminal acid-dependent and independent effects. *Am J Physiol* 1994; 267: G94-104.
- 23 Goldenberg MM, Honkomp LJ, Castellion AW. Prevention of bismuth subsalicylate of gastric mucosal lesions in response to noxious stimuli in rats. *Pharmacol Res Comm* 1978; 10: 13-20.
- 24 Slomiany BL, Nishikawa H, Bilski J, Slomiany A. Colloidal bismuth subcitrate inhibits peptic degradation of gastric mucus and epidermal growth factor in vitro. *Am J Gastroenterol* 1990; 85: 390-3.
- 25 Koo J, Ho J, Lam SK, Wong J, Ong GB. Selective coating of gastric ulcer by tripotassium dicitrate bismuthate in the rat. *Gastroenterology* 1982; 82: 864-70.
- 26 Konturek SJ, Bilski J, Kwiecien N, Obtulowicz W, Kopp B, Oleksy J. De-Nol stimulates gastric and duodenal alkaline secretion through prostaglandin dependent mechanism. *Gut* 1987; 28: 1557-63.
- 27 Mertz-Nielsen A, Steenberg P, Neumark T, Bukhave K, Rask-Madsen J. Colloidal bismuth subcitrate causes sustained release of gastric mucosal prostaglandin E₂. *Aliment Pharmacol Ther* 1991; 5: 127-33.
- 28 Manhart MD. In vitro antimicrobial activity of bismuth subsalicylate and other bismuth salts. *Rev Infect Dis* 1990; 12: S11-5.
- 29 Lambert JR. Pharmacology of bismuth-containing compounds. *Rev Infect Dis* 1991; 13 (suppl 8): S691-5.
- 30 Navarova J, Nasalova V. Effect of H₂-receptor antagonists on indomethacin-induced lysosomal enzyme release from rat gastric mucosa. *Methods Find Exp Clin Pharmacol* 1994; 16: 119-24.
- 31 Torii A, Kameda H, Kawamura T, Onizawa N, Nozawa H, Ariizumi M, et al. Effect of gastric mucus on the uptake of the carcinogen MNNG by gastric mucosal DNA. *J Clin Gastroenterol* 1990; 12 (suppl 1): S116-24.
- 32 Slomiany BL, Piotrowski J, Ismail A, Rajiyah G, Tamura S, Bielanski W, et al. Protection against alcohol-induced gastric mucosal injury by nitecapone. *Gen Pharmacol* 1991; 22: 1055-62.
- 33 Ohara S, Hotta K. Effects of fasting on mucus glycoprotein biosynthesis in rat stomach. *Comp Biochem Physiol* 1985; 82B: 207-10.
- 34 Mersereau WA, Hinchey EJ. Relationship between the gastric myoelectric and mechanical activity in the genesis of ulcers in indomethacin-insulin-treated rats. *Dig Dis Sci* 1988; 33: 200-8.
- 35 Ueki S, Takeuchi K, Okabe S. Gastric motility is an important factor in the pathogenesis of indomethacin-induced gastric mucosal lesions in rats. *Dig Dis Sci* 1988; 33: 209-16.
- 36 Gerkens JF, Shand DG, Flexner C, Nies AS, Oates JA, Data JL. Effect of indomethacin and aspirin on gastric blood flow and acid secretion. *J Pharmacol Exp Ther* 1977; 203: 646-52.
- 37 Holm L, Jägar A. Role of prostaglandins in regulation of gastric mucosal blood flow and acid secretion. *Am J Physiol* 1992; 263: G446-51.
- 38 Kiel JW, Riedel GL, DiResta GR, Shepherd AP. Gastric mucosal blood flow measured by laser-Doppler velocimetry. *Am J Physiol* 1985; 249: G539-45.
- 39 Engel E, Guth PH, Nishizaki Y, Kaunitz JD. Barrier function of the gastric mucus gel. *Am J Physiol* 1995; 269: G994-9.
- 40 Kaneko K, Guth PH, Kaunitz JD. NA⁺/H⁺ exchange regulates intracellular pH of rat gastric cells in vivo. *Pflügers Arch* 1992; 421: 322-8.
- 41 Seidler U, Carter K, Ito S, Silen W. Effect of CO₂ on pH in rabbit parietal, chief, and surface cells. *Am J Physiol* 1989; 256: G466-75.
- 42 Crowther RS, Marriott C, James SL. Cation induced changes in the rheological properties of purified mucus glycoproteins. *Biorheology* 1984; 21: 253-63.
- 43 Forstner JF, Jabbal I, Findlay BP, Forstner GG. Interaction of mucins with calcium, H⁺ ion and albumin. *Mod Probl Pediatr* 1976; 19: 54-65.
- 44 Marriott C, Shih CK, Litt M. Changes in the gel properties of tracheal mucus induced by divalent cations. *Biorheology* 1979; 16: 331-7.
- 45 Lee SP. A potential mechanism of action of colloidal bismuth subcitrate: diffusion barrier to hydrochloric acid. *Scand J Gastroenterol* 1982; 17 (suppl 80): 17-21.
- 46 Takeuchi K, Ueshima K, Hironaka Y, Fujioka Y, Matsumoto J, Okabe S. Oxygen free radicals and lipid peroxidation in the pathogenesis of gastric mucosal lesions induced by indomethacin in rats. *Digestion* 1991; 49: 175-84.

- 47 Takeuchi K, Ueki S, Okabe S. Importance of gastric motility in the pathogenesis of indomethacin-induced gastric lesions in rats. *Dig Dis Sci* 1986; **31**: 1114–22.
- 48 Stein J, Zeuzem S, Uphoff K, Laube H. Effects of prostaglandins and indomethacin on gastric emptying in the rat. *Prostaglandins* 1994; **47**: 31–40.
- 49 Kohut A, Majzis J. Effect of allopurinol and superoxide dismutase on indomethacin-induced gastric lesions in the rat. *Physiol Res* 1993; **42**: 273–6.
- 50 Slomiany A, Mizuta K, Piotrowski J, Nishikawa H, Slomiany BL. Gastric mucosal; protection by sucralfate involves phosphoinositides participation. *Int J Biochem* 1995; **22**: 1179–83.
- 51 Goddard PJ, Kao Y-CJ, Lichtenberger L. Luminal surface hydrophobicity of canine gastric mucosa is dependent on a surface mucous gel. *Gastroenterology* 1990; **98**: 361–70.
- 52 Kao Y-C, Goddard PJ, Lichtenberger LM. Morphological effects of aspirin and prostaglandin on the canine gastric mucosal surface. *Gastroenterology* 1990; **98**: 592–606.
- 53 McNulty CAM, Dent J, Wise R. Susceptibility of clinical isolates of *Campylobacter pyloridis* to 11 antimicrobial agents. *Antimicrob Agents Chemother* 1985; **28**: 837–8.
- 54 Rauws EAJ, Tytgat GN. *Campylobacter pylori*: treatment of gastritis. In: Rathbone BJ, Heatley RV, ed. *Campylobacter pylori and gastrointestinal disease*. Oxford: Blackwell Scientific, 1989: 225–32.
- 55 Coghlan JG, Tobin A, O'Morian C. *Campylobacter pylori* and ulcer treatment. In: Rathbone BJ, Heatley RV, ed. *Campylobacter pylori and gastroduodenal disease*. Oxford: Blackwell Scientific, 1989: 232–45.