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Gastric Motility Is an Important Factor in the Pathogenesis of Indomethacin-Induced Gastric Mucosal Lesions in Rats

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Effects of atropine, cimetidine, and 16,16-dimethyl prostaglandin E_2 (16,16-dmPGE$_2$) on indomethacin-induced gastric lesions were investigated in rats by correlating their effects on gastric acid and HCO^-_3 secretion and motility. Subcutaneously administered indomethacin (25 mg/kg) produced gastric mucosal lesions within 4 hr. In parallel studies, an equivalent dose of indomethacin inhibited gastric HCO^-_3 secretion, and stimulated gastric motor activity measured as intraluminal pressure recordings, whereas acid secretion was unaffected. The lesions induced by indomethacin were significantly prevented by three agents: cimetidine (100 mg/kg), which reduced acid secretion; atropine (1 mg/kg), which reduced acid secretion and gastric motility; and 16,16-dmPGE$_2$ (10 μg/kg), which reduced acid secretion and motility and increased gastric HCO^-_3 secretion. If acid (150 mM HCl) was infused into the stomach (1.2 ml/hr) during indomethacin treatment, only the latter two agents significantly prevented the formation of gastric lesions in response to indomethacin. Since only the effect on gastric motility was common to these two agents (atropine and 16,16-dmPGE$_2$), the increased gastric motility may be an important pathogenetic factor in indomethacin-induced gastric lesions. The presence of acid as well as a deficiency of endogenous PGs may be prerequisite for later extension of the lesions but cannot account for the induction of mucosal lesions in rats following administration of indomethacin.

KEY WORDS: indomethacin; gastric lesion; acid; motility; rat.

Indomethacin, a nonsteroidal antiinflammatory drug, is known to produce erosions and ulcers in the stomach as a side effect (1, 2). Since antacids, antisecretory agents, or various analogs of prostaglandins (PGs) potently inhibited the development of these lesions (3–6), luminal acid as well as a deficiency of endogenous PGs are thought to be involved in the mechanisms underlying formation of the lesions. Recently, we reported on the importance of gastric motility in the pathogenesis of indomethacin-induced gastric lesions in rats, by demonstrating a relationship between the increased gastric motility and the severity of lesions (7). However, to define a role of gastric motility in the pathogenetic mechanism of these lesions, (1) the effects of antiulcer drugs must be correlated with their effects on gastric motility in the presence of indomethacin, and (2) the influence of luminal acid

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must be separated from that of gastric motility during lesion formation caused by indomethacin.

In the current study, we therefore investigated the influences of atropine, cimetidine, and 16,16-dimethyl prostaglandin E_2 (16,16-dmPGE$_2$) on gastric acid and HCO^-_3 secretion, and motor activity in the presence of indomethacin in rats, and examined the effects of these agents on formation of gastric lesions induced by indomethacin in the absence or presence of exogenous acid. We discuss factor(s) responsible for their protective action against mucosal injury caused by indomethacin.

MATERIALS AND METHODS

Male Sprague-Dawley rats (230–250 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to tap water for 24 hr prior to the experiments. Each study was carried out using 5–10 rats per group.

Induction of Gastric Lesions. Indomethacin (Sigma, St. Louis, Missouri), suspended in saline with a few drops of Tween 80 (Nakarai, Kyoto, Japan), was given subcutaneously to rats in a dose of 25 mg/kg in a volume of 0.5 ml/100 g body weight. The animals were killed 4 hr later under deep ether anesthesia, and the stomachs were removed, inflated by injecting 12 ml of 2% formalin, immersed in 2% formalin for 10 min to fix both the inner and outer layers of gastric walls, and opened along the greater curvature. The stomachs were examined for lesions under a dissecting microscope with a square grid (×10), and the total length of macroscopically visible lesions (mm) was calculated for each stomach. Atropine sulfate (Merck, Darmstadt, West Germany; 0.3 and 1 mg/kg), cimetidine (Sigma, 30 and 100 mg/kg), or 16,16-dmPGE$_2$ (Funakoshi, 3 and 10 μg/kg) was given subcutaneously 30 min before administration of indomethacin. Atropine or cimetidine was dissolved or suspended in saline, respectively, and 16,16-dmPGE$_2$ was first dissolved in absolute ethanol and diluted with saline. Control animals were given the vehicle alone. In these studies, the person measuring the lesions did not know the treatment given to the animals.

Determination of Gastric Acid Secretion. While the rat was under ether anesthesia, the abdomen was incised and the pylorus ligated. An acute fistula, prepared using a polyethylene tube, was provided in the forestomach, and a fine polyethylene tube was inserted into the tail vein. The rats were kept in individual cages, and the gastric contents were collected hourly through the fistula by gravity drainage. During the experimental period, Hartman-Ringer solution (Midori-Juji, Osaka, Japan) was continuously infused at a rate of 1.2 ml/hr into the tail vein to compensate for the loss of body fluid due to collections of the gastric contents. After centrifugation, the samples were measured for volume and titrated for acidity against 0.1 N NaOH to pH 7.0 (Autoburette, Radiometer, Copenhagen). Acid output was calculated and expressed as microequivalents per hour. Indomethacin (25 mg/kg) was given subcutaneously at least 3 hr after basal acid secretion had stabilized. In some experiments, atropine (1 mg/kg), cimetidine (100 mg/kg), or 16,16-dmPGE$_2$ (10 μg/kg) was given 1 hr before administration of indomethacin. Acid secretion was measured for total period of 7 hr.

Determination of HCO^-_3 Secretion. Under ether anesthesia, the abdomen was incised, and the stomach and duodenum were exposed. An acute fistula was provided in the forestomach by means of a polyethylene tube. Another polyethylene tube was inserted into the stomach through a slit in the duodenum and was held in place by a ligature around the pylorus. Both cannulas were pulled out through the lateral abdominal wall and held in place with a ligature. The animals were then kept in the individual cages, and the stomachs were lavaged with saline, perfused at a flow rate of 1 ml/min with saline which was adjusted to pH 7.0, gassed with 100% O_2, heated at 37°C, and kept in a reservoir. To unmask HCO^-_3 in the lumen, omeprazole (Hassle, Mölndal, Sweden; 30 mg/kg), suspended in 1% carboxymethylcellulose solution, was given subcutaneously to inhibit acid secretion (8). HCO^-_3 secretion was measured at pH 7.0 using a pH-stat method (Hiranuma Comtite-7, Mito, Japan) and by adding 10 mM HCl to the reservoir. To stimulate HCO^-_3 secretion, the stomach was perfused for 10 min with 100 mM HCl made isotonic with NaCl. Approximately 1 hr after basal HCO^-_3 secretion had stabilized, indomethacin was given subcutaneously in a dose of 25 mg/kg, and in half the number of those rats the stomach was perfused for 10 min with 100 mM HCl 1 hr after administration of indomethacin. In another experiment, the effects of atropine (1 mg/kg), cimetidine (100 mg/kg), and 16,16-dmPGE$_2$ (10 μg/kg) on gastric HCO^-_3 secretion were investigated in the presence of indomethacin (25 mg/kg). These agents were given subcutaneously 30 min after administration of indomethacin. In every study, HCO^-_3 secretion was determined every 15 min, and the results were expressed as microequivalents per 15 min.

Determination of Gastric Motor Activity. Gastric motor activity was measured as intraluminal pressure recordings in conscious rats using a balloon according to a previous paper (7). Under ether anesthesia, a balloon (1 cm in diameter) was placed in the glandular part of the stomach, and the balloon and the support catheter were pulled out through abdominal incision and held in place by a ligature. The animals were then placed in Bollman cages, and gastric motility was monitored on a Hitachi recorder (Ibaragi, Japan; model 056) using a pressure transducer (Narco Tele Care, Houston, Texas; model 151-T) and a polygraph (San-Ei, Tokyo, Japan; model 6M-72) for 5 hr after complete recovery from anesthesia. Indomethacin was given subcutaneously in a dose of 25 mg/kg 1 hr after basal motor activity had well stabilized. Atropine (1 mg/kg), cimetidine (100 mg/kg), and 16,16-dmPGE$_2$ (10 μg/kg) were given subcutaneously 30 min before administration of indomethacin. In some cases, the effects of these agents on the increased gastric contractions caused by indomethacin were investigated by administering these agents 2 hr after indomethacin treatment. Quantitation of gastric motor activity was per-

formed by counting the number of contractions with an amplitude of 15 cm H_2O or greater and by measuring the amplitude of each contraction (clear spike) over a 10-min period, determining the mean for this period of a rat from these values, and by calculating the mean ± SEM for each time period from five different rats.

Intragastric Infusion of Exogenous Acid during Indomethacin Treatment. Since atropine, cimetidine, and 16,16-dmPGE$_2$ significantly affected gastric acid secretion, the effects of these agents on gastric lesions induced by indomethacin were examined in the presence of exogenous acid, in an attempt to separate the protective effects of these drugs from their antisecretory actions. Under ether anesthesia, the abdomen was incised and the stomach exposed. A fine polyethylene tube was inserted into the forestomach for infusing the stomach with acid. The animals were placed in Bollman cages, and 150 mM HCl was continuously infused into the stomach through the tube at a rate of 1.2 ml/hr during a 4-hr test period. Indomethacin was given subcutaneously in a dose of 25 mg/kg, and the acid infusion was started immediately after indomethacin treatment. Atropine (1 mg/kg), cimetidine (100 mg/kg), and 16,16-dmPGE$_2$ (10 μg/kg) were given subcutaneously 30 min before administration of indomethacin. The animals were killed 4 hr after indomethacin treatment, and the stomachs were removed, treated with 2% formalin, and examined for lesions under a dissecting microscope as described previously.

Statistics. Data are presented as the mean ± SEM from 5–10 rats per group. The values were analyzed using Dunnet's multiple comparison test (9), and considered to be statistically significant if $P < 0.05$.

RESULTS

Gastric Lesions Induced by Indomethacin. Subcutaneously administered indomethacin (25 mg/kg) produced gross mucosal lesions in the stomach within 4 hr, exclusively in the corpus mucosa, and the lesion index was 17.1 ± 1.8 mm (N = 30). Pretreatment of the animals with subcutaneously administered atropine (0.3 and 1 mg/kg), cimetidine (30 and 100 mg/kg), or 16,16-dmPGE$_2$ (3 and 10 μg/kg) dose dependently reduced the formation of gastric lesions induced by indomethacin (Figure 1.). The inhibition obtained by atropine (1 mg/kg), cimetidine (100 mg/kg), or 16,16-dmPGE$_2$ (10 μg/kg) was 93.3%, 89.7%, or 90.6%, respectively.

Gastric Acid Secretion. Control animals secreted acid in the rates of about 100–130 μeq/hr in acute fistula preparations under unanesthetized conditions. Subcutaneously administered indomethacin (25 mg/kg) had no effect on these rates of acid secretion during a 4-hr test period (Figure 2). Gastric acid secretion was markedly reduced (75%) within 1 hr after subcutaneous administration of

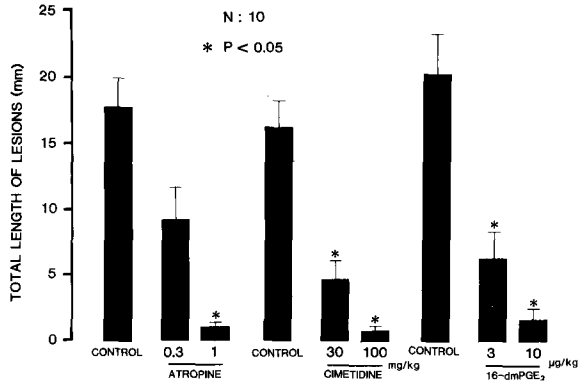

Fig 1. Effects of atropine, cimetidine, and 16,16-dmPGE$_2$ on gastric lesions induced by indomethacin in rats. Either agent was given subcutaneously 30 min before indomethacin treatment (25 mg/kg, subcutaneously). The animals were killed 4 hr after administration of indomethacin. Data are presented as the mean ± 1 SEM from 10 rats per group. *Statistically significant from controls at $P < 0.05$.

atropine (1 mg/kg), and almost abolished during the rest of experimental period in the presence of indomethacin. In the animals treated with cimetidine (100 mg/kg), acid secretion was significantly inhibited during a 4-hr test period, the maximal inhibition being 78.3% obtained at 2 hr after administration. Subcutaneous administration of 16,16-dmPGE$_2$ (10 μg/kg) also had a significant effect on acid secretion in the presence of indomethacin, and acid secretion was significantly reduced for the first

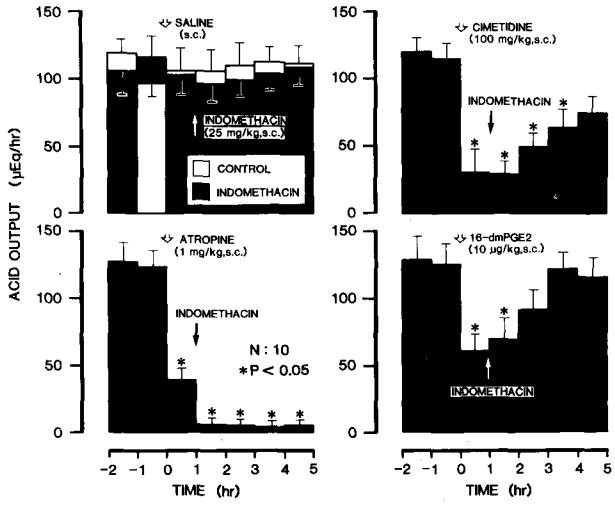

Fig 2. Effects of atropine, cimetidine, and 16,16-dmPGE$_2$ on gastric acid secretion in acute fistula rats in the presence of indomethacin. Indomethacin was given subcutaneously in a dose of 25 mg/kg. Either agent was given subcutaneously 1 hr before indomethacin treatment. Data are presented as the mean ± 1 SEM from 10 rats per group. *Statistically significant from control (indomethacin alone) at $P < 0.05$.

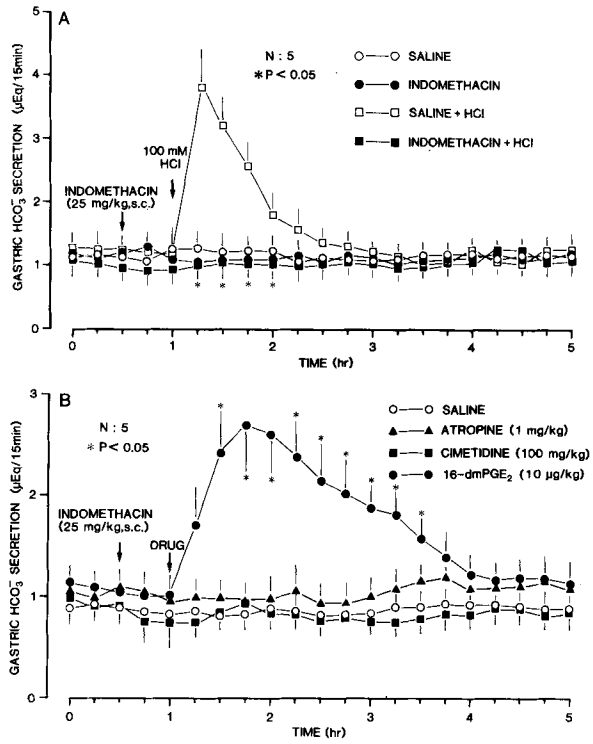

Fig 3. Effects of indomethacin on basal and acid-induced HCO^-_3 secretion in the stomachs of conscious rats (A) and those of atropine, cimetidine, and 16,16-dmPGE$_2$ on gastric HCO^-_3 secretion in the presence of indomethacin (B). Indomethacin was given subcutaneously in a dose of 25 mg/kg. (A) The stomach was exposed for 10 min to 100 mM HCl 1 hr after indomethacin treatment. (B) Either agent was given subcutaneously 30 min after administration of indomethacin. Data are presented as the mean ± 1 SEM from six rats per group. *Statistically significant from control at $P < 0.05$.

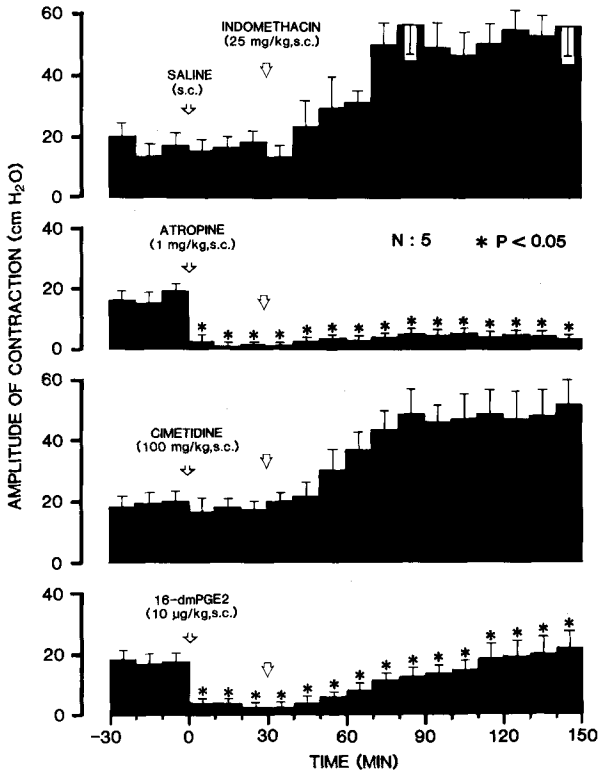

Fig 4. Effects of atropine, cimetidine, and 16,16-dmPGE$_2$ on gastric hypercontractions induced by indomethacin in rats. Indomethacin was given subcutaneously in a dose of 25 mg/kg. Either agent was given subcutaneously 30 min before administration of indomethacin. Values indicate the amplitude of contractions determined every 10 min and are presented as the mean ± 1 SEM from five rats per group. *Statistically significant from controls at $P < 0.05$.

2 hr, followed by a gradual return to control levels 4 hr later.

HCO^-_3 Secretion. The whole stomach preparations secreted HCO^-_3 at the rates of 1.0–1.4 μeq/15 min under unanesthetized conditions and responded to luminal acid (100 mM HCl for 10 min) by a significant rise in HCO^-_3 output from 0.9 ± 0.2 μeq/15 min to 4.0 ± 0.6 μeq/15 min (Figure 3A). Subcutaneous administration of indomethacin (25 mg/kg) had no effect on basal HCO^-_3 secretion but significantly attenuated the increased HCO^-_3 response caused by acid. In the presence of indomethacin, both atropine (1 mg/kg) and cimetidine (100 mg/kg) had no effect on gastric HCO^-_3 secretion, while subcutaneously administered 16,16-dmPGE$_2$ (10 μg/kg) significantly increased HCO^-_3 secretion from 0.9 ± 0.2 μeq/15 min to 2.7 ± 0.5 μeq/15 min, which remained elevated for roughly 2 hr and returned to the baseline values 3 hr later (Figure 3B).

Gastric Motor Activity. In agreement with a previous finding (7), subcutaneously administered indomethacin (25 mg/kg) produced a marked, persistent augmentation of gastric motor activity as measured with an intraluminal balloon in conscious rats (Figure 4). The amplitude and frequency of contractions were increased from 18.4 ± 2.1 cm H_2O to 55.6 ± 10.1 cm H_2O and 14.6 ± 2.3/10 min to 22.6 ± 4.3/10 min, respectively, within 1 hr after indomethacin treatment, and remained elevated during the rest of experimental period. Subcutaneous administration of atropine (1 mg/kg) and 16,16-dmPGE$_2$ (10 μg/kg) significantly inhibited not only the basal motor activity but the increased contractions induced by indomethacin as well. The effect of atropine lasted for 4 hr, while in the animals given 16,16-dmPGE$_2$ the amplitude of contractions were gradually restored within 2 hr following indometh-

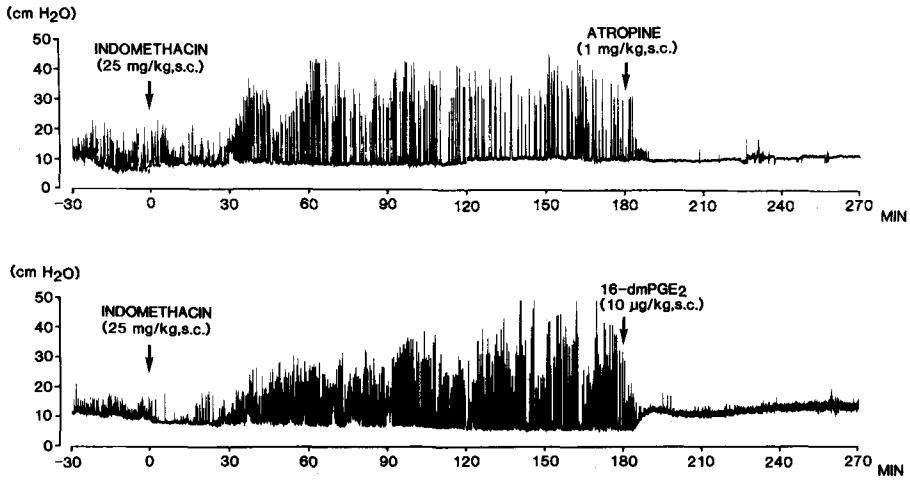

Fig 5. Representative figures showing the effects of atropine (1 mg/kg, subcutaneously) and 16,16-dmPGE$_2$ (10 μg/kg, subcutaneously) on the increased gastric motor activity caused by indomethacin (25 mg/kg, subcutaneously) in rats.

acin treatment but were still significantly lower at 4 hr as compared to control animals given indomethacin alone. As shown in Figure 5, the enhanced gastric motor activity caused by indomethacin was all but completely abolished by later administration of atropine or 16,16-dmPGE$_2$, resulting in fluctuations at the baselines which were difficult to characterize as the contraction spikes. On the other hand, cimetidine (100 mg/kg) did not significantly affect either the basal motor activity or the enhanced motor activity caused by indomethacin (Figure 4).

Gastric Lesions Induced by Indomethacin in the Presence of Exogenous Acid. Infusion of the stomachs of conscious rats with 150 mM HCl (1.2 ml/hr) produced severe hemorrhagic lesions in the corpus

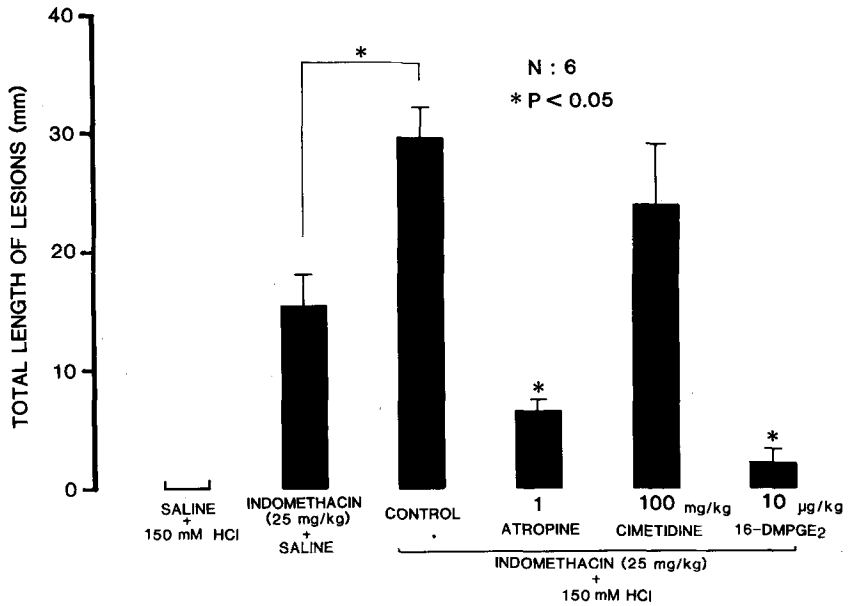

Fig 6. Effects of atropine, cimetidine, and 16,16-dmPGE$_2$ on the formation of gastric lesions induced by indomethacin in rats in the presence of exogenous acid. Either agent was given subcutaneously 30 min before indomethacin treatment (25 mg/kg, subcutaneously), and the stomach was infused with 150 mM HCl at a rate of 1.2 ml/hr during indomethacin treatment. The animals were killed 4 hr later. Data are presented as the mean ± 1 SEM from six rats per group. *Statistically significant from the corresponding control group at $P < 0.05$.

mucosa in response to indomethacin (25 mg/kg), the lesion index being 31.4 ± 4.3 mm, which was significantly greater when compared to the lesion index (16.9 ± 2.5 mm) induced by this agent in sham-operated animals without acid infusion (Figure 6). Pretreatment of the animals with atropine (1 mg/kg) or 16,16-dmPGE$_2$ (10 μg/kg) significantly reduced the development of gastric lesions in response to indomethacin under these conditions, the degree of inhibition being 75.6% or 89.2%, respectively. On the other hand, cimetidine at 100 mg/kg, which significantly inhibited the development of gastric lesions induced by indomethacin without acid infusion, failed to affect the formation of lesions in the presence of exogenous acid, the inhibition being 7.3%.

DISCUSSION

Many studies have demonstrated that nonsteroidal antiinflammatory drugs such as indomethacin and aspirin induce damage in the gastrointestinal mucosa of experimental animals and humans (1–6). Since these agents inhibit cyclooxygenase activity to reduce PG biosynthesis (10, 11), and since exogenous PGs prevent the formation of lesions (3, 5), a deficiency of endogenous PGs is considered to be a causative factor for the lesions. However, recent studies demonstrated that a low dose of indomethacin (5 mg/kg) or aspirin (25 mg/kg) exhibited over 90% inhibition of cyclooxygenase activity in the gastric mucosa without causing any gross damage in the rat (7, 12). Furthermore, a deficiency of endogenous PGs cannot account for the characteristics and localization of the damage induced by these agents: bandlike lesions largely confined to the crest of the rugae in the corpus mucosa (13, 14). Thus, there must be other factors responsible for the genesis of gastric lesions following administration of nonsteroidal antiinflammatory drugs.

We confirmed the findings by others that indomethacin alone had no effect on acid secretion and inhibited the increased HCO^-_3 secretion caused by acid in the stomach (7, 15, 16). The latter phenomenon may result from a deficiency of PGs (17) and can be elicited by a low dose of indomethacin (5 mg/kg) which does not induce any damage in the gastric mucosa (7). Therefore, the secretory disorders caused by indomethacin cannot account for the development of lesions. Nevertheless, since the lesions were markedly prevented by means of decreasing acid secretion with cimetidine, it may be assumed that gastric lesions induced by indomethacin do not result from acid hypersecretion but require the presence of acid in the lumen.

Subcutaneous administration of indomethacin (25 mg/kg) increased both the amplitude and frequency of contractions of the stomach as measured with an intraluminal balloon system. Mersereau and Hinchey (13, 18, 19) showed abnormal hypergastric contractions following administration of indomethacin and phenylbutazone, and they postulated that the mucosal compression may be associated with formation of these localized lesions. Indomethacin induced gastric hypercontractions and mucosal lesions at similar dose levels (≥10 mg/kg) (7). As expected from previous observations (18, 20), both atropine and 16,16-dmPGE$_2$ significantly inhibited spontaneous gastric motor activity as well as the increased motor responses caused by indomethacin. These agents markedly reduced the formation of gastric lesions induced by indomethacin, even in the stomach infused with exogenous acid (150 mM HCl). In contrast, cimetidine failed to affect the increased gastric motility and the development of gastric lesions in the presence of exogenous acid during indomethacin treatment. Since the gastric mucosa was bathed in excess amounts of acid during an experimental period, it is unlikely that the mucosal protection afforded by the former two agents was accounted for by their antisecretory and/or HCO^-_3 stimulating action. Since only the effect on gastric motility was common to these two agents (atropine and 16,16-dmPGE$_2$), the motor alterations might be an important pathogenetic factor in indomethacin-induced gastric lesions.

It is known that 16,16-dmPGE$_2$ protected the surface epithelial cell of the stomach and intestine from injury caused by indomethacin (21, 22). The damage in the surface cells of the stomach occurred within 1 hr after indomethacin treatment (14). Since the amplitude of gastric contractions reached the maximal levels within 1 hr following indomethacin (7), it may be assumed that the high-amplitude contractions are responsible for damage in the surface cells (initial damage). Similar findings have been reported by Ohno et al (23), who showed that 16,16-dmPGE$_2$ but not timoprazole prevented both the increased gastric contractions and the surface cell damage in the stomach during vagal nerve stimulation. These results suggest that the initially formed mucosal damage may be strongly associated with motility factors of the stomach but not with secretory alterations.

The detailed mechanisms by which gastric motor alterations induce the initial damage to the mucosa remain unknown. Mersereau and Hinchey (24, 25) recently postulated that a deficiency of PGs may render the mucosal folds more vulnerable to compression injury at stress concentrating sites. Small amounts of acid may be corrosive to such vulnerable mucosa and contribute to evolution of the initial damage to macroscopically visible lesions. This contention was supported by the definite decrease in lesion formation in animals treated with cimetidine or antacid solution during indomethacin treatment (4). Kauffman et al (26) reported that intravenous administration of indomethacin reduced gastric mucosal blood flow in the canine stomach. Rainsford (27) showed the vascular damage in the early stage of gastric mucosal lesions after indomethacin treatment in rats and pigs. These events may occur independently from the motor alterations and contribute to the formation of gastric lesions in response to indomethacin. However, since the tightly contracted muscle in the stomach wall would block the small vessels traversing through it, and since the restriction of blood flow might involve vascular contractions or the shunting of blood away from the mucosa (28), the vascular damage observed in the area destined to ulcerate may be secondary to alterations in the gastric motor activity.

Taken together, the present data suggest that gastric contractions characterized by high amplitude may play an important role in the pathogenesis of indomethacin-induced gastric lesions in the rat. Both atropine and 16,16-dmPGE$_2$ protect the mucosa from the lesions by inhibiting gastric motility, while cimetidine reduces the severity of lesions by preventing the later extension of damage through inhibition of acid secretion. Certainly, as PGs are known to have a variety of actions, such as an increase of mucosal blood flow and mucus secretion (29, 30), a part of the protection afforded by 16,16-dmPGE$_2$ may be ascribed to these effects as well as a replacement of deficient endogenous PGs caused by indomethacin.

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