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Gastric mucosal protection by YM638, a novel leukotriene D_4 receptor antagonist, in rats

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Abstract

YM638 ([[5-[[3-(4-acetyl-3-hydroxy-2-propylphenoxy)propyl]thio]-1,3,4-thiadiazol-2-yl]thio] acetic acid) is a novel leukotriene D_4 receptor antagonist. We investigated the involvement of the leukotriene D_4 receptor blocking activity of YM638 in the gastric mucosal protection of this drug in rats. YM638 significantly prevented gastric lesion formation induced by water-immersion restraint stress, indomethacin, absolute ethanol, 0.7 N HCl and the combination of 0.2 N HCl and hemorrhagic shock, with ED_{50} values of 26.4, 4.1, 4.7, 35.4 and 8.0 mg/kg p.o., respectively. Cetraxate and sofalcone showed inhibitory effects on most of these gastric lesions, but the inhibitory effects of these compounds were much weaker than those of YM638. In contrast, YM638 had no effect on gastric acid secretion and gastric lesion formation in pylorus-ligated rats, or on duodenal lesion formation in cysteamine-administered rats. YM638 competitively antagonized leukotriene D_4-induced contraction of the isolated stomach, with a pA_2 value of 7.63 ± 0.18. In anesthetized rats, intravenous YM638 inhibited leukotriene D_4-induced aggravation of gastric lesions caused by HCl, and leukotriene D_4 and HCl-induced reduction of the potential difference. In addition, oral YM638 significantly increased gastric mucosal blood flow and prevented ethanol-induced increase in gastric vascular permeability. Endogenous prostaglandins, sulfhydryls and nitric oxides were not involved in this inhibitory effect on absolute ethanol-induced gastric lesion. YM638 did not react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl in vitro, indicating that YM638 does not have potential as free radical scavenger. These results suggest that the preventive effect of YM638 on gastric lesions is attributable not only to its leukotriene D_4 receptor blocking activity but also to the activation of gastric mucosal defensive mechanisms such as mucosal blood flow and vascular permeability.

Keywords: YM638 ([[5-[[3-(4-acetyl-3-hydroxy-2-propylphenoxy)propyl]thio]-1,3,4-thiadiazol-2-yl]thio] acetic acid); Leukotriene D_4 receptor antagonist; Gastric mucosal protection; Gastric lesion

1. Introduction

The sulfidopeptide leukotrienes C_4, D_4 and E_4 are synthesized from arachidonic acid by 5-lipoxygenese (Konturek and Pawlik, 1986) in many cells such as leukocytes, macrophages, mast cells and eosinophils. These peptidyl leukotrienes are potent contractile agonists of respiratory, vascular and gastrointestinal smooth muscle (Dahlen et al., 1980; Drazen et al., 1980; Goldenberg and Subers, 1983; Krilis et al., 1983).

Peptic ulcer and gastritis are generally considered to result from an imbalance between the mucosal aggressive factors of acid and pepsin and the defensive factors of gastric mucosal blood flow, mucus and alkaline secretion. Recent investigations have indicated that leukotrienes may have an important role as one of the mucosal aggressive factors. In rats, leukotrienes have been shown to be released in response to necrotizing agents such as ethanol (Peskar et al., 1986) and hydrochloric acid (Osada et al., 1990). Exogenous leukotrienes have been reported to cause potent vasoconstriction, vascular stasis and an increase in vascular permeability (Dahlen et al., 1981; Whittle et al., 1985). Furthermore, leukotrienes C_4 and D_4, although not ulcerogenic themselves, aggravated mucosal injury induced by various noxious agents (Konturek et al., 1988; Pihan et al., 1988).

A number of reports have suggested that leukotriene

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synthesis inhibitors and leukotriene D_4 receptor antagonists can protect against the formation of gastric lesions (Konturek et al., 1988; Nielsen et al., 1987; Rainsford, 1987; Wallace and Whittle, 1985; Vaananen et al., 1992). Among these, YM638 has been shown to be a potent and orally active leukotriene D_4 receptor antagonist in several in vitro and in vivo test systems (Tomioka et al., 1988).

In the present study, we investigated the effects of YM638 on several types of gastric and duodenal lesions in rats, and compared results with those for cetraxate and sofalcone. We also examined the role of leukotriene D_4 in the formation of gastric lesions and the effect of YM638 on gastric acid secretion, transgastric potential difference, mucosal blood flow and vascular permeability in rat stomach.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 200–300 g were used. The animals were maintained on ordinary chow and tap water ad libitum under a constant 12-h light-dark cycle.

2.2. Leukotriene D_4-induced contraction of isolated rat stomach

Rats were killed by cervical dislocation. The stomach was excised and longitudinal smooth muscle strips of fundus were prepared according to the method of Goldenberg and Subers (1983). Preparations were suspended under 1.0-g tension in an organ bath containing 10 ml of Krebs-bicarbonate solution equilibrated with a mixture of 95% O_2 and 5% CO_2 at 37°C. The composition of the Krebs solution was (mM): NaCl, 118.2; KCl, 4.6; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 24.8; $MgSO_4$, 1.2 and glucose, 10.0. The tissues were equilibrated for 90 min, during which time the Krebs-bicarbonate solution was replaced every 30 min and the loading tension was adjusted to maintain tension at 1.0 g. The developed tension of the tissue was measured isometrically with a strain gauge transducer (SB-1T, Nihon Kohden, Tokyo, Japan), and recorded on a recticorder (RJG-4008, Nihon Kohden) through a carrier amplifier (RP-5, Nihon Kohden). After equilibration, submaximal contractions were elicited by repeated concentrations of 1×10^{-9} M leukotriene D_4 until constant responses were obtained. Cumulative concentration-response curves for leukotriene D_4 were then constructed by increasing bath concentrations of the agonist approximately 3-fold. Antagonists were added to the bath after a concentration-response curve to leukotriene D_4 had been obtained. The tissue was exposed to the antagonist for 30 min before rechallenge with leukotriene D_4. Since the cumulative concentration-response curves for leukotriene D_4 (control) under these conditions could be constructed twice in the same preparation without significantly changing the maximal effect (E_{max}) or the concentration causing 50% of the E_{max} (EC_{50}) values (data not shown), each antagonist was examined at one concentration in the same preparation.

2.3. Measurement of transgastric potential difference

The experimental procedure was essentially the same as that described by Takeuchi and Okabe (1983). Rats were fasted for 24 h before the experiment with free access to water. Under anesthesia with urethane (1.25 g/kg i.p.), the trachea of the rat was cannulated, the abdomen opened and the stomach exposed. The esophagus was ligated without disturbing the vagus nerves. A cannula for a close intraarterial (i.a.) injection of drugs was retrogradely inserted into the splenic artery and the tip of the cannula was positioned at the origin of the left gastric artery. The associated branch vessels and the common hepatic artery were ligated to cause a solution to drain into the left gastric artery. One catheter filled with 4% agar in saturated KCl was inserted into the stomach through an incision in the duodenum to serve as the intragastric electrode. A temporary gastric fistula was prepared using a polyethylene tube in the forestomach. The tube was connected to a three-way valve and used for intragastric instillation and removal of gastric contents. A second catheter filled with 4% agar and saturated KCl was inserted into the peritoneal cavity to serve as the ground electrode. The two electrodes were placed in separate beakers containing a saturated KCl solution in which a balanced Ag-AgCl electrode was positioned. The whole interior of the stomach was gently rinsed with warm saline 3–4 times, and then 2 ml of saline was instilled into the stomach. Changes in potential difference were continuously monitored using a recorder (MC6621, Graphtec, Tokyo, Japan) connected to a millivoltometer (HM-16S, TOA Electronics, Tokyo, Japan).

Approximately 1 h after a stable potential difference had been obtained, the perfusion system was interrupted and the solution in the stomach was withdrawn. The stomach was then exposed for 10 min to 2 ml of 0.2 N HCl. After HCl exposure, the stomach was gently rinsed again, another 2 ml of saline was instilled, and the perfusion system was resumed. Leukotriene D_4 or saline infusion, started at the same time as HCl exposure, was carried out over 15 min at 0.03 ml/min/rat through the catheter. Test drugs were intravenously administered 5 min before exposure to HCl.

2.4. Enhancement of HCl-induced gastric lesion by leukotriene D_4

Rats were fasted for 24 h before the experiment with free access to water. Under anesthesia with pentobarbital (50 mg/kg i.p.), the trachea was cannulated and a midline laparotomy was performed to expose the stomach. Cannulation of the left gastric artery for injection of drugs was as described in Section 2.3. After stabilization for 30 min, 0.4 N HCl (2 ml/rat) was intragastrically administered and leukotriene D_4 or saline infusion was carried out for 15 min at 0.03 ml/min/rat through the catheter. Just after termination of the local i.a. infusion, the stomachs were removed and opened along the greater curvature for the measurement of lesion index. Test drugs were intravenously administered 5 min before the administration of HCl.

2.5. Gastric and duodenal lesion production in conscious rats

Water immersion- and restraint stress-induced gastric lesion

Rats were fasted for 18 h before the experiment with free access to water. Stress ulcerations were induced by placing the animals individually in compartments of a special stress cage and immersion in a water bath at 23°C for 7 h to the xyphoid level, as described by Takagi and Okabe (1968). The animals were then killed, the stomach was removed and opened along the greater curvature. The limiting ridge between the rumen and the corpus was stretched to approximately 3.5 cm in diameter, and the ulcerated area (mm^2) of the glandular stomach was measured macroscopically as the lesion index. Test drugs were orally administered 1 h before restraint and water immersion.

Indomethacin-induced gastric lesion

Rats were fasted for 18 h before the study. Indomethacin (20 mg/2 ml/kg) was subcutaneously administered at 1 h after oral administration of test drugs. Five hours later, the animals were killed for the macroscopical measurement of gastric lesions.

HCl- or ethanol-induced gastric lesion

Gastric lesions were produced according to the method of Robert et al. (1979). Rats were deprived of food for 24 h and water for 18 h prior to experiments. They were individually housed in cages to prevent coprophagy. One hour after oral administration of test drugs, 0.7 N HCl or absolute ethanol was introduced intragastrically at a volume of 1 ml. The animals were killed 1 h after HCl or ethanol administration, the stomach was removed, and the lesion index was measured macroscopically.

In another series of experiments to evaluate the involvement of endogenous prostaglandins, sulfhydryls and nitric oxides on the effect of the drug, subcutaneous indomethacin (5 mg/kg), a cyclooxygenase inhibitor, *N*-ethylmaleimide (10 mg/kg), a sulfhydryl blocker, and intravenous *N*-nitro-L-arginine methyl ester (5 mg/kg), a nitric oxide synthase inhibitor, were administered to rats 75, 30 and 10 min before drug administration, respectively.

Pylorus ligation-induced gastric lesion

Rats housed individually were fasted for 48 h but allowed free access to water. The pylorus was ligated after abdominal midline incision under ether anesthesia. Eighteen hours later, the animals were killed for the measurement of the lesion index. The test drugs were orally administered 1 h before pylorus ligation.

Cysteamine-induced duodenal lesion

Rats were fasted for 18 h with free access to water. Animals were killed 18 h after subcutaneous injection of cysteamine (300 mg/kg), and the duodenum was examined for lesions. The drugs were orally given 1 h before the administration of cysteamine.

2.6. Enhancement of HCl-induced gastric lesion by hemorrhagic shock

Rats were fasted for 24 h with free access to water. After anesthesia with pentobarbital (50 mg/kg i.p.), the trachea was cannulated, a midline laparotomy was performed to expose the stomach, and the right carotid artery was canulated. Approximately 30 min after anesthesia, 2–2.5 ml of blood (equivalent to 1% of body weight) was slowly removed over 5 min from the carotid artery with a heparinized syringe. Just after blood removal, 1 ml of 0.2 N HCl was intragastrically administered through the forestomach. The temperature of the removed blood was kept at 37°C for 20 min and then reinfused into the donating rats over 5 min. Thirty minutes later, the stomachs were removed and opened along the greater curvature for the mesurement of the lesion index. Test drugs were orally administered 30 min before anesthesia.

2.7. Gastric acid secretion

Rats housed individually in stainless cages with wide-mesh grids to prevent coprophagy were fasted for 24 h with free access to water. The pylorus was ligated after abdominal midline incision under ether anesthesia. Four hours later, the animals were killed for the collection of gastric juice. The test drugs were administered orally 1 h before the pylorus ligation. Gastric juice (0.5 ml) was titrated against 0.05 N NaOH up to

pH 7 to determine acid concentration using an automatic titrator (Comtite-7, Hiranuma, Mito, Japan). Total acid output was calculated as the product of gastric volume by acid concentration.

2.8. Measurement of gastric mucosal blood flow

Rats were fasted for 24 h with free access to water. Under pentobarbital (50 mg/kg i.p.) anesthesia, the trachea was cannulated and the abdomen was opened. Gastric mucosal blood flow was determined by means of the hydrogen gas clearance technique (Murakami et al., 1982). The tip of a platinum electrode was implanted into the gastric wall of the glandular portion and an indifferent electrode was placed under the skin of the femoral portion. The terminals of the electrodes were connected to an amplifier (PHG-300, MT Giken, Tokyo, Japan). At 30-min intervals, hydrogen gas was inhaled with air for half to one minute via the tracheal cannula to achieve the saturation of the tissue. The concentration of hydrogen gas in the gastric mucosa was recorded as an amplification of the output current. The half time ($t_{1/2}$) for the decay of current was obtained from the hydrogen gas clearance curve. Gastric mucosal blood flow was calculated by the rate of disappearance of hydrogen gas (blood flow = $0.693/t_{1/2} \times 100$ ml/min/100 g). Drugs were orally administered to the rats 30 min before anesthesia.

2.9. Measurement of vascular permeability in gastric mucosa

Rats were deprived of food for 24 h and water for 18 h prior to experiments. Evans blue (10 mg/kg) was injected into the jugular vein 15 min before the rats were killed. Fourteen minutes after the Evans blue (1 min before death), 1 ml of absolute ethanol was intragastrically administered. Drugs were orally administered 1 h before Evans blue. Rats were killed by decapitation and the glandular stomach was removed, weighed, and digested in concentrated 32% HCl. After tissue digestion, Evans blue was extracted into chloroform and its concentration was measured spectrophotometrically in a spectrophotometer (UV-160A, Shimadzu Corp., Tokyo, Japan) at a wavelength of 610 nm. Results were compared to those for standards of known concentrations (Bernauer, 1980).

2.10. Reactivity with 1,1-diphenyl-2-picrylhydrazyl

The reactivity of test drugs with 1,1-diphenyl-2-picrylhydrazyl was examined according to the method of Smith and Reeves (1987). 1,1-Diphenyl-2-picrylhydrazyl was dissolved in 95% ethanol at a concentration of 0.11 mM. After 2.7 ml of 1,1-diphenyl-2-picrylhydrazyl solution was placed in a cuvette, 0.3 ml of test drugs was added. The mixed solution was placed at room temperature for 15 min, and the coloring reaction was spectrophotometrically determined by measuring the absorbance at a wavelength of 517 nm in a spectrophotometer (UV-160A).

2.11. Statistical evaluation

Results are expressed as the mean ± S.E.M. or means with 95% confidence limits. In the isolated rat stomach, the dose ratio was obtained from the ratio of the EC_{50} value (concentration required to contract the stomach to 50% as determined by log-probit analysis) of leukotriene D_4 in the presence and absence of an antagonist. Antagonist dissociation constants (K_B; ([antagonist (M)]/[dose ratio] − 1)) were determined at each concentration of the antagonist. The pA_2 values were then expressed as the negative logarithm of the K_B value. The inhibitory effects of test drugs on gastric lesions were expressed as the dose required to reduce gastric lesion formation by 50% (ED_{50}). Statistical significance of values was determined by one-way analysis of variance (ANOVA). Differences between treatment groups were compared by the Newman-Keuls multiple range test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

2.12. Drugs

YM638 ([[5-[[3-(4-acetyl-3-hydroxy-2-propyl-phenoxy)propyl]thio]-1,3,4-thiadiazol-2-yl]thio] acetic acid) and indomethacin were prepared by Yamanouchi Pharmaceutical Co. Cetraxate hydrochloride and sofalcone were extracted and purified from Neuer (Daiichi Pharmaceutical, Tokyo, Japan) and Solon (Taisho Pharmaceutical, Tokyo, Japan), respectively. Leukotriene D_4 solution, *N*-ethylmaleimide and cysteamine hydrochloride were purchased from Wako Pure Chemical (Osaka, Japan), Evans blue was from Tokyo Kasei (Tokyo, Japan), *N*-nitro-L-arginine methyl ester hydrochloride was from Sigma Chemical Co. (St. Louis, MO, USA), ascorbic acid was from Tokyo Tanabe Pharmaceutical Co. (Tokyo, Japan) and 1,1-diphenyl-2-picrylhydrazyl was from Nacalai Tesqui (Kyoto, Japan). YM638 was dissolved in a vehicle of 0.2 M Na_2CO_3 and injected to rats in volumes of 2 ml/kg. In the case of oral administration, YM638, cetraxate and sofalcone were suspended in 0.5% methylcellulose solution and given to rats in volumes of 5 ml/kg. Leukotriene D_4 solution was diluted with 0.9% w/v sodium chloride solution (saline). Indomethacin was suspended with saline containing 0.2% Tween 80. *N*-Ethylmaleimide and cysteamine were dissolved in saline.

3. Results

3.1. Blockade of leukotriene D_4-induced contraction of isolated rat stomach strips

Leukotriene D_4 caused a concentration-dependent contraction of fundus strips over a concentration range of 3×10^{-11} to 10^{-7} M. EC_{50} and E_{max} values were 9.3 $(6.0–14.6) \times 10^{-10}$ M and 1.39 ± 0.08 g, respectively. The maximal response of contraction by leukotriene D_4 was closely similar to that obtained by acetylcholine 10^{-6} M (data not shown). YM638 (3×10^{-8} to 3×10^{-7} M) produced concentration-dependent shifts to the right of the leukotriene D_4 concentration-response curves without a decrease in the maximal response, with a pA_2 value of 7.63 ± 0.18 ($n = 12$) (Fig. 1). YM638 had no effect on baseline tension of the preparation at the concentrations used.

3.2. Effect on the decrease in transgastric potential difference caused by HCl and leukotriene D_4

Gastric transmucosal potential difference showed no significant reduction by exposure to 0.2 N HCl (2 ml/rat) for 10 min in anesthetized rats, and had been maintained stable in the range of -37.2 ± 1.6 to -41.0 ± 2.0 mV ($n = 5$) throughout the experimental period (mucosa negative). After intraarterial infusion of leukotriene D_4 (1 μg/rat/min) for 15 min, the potential difference immediately fell from -36.8 ± 2.0 to -27.3 ± 1.4 mV ($n = 4$) in HCl-treated rats. YM638 injected intravenously at 1 mg/kg 5 min before the start of HCl exposure and leukotriene D_4 infusion completely prevented the decrease in potential difference (Fig. 2).

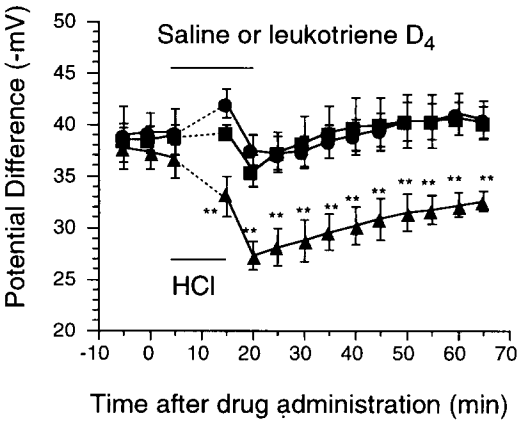

Fig. 2. Inhibitory effect of YM638 on HCl- and leukotriene D_4-induced reduction of potential difference in anesthetized rats. Each point represents the mean ± S.E.M. of values recorded every 5 min from four or five animals in the saline-control group (●), leukotriene D_4-control group (▲) and YM638 (1 mg/kg)-treated group (■). Test drug was intravenously injected 5 min before exposure to HCl and intraarterial infusion of leukotriene D_4. * $P < 0.05$; ** $P < 0.01$ compared with the saline-control group (ANOVA).

3.3. Effect on HCl- and leukotriene D_4-induced gastric lesion

Intraarterial infusion of leukotriene D_4 (1 μg/rat/min) without HCl did not cause conspicuous lesions in the glandular stomach of the anesthetized rats. HCl (0.4 N) alone induced gastric damage with a lesion index of 30.2 ± 9.5 mm^2 ($n = 12$), while after infusion of leukotriene D_4 (1 μg/rat/min) the damage was enhanced significantly ($P < 0.05$) and the lesion index was 62.4 ± 10.1 mm^2 ($n = 12$). No significant changes in gastric damage were caused by 0.1 μg/rat/min

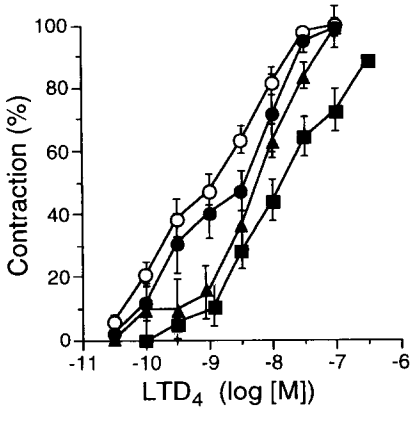

Fig. 1. Antagonism by YM638 of the contractile effect of leukotriene D_4 in the isolated rat stomach. Concentration-response curves show control responses to leukotriene D_4 (○) and the effect of YM638 3×10^{-8} (●), 10^{-7} (▲) and 3×10^{-7} (■) M. Each point represents the mean ± S.E.M. for four experiments. The stomach was exposed to the test drug for 30 min before rechallenge with leukotriene D_4.

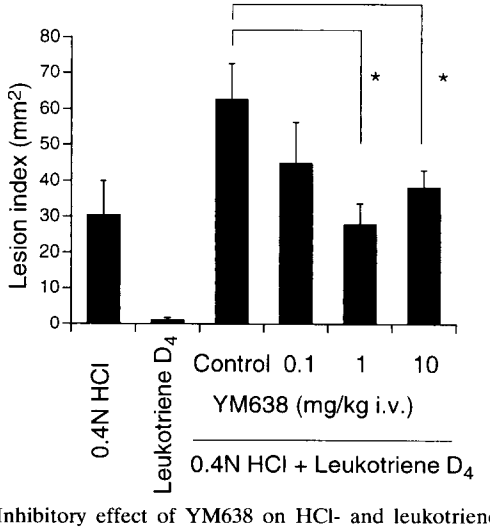

Fig. 3. Inhibitory effect of YM638 on HCl- and leukotriene D_4-induced gastric lesion in anesthetized rats. Each bar represents the mean ± S.E.M. for 6–12 rats. Test drugs were intravenously injected 5 min before exposure to HCl and intraarterial infusion of leukotriene D_4. * $P < 0.05$ compared with the control group (ANOVA).

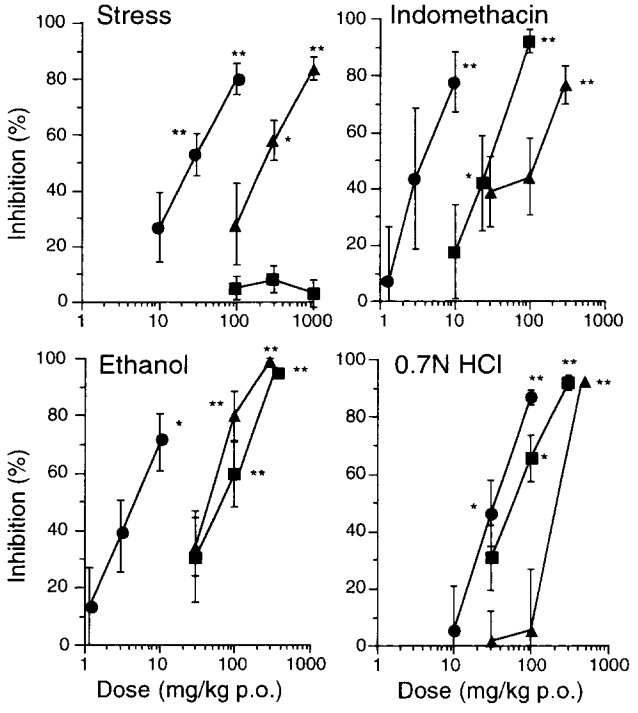

Fig. 4. Inhibitory effect of YM638 (●), cetraxate (▲) and sofalcone (■) on water immersion and restraint stress-, indomethacin-, absolute ethanol- and 0.7 N HCl-induced gastric lesions in conscious rats. Each point represents the mean ± S.E.M. for 7–10 rats. Test drugs were given orally 1 h before restraint stress, indomethacin, ethanol or HCl. * $P < 0.05$; ** $P < 0.01$ compared with the control group (ANOVA).

leukotriene D_4 (data not shown). Intravenous administration of YM638 (1 and 10 mg/kg) inhibited the gastric lesion induced by the combination of leukotriene D_4 with HCl. The inhibitory effect of YM638 on HCl- and leukotriene D_4-induced gastric lesion was maximal at the dose of 1 mg/kg (Fig. 3).

3.4. Effect on gastric and duodenal lesions in conscious rats

Water immersion- and restraint stress-induced gastric lesion

Water immersion and restraint stress in rats resulted in hemorrhagic change along the long axis of the stomach, with a lesion index of 13.1 ± 1.6 mm^2 ($n = 10$). Oral treatment with YM638 (30 and 100 mg/kg) and cetraxate (300 and 1000 mg/kg) significantly and dose-dependently suppressed this lesion formation with ED_{50} values (95% confidence limit) of 26.4 (23.7–29.4) and 231.7 (209.5–256.1) mg/kg, respectively (Fig. 4). Sofalcone had no effect on lesion formation at doses up to 1000 mg/kg p.o.

Indomethacin-induced gastric lesion

Administration of indomethacin at 20 mg/kg s.c. produced gastric lesions with a lesion index of 8.6 ± 1.5 mm^2 ($n = 10$). As shown in Fig. 4, oral treatment with YM638 (10 mg/kg), cetraxate (300 mg/kg) and sofalcone (100 mg/kg) significantly inhibited this lesion formation, with ED_{50} values of 4.1 (3.2–5.1), 78.1 (32.8–186.0) and 29.6 (20.1–43.7) mg/kg p.o., respectively.

Ethanol-induced gastric lesion

Administration of absolute ethanol to the control animals induced multiple severe lesions in the glandular portion of the stomach, with a lesion index of 44.0 ± 7.4 mm^2 ($n = 10$). Oral YM638 (10 mg/kg), cetraxate (100 and 300 mg/kg) and sofalcone (100 and 300 mg/kg) significantly suppressed this ethanol-induced gastric lesion formation, with ED_{50} values of 4.7 (4.2–5.1), 44.2 (39.6–49.4) and 60.5 (40.2–91.0) mg/kg, respectively (Fig. 4).

HCl-induced gastric lesion

Intragastric administration of 0.7 N HCl produced hemorrhagic damage along the long axis of the stomach, with a lesion index of 58.0 ± 7.6 mm^2 ($n = 10$). Oral treatment with YM638 (30 and 100 mg/kg) and sofalcone (100 and 300 mg/kg) significantly and dose-dependently suppressed this gastric lesion formation with ED_{50} values of 35.4 (29.8–42.1) and 57.3 (51.6–63.6) mg/kg, respectively. Cetraxate had no effect on these lesions at oral doses of 30 and 100 mg/kg, but inhibited them by 92.8 ± 1.2% at 300 mg/kg p.o. (Fig. 4).

Pylorus ligation-induced gastric lesion

Pylorus ligation for 18 h produced gastric mucosal damage, with a lesion index of 25.5 ± 4.6 mm^2 ($n = 14$). Oral treatment with YM638, cetraxate and sofalcone did not inhibit this pylorus ligation-induced gastric lesion formation at doses up to 100, 300 and 300 mg/kg, respectively.

Cysteamine-induced duodenal lesion

In the control animals, cysteamine induced severe hemorrhagic lesions in duodenal mucosa, with a lesion index of 29.2 ± 4.8 mm^2 ($n = 13$). YM638 (10–100 mg/kg), cetraxate (30–300 mg/kg) and sofalcone (30–300 mg/kg) did not inhibit this cysteamine-induced duodenal lesion formation.

3.5. Effect on HCl- and hemorrhagic shock-induced gastric lesion

Hemorrhagic shock by blood loss equivalent to 1% of body weight or 0.2 N HCl (1 ml/rat) alone resulted in only small areas of lesions, with indices of 1.0 ± 0.6 ($n = 7$) and 0.3 ± 0.2 mm^2 ($n = 7$), respectively. When 0.2 N HCl was intragastrically administered to rats in combination with hemorrhagic shock, however, marked

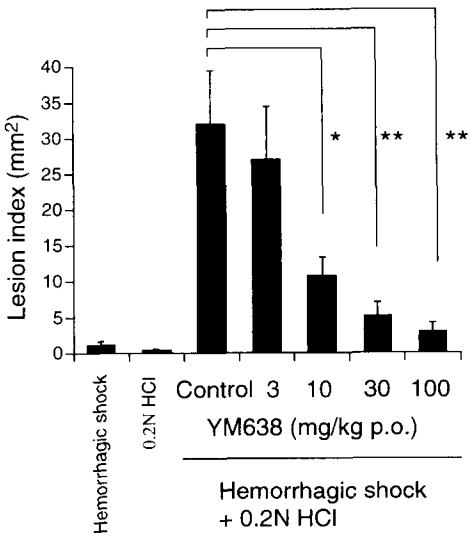

Fig. 5. Inhibitory effect of YM638 on HCl- and hemorrhagic shock-induced gastric lesions in anesthetized rats. Each bar represents the mean ± S.E.M. for seven rats. Test drugs were orally administered 30 min before anesthesia. * $P < 0.05$; ** $P < 0.01$ compared with the control group (ANOVA).

gastric lesions were observed in the glandular part of the stomach with a lesion index of 31.9 ± 7.5 mm^2 ($n = 7$). As shown in Fig. 5, YM638 significantly and dose-dependently inhibited HCl- and hemorrhagic shock-induced gastric lesion formation at doses of 10–100 mg/kg p.o., with an ED_{50} value of 8.0 (5.3–12.1) mg/kg.

3.6. Effect on gastric acid secretion

Basal gastric acid secretion in pylorus-ligated rats was 249.2 ± 23.5 μEq ($n = 15$) over 4 h. YM638, cetraxate and sofalcone did not affect the volume, acidity and total acid output of gastric juice in these rats at a dose range of 30–300 mg/kg p.o.

3.7. Effect on gastric mucosal blood flow

The basal gastric mucosal blood flow in anesthetized rats was 76.7 ± 9.6 to 99.0 ± 19.3 ml/min/100 g ($n = 6$). YM638 did not affect gastric mucosal blood flow in an oral dose of 10 mg/kg, but significantly increased it at doses of 30 and 100 mg/kg with a maximal response of 78% and 94%, respectively. The increase in mucosal blood flow in the 30 and 100 mg/kg groups lasted approximately 90 min after oral administration (Fig. 6).

3.8. Effect on enhancement of vascular permeability induced by ethanol

In control rats that received saline intragastrically, the concentration of Evans blue in the glandular stomach was 5.5 ± 0.4 μg/g tissue ($n = 10$). Absolute ethanol (1 ml/rat), given intragastrically 1 min before killing, significantly ($P < 0.01$) increased the concentration of Evans blue in the glandular stomach to 16.7 ± 0.8 μg/g tissue ($n = 10$). As shown in Fig. 7, administration of YM638 (30 and 100 mg/kg p.o.) 1 h before absolute ethanol (corresponding to 46 min before Evans blue) significantly and dose-dependently reduced the ethanol-induced increase in vascular permeability of the rat.

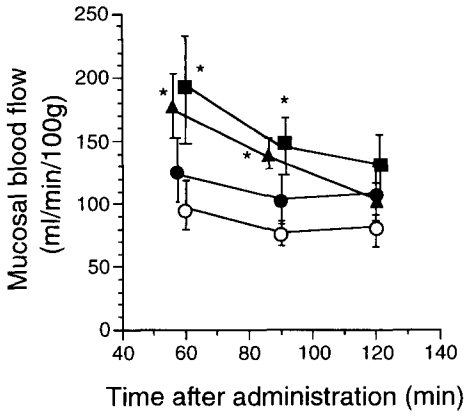

Fig. 6. Time course of gastric mucosal blood flow in the control group (○) and the effect of YM638 in oral doses of 10 (●), 30 (▲) and 100 (■) mg/kg in anesthetized rats. Each point represents the mean ± S.E.M. for six animals. Test drugs were orally administered 30 min before anesthesia. * $P < 0.05$ compared with the control group (ANOVA).

3.9. Involvements of endogenous prostaglandins, sulfhydryls and nitric oxides

To evaluate the involvement of endogenous prostaglandins, sulfhydryls and nitric oxides with the activity

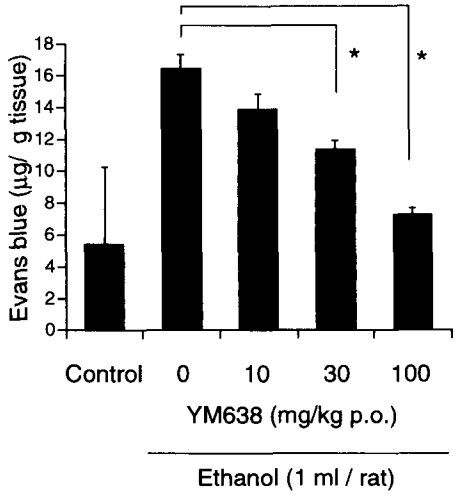

Fig. 7. Inhibitory effect of YM638 on the enhancement of vascular permeability by ethanol in rats. Each bar represents the mean ± S.E.M. of the concentration of Evans blue in the glandular stomach from ten animals. Test drugs were orally administered 1 h before absolute ethanol exposure. * $P < 0.05$ compared with the control group (ANOVA).

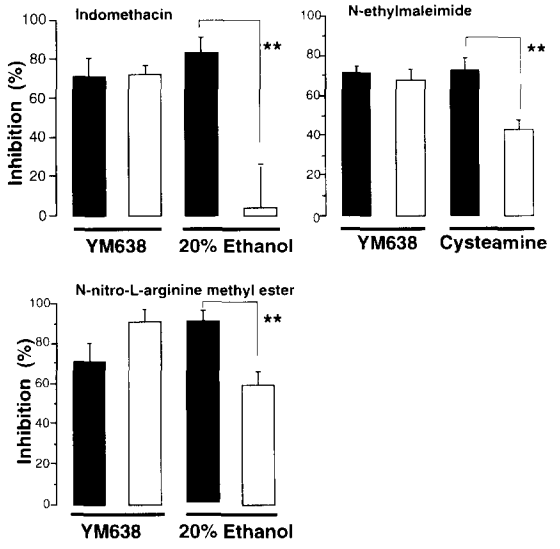

Fig. 8. Inhibitory effect of YM638 (10 mg/kg p.o.), 20% ethanol (1 ml/rat p.o.) or cysteamine (100 mg/kg p.o.) on absolute ethanol-induced gastric lesion with (open column) or without (closed column) pretreatment with indomethacin (5 mg/kg s.c.), *N*-ethylmaleimide (10 mg/kg s.c.) or *N*-nitro-L-arginine methyl ester (5 mg/kg i.v.) in rats. Each bar represents the mean ± S.E.M. for 5–10 rats. Test drugs were orally administered 1 h before absolute ethanol. Subcutaneous indomethacin or *N*-ethylmaleimide, or intravenous *N*-nitro-L-arginine methyl ester were administered to rats 75, 30 and 10 min before test drugs, respectively. ** $P < 0.01$ compared between groups with or without pretreatment with indomethacin, *N*-ethylmaleimide or *N*-nitro-L-arginine methyl ester (ANOVA).

of YM638, the inhibitory effect of this drug on absolute ethanol-induced gastric lesion formation was examined in the presence and absence of indomethacin (5 mg/kg s.c.), *N*-ethylmaleimide (10 mg/kg s.c.) and *N*-nitro-L-arginine methyl ester (5 mg/kg i.v.). Premedication

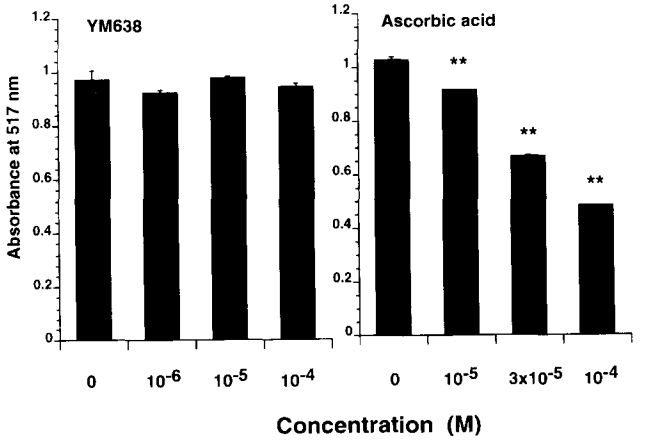

Fig. 9. Effect of YM638 and ascorbic acid on absorbance at a wavelength of 517 nm produced by 1,1-diphenyl-2-picrylhydrazyl in vitro. Each bar represents the mean ± S.E.M. for four samples. Test drugs (0.3 ml) were reacted with 2.7 ml of 0.11 mM 1,1-diphenyl-2-picrylhydrazyl solution at room temperature for 15 min. ** $P < 0.01$ compared with the control group (ANOVA).

with indomethacin, *N*-ethylmaleimide and *N*-nitro-L-arginine methyl ester did not affect the inhibitory effect of YM638 on gastric lesion induced by absolute ethanol. In contrast to YM638, the protection afforded by 20% ethanol (1 ml/rat) was significantly reduced by indomethacin and *N*-nitro-L-arginine methyl ester, and that afforded by cysteamine (100 mg/kg p.o.) was also significantly reduced by *N*-ethylmaleimide (Fig. 8).

3.10. Effect on free radical

YM638 at concentrations of 10^{-6} to 10^{-4} M did not react with 1,1-diphenyl-2-picrylhydrazyl and did not change absorbance at 517 nm at all. In contrast, ascorbic acid significantly decreased absorbance produced by 1,1-diphenyl-2-picrylhydrazyl in a concentration-dependent manner, with an EC_{50} value of 7.7 (5.3–11.4) $\times 10^{-5}$ M (Fig. 9).

4. Discussion

YM638, an acetophenone derivative, is reported to be an orally active leukotriene D_4 receptor antagonist (Tomioka et al., 1988). The present studies were carried out to examine the effect of YM638 on gastric lesions and to evaluate the mechanism for gastric mucosal protection by YM638 in rats.

While leukotrienes are classically considered to be mediators of inflammation (Samuelsson, 1983), their biological actions and the observation of increased production in some experimental models have led to the suggestion that they play an important role in the pathogenesis of gastric lesion. In 1983, Goldenberg and Subers reported that leukotriene D_4 induced contraction of the isolated rat stomach directly at specific smooth muscle receptor sites (Goldenberg and Subers, 1983). In addition to its contractile effect on gastric smooth muscle, leukotriene D_4 has been demonstrated to reduce gastric mucosal blood flow (Pawlik et al., 1987; Wallace et al., 1990), decrease the transgastric electrical potential difference across the gastric mucosa (Pendleton et al., 1986; Pendleton and Stavorski, 1986), and increase the vascular permeability (Pihan et al., 1988) and pepsin secretion (Pendleton et al., 1986) associated with the development of gastric mucosal damage. Nonsteroidal antiinflammatory drugs are also well known causes of gastric lesions; the pathogenesis of these lesions is suggested to be an imbalance in the mucosal production of protective prostaglandins and potentially harmful leukotrienes (Peskar, 1991; Rainsford, 1987). In the present study, leukotriene D_4 produced concentration-dependent contraction in isolated rat stomach, reduced gastric transmucosal potential difference and aggravated gastric lesion formation caused by HCl in anesthetized rats. These findings

therefore confirmed that leukotriene D_4 is involved in the pathogenesis of gastric lesion formation in rats, and that it is a mucosal aggressive factor.

The leukotriene D_4 receptor blocking activity of YM638 is reported to be closely similar in vitro to that of FPL55712 (Tomioka et al., 1988), the first leukotriene D_4 receptor antagonist synthesized (Augstein et al., 1973). Unlike FPL55712, however, YM638 is an orally active compound. In this investigation, YM638 competitively antagonized leukotriene D_4-induced contraction of isolated rat stomach at doses that inhibit leukotriene D_4-induced contraction of isolated guinea pigs ileum and trachea (Tomioka et al., 1988). In anesthetized rats, YM638 inhibited a leukotriene D_4-induced decrease in gastric transmucosal potential difference and aggravation of gastric lesion formation caused by HCl. Taken together, these data suggest that the leukotriene D_4 receptor blocking activity of YM638 is involved, at least in part, in the preventive effect of it on gastric lesions in rats.

To clarify the pharmacological profile of YM638, its effect on gastric lesion formation was compared with those of cetraxate and sofalcone. YM638 significantly prevented the gastric lesion formation induced by water immersion-restraint stress, indomethacin, absolute ethanol and 0.7 N HCl in rats. Cetraxate and sofalcone also inhibited almost all types of gastric lesions, but were much less potent than YM638. In contrast to these findings, YM638, cetraxate and sofalcone did not affect the formation of gastric and duodenal lesions by pylorus ligation or cysteamine. Gastric acid is well known to play an important role in the pathogenesis of these experimental lesions. In the present study, none of the three test drugs prevented gastric acid secretion in pylorus-ligated rats. Further, ethanol-induced mucosal injury is independent of gastric acid, and histamine H_2 receptor antagonists do not inhibit the gastric lesions induced by such severe conditions as 0.7 N HCl and absolute ethanol (Robert et al., 1979; Tarnawski et al., 1985). Moreover, although antisecretory drugs such as cimetidine and propantheline have no effect on the development of lesions induced by hemorrhagic shock (Takeuchi et al., 1986), YM638 significantly inhibited gastric lesion formation during hemorrhagic shock. We therefore suggest that the prevention of experimental gastric lesions by YM638, like that by cetraxate and sofalcone, is attributable to its ability to reinforce gastric mucosal defensive factors.

In the next series of experiments, therefore, the effect of YM638 on mucosal defensive factors was examined in rats. The gastric mucosa has a rapid turnover, and is damaged when its blood supply is reduced by hypovolemic shock or by stress. Vascular damage is thought to play a primary role in the development of gross hemorrhagic erosions induced by exposure to ulcerogenic stimuli. Thus, gastric blood flow and vascular permeability are closely associated with physiological changes in gastric mucosa subjected to ulcerogenic conditions. In this study, YM638 given orally increased gastric mucosal blood flow in a dose-dependent manner in anesthetized rats. Evans blue binds largely to albumin; this dye normally escapes into extravascular spaces only in small quantities, but leaks out in larger amounts if vascular permeability is increased (Bernauer, 1980). Pretreatment of rats with YM638 dose-dependently decreased the ethanol-enhanced vascular permeability to Evans blue. The protective effects of cetraxate and sofalcone on gastric mucosa are reported to be mediated by an increase in endogenous prostaglandins (Kurebayashi et al., 1988; Suwa et al., 1984). In addition to prostaglandins, moreover, endogenous sulfhydryls and nitric oxides may also play an important role in the mechanisms of gastric mucosal protection (Kitagawa et al., 1990; MacNaughton et al., 1989; Szabo et al., 1981). In the present study, indomethacin or *N*-nitro-L-arginine methyl ester reduced the protective effect of 20% ethanol (mild irritant), and *N*-ethylmaleimide reduced that of cysteamine on absolute ethanol-induced gastric lesions in rats, confirming that endogenous prostaglandins or nitric oxides, and sulfhydryls are involved in the protection afforded by 20% ethanol and cysteamine, respectively. Our data show that the inhibitory effect of YM638 on absolute ethanol-induced gastric lesions was not influenced by prior administration with indomethacin, *N*-ethylmaleimide and *N*-nitro-L-arginine methyl ester, indicating that neither endogenous prostaglandins, sulfhydryls nor nitric oxides are involved in the mechanism by which YM638 protects the gastric mucosa against the injurious action of absolute ethanol. Recently, free radical has been suggested to be involved in the pathogenesis of gastric lesion caused by indomethacin (Vaananen et al., 1991; Yoshikawa et al., 1993) and acidified ethanol (Matsumoto et al., 1993). YM638, in the present study, did not react with the stable free radical 1,1-diphenyl-2-picrylhydrazyl in vitro. Therefore, it is suggested that the inhibitory effect of YM638 on gastric lesion formation is not attributable to free radical scavenging activity.

In summary, YM638, a novel leukotriene D_4 receptor antagonist without free radical scavenging property, prevented gastric mucosal lesion formation induced by water immersion-restraint stress, indomethacin, absolute ethanol, 0.7 N HCl and the combination of 0.2 N HCl and hemorrhagic shock in rats. This effect of YM638 was much more potent than that of cetraxate and sofalcone. The preventive effect of YM638 on gastric lesions is thought to be attributable to both its leukotriene D_4 receptor blocking activity and to the activation of gastric mucosal defensive mechanisms, such as mucosal blood flow and vascular permeability.

Furthermore, the results of this study indicate that the mucosal protection by YM638 is not mediated through either endogenous prostaglandins, sulfhydryls or nitric oxides.

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