Bile Reflux due to Disturbed Gastric Movement Is a Cause of Spontaneous Gastric Ulcer in $W/W^v$ Mice

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$c$-Kit is a receptor tyrosine kinase, and it is encoded by the mouse $W$ locus. Mutant $W/W^v$ mice develop spontaneous gastric antral ulcers. The aim of the present study was to investigate the pathogenesis of these gastric ulcers and to examine the effects of two antiulcer drugs; a proton pump inhibitor (2-[[4-(3-methoxypropoxy)-3-methylpyridine-2-yl]methyl-sulfonyl]-1H-benimidazole sodium salt, rabeprazole) and a mucosal protective drug (geranylgeranylace tone, GGA), on the gastric ulcers. The inhibition of the gastric acid secretion by rabeprazole (30 mg/kg body weight, subcutaneous injection once a day for six weeks) significantly increased the gastric ulcer formation compared to the controls. In contrast, the GGA treatment (100 mg/kg body weight, oral administration for six weeks) significantly inhibited the ulcer formation. Bile reflux was seen in these mutant mice, and they showed no cyclic intense contractions in the gastric antrum. These results suggest that bile reflux due to the disturbance of gastric antral movement is a cause of the spontaneous gastric ulcers in $W/W^v$ mice.

KEYWORDS: $c$-kit, gastric ulcer; proton pump inhibitor; mucosal protective drugs; bile reflux.

$c$-Kit is a proto-oncogene encoding a receptor tyrosine kinase in the platelet-derived growth factor (PDGF)/colony-stimulating factor 1 (CSF-1) receptor family (1, 2). The finding that the dominant white spotting ($W$) locus is allelic with $c$-kit and the subsequent molecular analyses of the $c$-kit gene in a number of $W$ mutants have facilitated the understanding of the in vivo function of $c$-kit (3–6). Previous studies have demonstrated that the developmental failure of three cell lineages, ie, hematopoietic cells, melanocytes, and germ cells is a characteristic of $W$ mutant mice (7). The mice of the $W/W^v$ genotype show macroscopic anemia, sterility, lack of hair pigmentation, and lack of tissue mast cells (7, 8). Mast cells contain histamine, which has been implicated in the pathogenesis of peptic ulcers (9, 10). The development of gastroduodenal ulcers was reported in animals with mastocytoma (11). Interestingly, $W/W^v$ mice develop spontaneous gastric antral ulcers despite their lack of mast cells (12). It has been reported that bile reflux is a cause of the gastric ulcers in these mutant mice (13). Bile reflux is considered to be an important cause of human gastric ulcers. The presence of bile has been shown to facilitate the back-diffusion of hydrogen...
ions into the gastric mucosa (14, 15). W/W* mice thus appear to be a useful model for studying the pathogenesis of gastric ulcers. In the present study, we investigated the pathogenesis of the spontaneous gastric antral ulcers of W/W* mice and examined the effects of antiulcer drugs on these gastric ulcers.

**MATERIALS AND METHODS**

**Animals.** Four-week-old male WBB6F1-W/W* were purchased from Japan SLC Inc. (Shizuoka, Japan). The mice were fed tap water and Oriental CMF laboratory food (Oriental Yeast, Tokyo, Japan) ad libitum. The composition of the CMF food has been described (16). The mice were housed in polycarbonate cages that were changed weekly.

**Effects of Antiulcer Drugs on Gastric Ulcers.** Proton pump inhibitors, which are potent inhibitors of gastric acid secretion, are widely used for the treatment of peptic ulcer. It has been shown that the inhibition of gastric acid secretion by a proton pump inhibitor has antiulcer activities in experimental ulcer models, such as Shay's ulcer, cold-restraint stress ulcer, and acetic acid-induced gastric ulcer (17, 18). A novel proton pump inhibitor, rabeprazole, [(4-(3-methoxypropoxy)-3-methylpyridine-2-yl)methyl-sulfanyl]-1H-benimidazole sodium salt, was used here to evaluate the effect of the inhibition of gastric acid secretion on the ulcer formation in W/W* mice (19). In a preliminary study, the effect of rabeprazole on gastric secretion was tested by pyloric ligation as described by Shaye et al (20). Rabeprazole was dissolved in physiological saline, and 2, 10, or 30 mg/kg body weight of the solution in a volume of 0.1 ml was injected subcutaneously once a day at 9 AM for one week. After the last injection, the animals were deprived of food but allowed free access to water for 24 hr. The mice were then anesthetized with ether, and the abdomen was opened, and the pylorus was ligated. Four hours after the ligation, the animals were killed by cervical dislocation, and the gastric contents were collected. After a brief centrifugation of the gastric contents, the acidity and volume of the supernatant were determined. Acidity was determined by titration against 100 mmol/liter NaOH to pH 7.0. The acid output (micromoles per hour) was calculated.

Four-week-old male W/W* mice were used to examine the effects of antiulcer drugs on the gastric ulcers. Rabeprazole was dissolved in physiological saline solution, and 30 mg/kg body weight of rabeprazole in a volume of 0.1 ml was injected subcutaneously once a day at 9 AM for six weeks. For controls, 0.1 ml of physiological saline was injected subcutaneously once a day at 9 AM for six weeks. After the last injection, the animals were deprived of food but allowed free access to water for 24 hr. They were then killed by cervical dislocation for the examination of the gastric mucosal pH, the concentration of total bile acid, and the ulcer formation as described below.

Geranylgeranilacetone (GGA; tetraprenylacetone), an acyclic polyisoprenoid, is an antiulcer agent developed by Murakami et al (21). It is reported to have a protective effect on gastric mucosa without inhibiting gastric acid secretion (22). It has been shown that the enhancement of the mucosal protective potential by GGA correlates with the enrichment of gastric mucus gel (23). A GGA diet in CMF food (each animal had approximately 100 mg/kg body weight of GGA daily) was given for six weeks. For controls, only CMF food was given for six weeks. After a 24-hr fast, the animals were killed by cervical dislocation for examination of gastric mucosal pH, concentration of total bile acid, and ulcer formation as described below.

**Histology.** W/W* mice were killed by cervical dislocation. The stomach and duodenum were removed as a whole; the stomach was split along the greater curvature. After macroscopic examination, the stomach and duodenum were fixed in 10% buffered formalin (pH 7.2). Ordinary paraffin sections (4 μm thick) were made and stained with hematoxylin and eosin to confirm the diagnosis. In most cases, subserial sections were made to determine the size of the ulcer when found. The diameter of the ulcer was measured in the largest cross-section, using an ocular micrometer attached to an eyepiece of the microscope.

**Measurement of Intragastric pH and Bile Acid in the Stomach.** After the 24-hr fast, the animals were killed by cervical dislocation. Both the oral and anal sides of the stomach were ligated, and the stomach was removed. One milliliter of distilled water was injected into the stomach by a syringe with a needle, and the gastric content was obtained after gentle pipetting. The pH of the gastric content was evaluated with a pH electrode and corrected to within 6.5–7.5 by adding 100 mmol/liter NaOH. The concentration of total bile acids was measured by the method using 3α-hydroxysteroid dehydrogenase (24). Briefly, part of the obtained solution was dried up, and the precipitate was dissolved in methanol. A reaction mixture containing 3α-hydroxysteroid dehydrogenase was added to the solution to convert bile acids to 3-oxo bile acids with a concomitant reduction of oxidized nicotinamide adenine dinucleotide to reduced nicotinamide adenine dinucleotide. The hydrogen in the reduced nicotinamide adenine dinucleotide generated was transferred to nitrotetrazolium blue by diaphorase to yield formazan. The color of the resulting formazan was measured at 540 nm to evaluate the concentration. The entire amount of total bile acids in a stomach was then calculated.

**Gastric Motility.** The contractile activity of the gastric antrum was analyzed with the use of a strain gauge force transducer with a telemetry recording system, which can be implanted in the abdominal cavity of a small animal (25). Ten-week-old W/W* and +/+ male mice were used in this experiment. The telemeter is a cylinder (10 × 35 mm) with a strain gauge force transducer (4 × 3 mm) connected by fine lead wires. The telemeter includes a battery and amplification, transmission, and power supply circuit to the transducer. The battery is designed to be turned on and off from outside the body by means of a magnetic switch. The device weighs 4 g and is waterproofed with silicon. In the anesthetized mouse, the force transducer (4 × 3 mm) was sutured onto the serosa in the gastric antrum, and the telemeter was fixed in a subcutaneous space of the abdomen. During the measurement, the mice were housed in individual cages under unrestrained conditions, and the cage was placed on the receiver. Gastric motility was continuously recorded for two days, although body movements sometimes affected the recordings slightly due to adhesion.