Effects of FK506, An Immunosuppressive Agent, on Genesis of Water-Immersion Stress-Induced Gastric Lesions in Rats

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We examined the effects of FK506, an immunosuppressive agent, on the genesis of water immersion stress-induced gastric lesions in rats. Using high-performance liquid chromatography, four kinds of prostaglandins, ie, 6-keto-prostaglandin F₁₂, prostaglandin F₂₀, prostaglandin E₂, and prostaglandin D₂, were detected, and no leukotrienes were detected in gastric mucosa in rats without stress. After 6 hr of stress, gastric lesions developed with decreases in all prostaglandin contents, and the emergence of peptide leukotrienes was observed. Intramuscular administration of FK506 (0.1, 0.25, 0.5, 1.0, and 2.0 mg/kg) reduced lesion index dose-dependently. Administration of FK506 at doses over 0.25 mg/kg decreased all prostaglandin contents, but did not affect the increase in leukotriene contents. Pretreatment with famotidine or omeprazole reduced lesion index, and the protective effects were equivalent to those of 1.0 mg/kg of FK506, although FK506 did not affect gastric secretion during water-immersion stress. Water-immersion stress did not change the activities of xanthine oxidase in either stomach or serum. Polyoxyethylene-modified superoxide dismutase did not prevent gastric lesions. Water-immersion stress significantly increased myeloperoxidase activity in gastric mucosa, and FK506 reduced the increase in myeloperoxidase activity induced by stress. From our results, other factors besides gastric acid secretion and tissue eicosanoid contents, such as chemoattractant factor, might also be involved in the genesis of water-immersion stress-induced gastric lesions in rats.

KEY WORDS: FK506; cold restraint; gastric lesions; eicosanoids; free radicals; myeloperoxidase.

One of the most common causes of upper gastrointestinal bleeding is stress ulceration, and management of stress ulcers is of primary importance. Water-immersion stress is widely used as an experimental model of multifocal, gastric corpus erosions in rats because of the reliable reproducibility (1).

Several pathogenic mechanisms have been suggested to account for stress-induced gastric lesions: alteration in gastric secretion, changes in mucosal prostaglandin (PG) and leukotriene (LT) contents, the production of oxygen free radicals, neural factors, disturbance of mucosal microcirculation, abnormal gastric motility, and the increase in blood viscosity, although details remain obscure (2-9).

Recent studies have suggested that cytokines play important pathophysiological roles in the gastrointestinal system. Various papers demonstrated favorable effects of interleukin (IL) -1 on gastric ulcers (10-13). Cyclosporin A, an immunosuppres-
sive agent, is reported to mitigate inflammatory bowel disease (14, 15). A potent, newly developed immunosuppressive agent, FK506 (17-allyl-1,14-dihydroxy-12-[2-(4-hydroxy-3-methoxycyclohexyl)]-1-methylvinyl)-23,25-dimethoxy-13,19,21,27-tetramethyl-1,28-dioxo-4-azatriycloc[22. 3. 1. 0^8]octacos-18-ene-2,3,10,16-tetraone) was isolated from Streptomyces tsukubaensis No. 9993 (16, 17), and its beneficial immunosuppressive effects against the rejection of organ transplants were reported (18, 19). FK506 has been shown to suppress a variety of immune functions both in vitro and in vivo (16–24), but the effects of FK506 on acute gastric mucosal lesions have hardly been evaluated.

In this study, we examined the effects of FK506 on the genesis of water-immersion stress-induced gastric lesions, in relation to gastric mucosal PG and LT production, xanthine oxidase (XOD) activities in stomach and serum, myeloperoxidase (MPO) activities in gastric mucosa, and gastric secretion.

**MATERIALS AND METHODS**

Experiments were carried out on male Wistar rats weighing 200–250 g. The rats were fasted for 24 hr before the experiments and were allowed free access only to water.

**Chemicals**

FK506 was kindly given by Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan), and polyoxyethylene-modified superoxide dismutase (SOD-POE) was kindly given by Ajinomoto Co., Inc. (Tokyo, Japan). FK506 or SOD-POE was dissolved in physiological saline, and 0.4 ml of the solutions were used in the following experiments. Famotidine was kindly given by Yamanouchi Pharmaceutical Co. Ltd. (Tokyo, Japan) and dissolved in physiological saline (1 ml). Omeprazole was kindly given by Fujisawa Pharmaceutical Co. Ltd. and suspended in carboxyl methyl cellulose (1 ml). Hexadecyltrimethylammonium bromide, and O-dianisidine dihydrochloride were purchased from Sigma Chemical Co. (St. Louis, Missouri).

**Effects of FK506, Antisecretory Drugs, or SOD-POE on Water-Immersion Stress-Induced Gastric Lesions**

The animals were divided into five groups, as follows: (1) the control group (N = 6): untreated rats; (2) the stress group (N = 6): 30 min after animals were injected intramuscularly with 0.4 ml of physiological saline, they were placed in a stress cage and immersed in a water bath (23°C) for 6 hr to the level of xiphoid process, in accordance with the method of Takagi and Okabe (1); (3) the stress + FK506 group: this group was divided into five subgroups of six rats each according to the dose of FK506 administered, ie, 30 min after animals were injected intramuscularly with 0.1, 0.25, 0.5, 1.0, and 2.0 mg/kg of famotidine, an H2-receptor antagonist (N = 6), or 20 mg/kg of omeprazole, a proton pump inhibitor (N = 6), they were immersed for 6 hr stress; (4) the stress + antisecretory drugs group: 30 min after animals were administered intragastrically with 10 mg/kg of famotidine, an H2-receptor antagonist (N = 6), or 20 mg/kg of omeprazole, a proton pump inhibitor (N = 6), they were immersed for 6 hr stress; (5) the stress + SOD-POE group: this group was divided into four subgroups of six rats each according to the dose of SOD-POE administered, ie, 30 min after animals were injected via a tail vein with 5,000, 10,000, 15,000, 30,000 units/kg of SOD-POE, respectively, they were immersed for 6 hr stress. Immediately after these preparations, all of the animals were cervically dislocated, and the stomachs were quickly removed. Stomachs were opened along the greater curvature and were examined macroscopically for gastric mucosal lesions in the fundic portion. In each group, the lesion index was measured. The lesion index, the degree of lesion formation, was calculated as the sum of the length (millimeters) of each lesion in the stomach by an independent observer blinded to the treatment. Then, gastric mucosal PG and LT contents were measured as explained below, excluding the stress + antisecretory drugs and the stress + SOD-POE groups (groups 4 and 5).

**Measurement of Gastric Mucosal PG and LT Contents.**

In groups 1–3, the fundic mucosal layer was separated from the submucosal layer by a freeze–clamp method. Mucosal PGs and LTs were extracted and measured according to the method described previously (25, 26). The high-performance liquid chromatography system was a FAMILIC 300-S (Japan Spectroscopic, Tokyo, Japan) equipped with reversed-phase columns of Develosil ODS (0.46 × 15 cm plus 0.46 × 25 cm) at 40°C. PGs were separated into the columns with a mobile phase of acetonitrile-water (40:60) adjusted to pH 2.0 with phosphoric acid. The column effluent was monitored at 192 nm with a UV detector, UVIDEC-100-V (Japan Spectroscopic), connected to a computerized recorder (Shimadzu Chromatopac C-R3A, Kyoto, Japan). LTs were separated into the columns with a mobile phase of acetonitrile-water (47:53) adjusted to pH 2.0 with phosphoric acid. The column effluent was monitored at 280 nm with a UV detector using the same procedure as in the measurement of PGs.

**Effects of FK506 on Gastric Mucosal PG and LT Contents in Rats without Stress**

The animals were divided into two groups: (1) the control group (N = 6): untreated rats; and (2) the FK506 group (N = 6): animals sacrificed by cervical dislocation 6.5 hr after administration of 1.0 mg/kg of FK506 intramuscularly. In each group, gastric mucosal PG and LT contents were measured as described in the previous section.

**Effects of FK506 on XOD Activities in Stomach and Serum**

The animals were divided into three groups: (1) the control group (N = 6): untreated rats; (2) the stress group...