Ranitidine Reduces Ischemia/Reperfusion-Induced Liver Injury in Rats by Inhibiting Neutrophil Activation

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ABSTRACT
We previously reported that ranitidine, an H2 receptor antagonist, inhibited neutrophil activation in vitro and in vivo, contributing to reduce stress-induced gastric mucosal injury in rats. In this study, we examined whether ranitidine would reduce ischemia/reperfusion-induced liver injury, in which activated neutrophils are critically involved, in rats. We also examined the effect of famotidine, another H2 receptor antagonist, on leukocyte activation in vitro and after ischemia/reperfusion-induced liver injury in rats to know whether inhibition of neutrophil activation by ranitidine might be dependent on its blockade of H2 receptors. Ranitidine inhibited the activation of neutrophils in vitro as reported previously, whereas famotidine significantly enhanced it. Ranitidine inhibited the production of tumor necrosis factor-α (TNF-α) in monocytes stimulated with lipopolysaccharide in vitro, whereas famotidine did not. Although hepatic ischemia/reperfusion-induced increases in hepatic tissue levels of TNF-α, cytokine-induced neutrophil chemoattractant, and hepatic accumulation of neutrophils were inhibited by intravenously administered 30 mg/kg ranitidine, these increases were significantly enhanced by 5 mg/kg i.v. famotidine. The decreases in both hepatic tissue blood flow and bile secretion and the increases in serum levels of transaminases seen after reperfusion were significantly inhibited by ranitidine, whereas these changes were more marked in animals given famotidine than in controls. These observations strongly suggested that ranitidine could reduce ischemia/reperfusion-induced liver injury by inhibiting neutrophil activation directly, or indirectly by inhibiting the production of TNF-α, which is a potent activator of neutrophils. Furthermore, the therapeutic efficacy of ranitidine might not be explained solely by its blockade of H2 receptors.

Ischemia/reperfusion is an important pathological mechanism that leads to hepatic damage after circulatory shock or major hepatic surgery (Keller et al., 1985; Hasselgren, 1987). Ischemia/reperfusion-induced liver injury is thought to be mediated by proinflammatory cytokines and other inflammatory mediators released from activated leukocytes (Jaeschke et al., 1990; Ishii et al., 1994; Farhood et al., 1995; Vollmar et al., 1995), suggesting that inhibition of leukocyte activation by some therapeutic agents might contribute to reduce ischemia/reperfusion-induced liver injury. Among the various inflammatory mediators released from activated neutrophils, neutrophil elastase damages endothelial cells, thereby playing an important role in ischemia/reperfusion-induced hepatic damage (Kushimoto et al., 1996). Because proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β have been shown to activate neutrophils (Klebanoff et al., 1986), inhibition of either neutrophil activation or proinflammatory cytokine production may contribute to prevent ischemia/reperfusion-induced liver injury.

Ranitidine, a well known H2 receptor antagonist, has proved effective in patients with gastric ulcers (Deakin and Williams, 1992). We have demonstrated previously that ranitidine prevents the release of neutrophil elastase and reactive oxygen species, the cell surface expression of CD11b and CD18, and the increase in intracellular calcium concentration in neutrophils stimulated with formyl-methionyl-leucyl-phenylalanine (fMLP) (Okajima et al., 2000). Such inhibitory activities of ranitidine on neutrophil activation may contribute to reduce stress-induced gastric mucosal injury in rats (Okajima et al., 2000). Ranitidine is frequently used for prophylaxis of acute gastric mucosal injury in patients with circulatory shock or sepsis (Messori et al., 2000). Because such patients frequently develop ischemia/reperfusion-induced liver injury (Weigand et al., 1999), it is possible that intravenously administered ranitidine will reduce hepatic injury by inhibiting neutrophil activation. In the present study, we examined this possibility using a rat model of ischemia/reperfusion-induced liver injury. We also compared the effects of ranitidine on both leukocyte activation in vitro and ischemia/reperfusion-induced liver injury in vivo with those of famotidine, another H2 receptor antagonist (Hudson et al., 1997), to investigate whether the effects of ranitidine were mediated by its blockade of H2 receptor.

ABBREVIATIONS: TNF-α, tumor necrosis factor-α; fMLP, formyl-methionyl-leucyl-phenylalanine; [Ca2+]i, intracellular Ca2+ concentration; I/R, ischemia/reperfusion; CINC, cytokine-induced neutrophil chemoattractant; PMN, polymorphonuclear leukocyte; O2−, superoxide.
Experimental Procedures

Materials
Pathogen-free male Wistar rats, weighing 220 to 280 g, were obtained from Nihon SLC (Hamamatsu, Japan). Ranitidine and famotidine were gifts from Sankyo Co. (Tokyo, Japan) and Yanamouchi Pharmaceutical Co. (Tokyo, Japan), respectively. Hexadecyltrimethyl-ammonium bromide, o-dianisidine, and fMLP were obtained from Sigma-Aldrich (St. Louis, MO). Other materials obtained for the experiment included the following: lipopolysaccharide (endotoxin, Escherichia coli, serotype 055:B5; Difco, Detroit, MI), Nyco Prep 1.077 (Nycomed Pharma AS, Oslo, Norway), and fetal bovine serum and RPMI 1640 medium (Invitrogen, Carlsbad, CA). All other reagents used were of analytical grade.

In Vitro Study
Release of Neutrophil Elastase in Vitro. Neutrophil elastase release was induced by activating neutrophils from healthy volunteers using fMLP, as described previously (Okajima et al., 2000). In brief, neutrophils were isolated using Nyco Prep 1.077. The preparation, which contained more than 95% neutrophils, was washed twice with phosphate-buffered saline. A cell viability of 95% or higher was confirmed by the trypan blue exclusion method. Cells were suspended in phosphate-buffered saline at a cell density of 5000 cells/μl. Ranitidine or famotidine was dissolved in distilled water by adding dimethyl sulfoxide and then diluted in phosphate-buffered saline, pH 7.4. The final concentration of dimethyl sulfoxide was less than 0.05%. In a preliminary study, dimethyl sulfoxide at a concentration of 0.05% sodium azide, and 2% rabbit serum. Phycoerythrin-labeled monoclonal antibodies (BD PharMingen, San Diego, CA) were used for the experiment. Isotype-matched immunoglobulin (mouse IgG1) was used to assess nonspecific binding as described previously (Okajima et al., 2000). The neutrophil population was distinguished from other leukocytes by gating of the regionalized cells according to size and granularity. Antibody binding to neutrophils was analyzed using an FACSscan flow cytometer (BD Biosciences, San Jose, CA) using the channel number (log scale) representing the mean fluorescence intensity of 10,000 cells. To assess the effects of stimulation, the percentage of changes in surface expression of CD11b or CD18 were calculated as indicated in the following formula: mean fluorescence intensity stimulated/mean fluorescence intensity unstimulated × 100, where “mean fluorescence intensity stimulated” was the mean fluorescent intensity of cells labeled after stimulation and “mean fluorescence intensity unstimulated” was that of the corresponding unstimulated cells that were labeled and analyzed at the same time and under the same conditions.

Measurement of Intracellular Concentration of Ionized Calcium ([Ca2+]i). [Ca2+]i, was measured as described previously (Simon et al., 1992). Briefly, neutrophils isolated as described above were suspended at a cell density of 5 to 10 × 10^6 cells/ml in RPMI 1640 medium (Invitrogen) containing 10% fetal calf serum and 2.5 μg/ml acetylated low-density lipoprotein (Invitrogen). The cells were then washed twice and resuspended in RPMI 1640 medium (1 × 10^6 cells/ml) containing 0.05% fetal calf serum. Immediately before analysis of [Ca2+]i, the cells were washed in buffer A, which had the following composition: 140 mM NaCl, 3 mM KCl, 1 mM MgCl2, 10 mM glucose, 1 mM CaCl2, and 20 mM Hepes, pH 7.23; 1 × 10^6 cells were suspended in 1.7 ml of the same buffer and transferred to a thermostatically controlled (37°C) cuvette. Fluorescence emission was measured in a spectrophotometer (Hitachi 850; Hitachi Co., Ltd., Tokyo, Japan) using an excitation wavelength of 331 nm and an emission wavelength of 410 nm. After equilibration of fluorescence to a stable baseline, 1 × 10^6 cells were stimulated with 0.3 μM fMLP and fluorescence assessment was continued. Fluorescence levels were calibrated in terms of [Ca2+]i after each experiment by lysing the cells with 0.1% Triton X-100 and measuring fluorescence in the presence (Fmax, buffer A containing 1 mM CaCl2) and absence (Fmin, buffer A containing 4 mM EDTA) of calcium. [Ca2+]i was calculated using the following formula: [Ca2+]i = Kd(F - Fmin)/((Fmax - Fmin) × Fmax), where Kd was 180 nM and F the fluorescence of the unknown.

Isolation and Cultivation of Human Monocytes. Peripheral blood mononuclear cells were isolated from theuffy coats obtained from the blood of healthy donors by isopycnic centrifugation on Nyco Prep 1.077 according to a method described previously (Murakami et al., 1999). Mononuclear cells were cultured in RPMI 1640 medium plus 1% calf serum (Hyclone Laboratories, Logan, UT) and incubated in plastic dishes (Falcon 1058; Falcon Plastics, Lincoln Park, NJ) for 2 h at 37°C in a humidified 5% CO2 incubator. Lymphocytes were removed from the adherent monocytes by repeated rinsing with serum-free RPMI 1640 medium. Staining with Turk’s solution and for nonspecific esterase activity confirmed that monocytes constituted >90% of the harvested cells. Cell density was adjusted to 500 cells/μl in RPMI 1640 medium/1% serum and then stimulated with 100 ng/ml lipopolysaccharide for 4 h at 37°C in a humidified 5% CO2 incubator in the presence or absence of various concentrations of the H2 receptor antagonists. After incubation, the cell suspensions were centrifuged at 10,000 g for 10 min. Levels of TNF-α in the supernatant fractions were determined using an enzyme-linked immunosorbent assay kit (BioSource International, Camarillo, CA).

In Vivo Study
Animal Model of Hepatic Ischemia/Reperfusion. Care and handling of the animals were in accordance with the National Institutes of Health guidelines. All experimental procedures described below were approved by the Kumamoto University Animal Care and Use Committee. All rats were deprived of food, but not of water, for 24 h before each experiment. The hepatic ischemia/reperfusion (I/R) protocol was performed as described previously (Harada et al., 1999a). Ranitidine (30 mg/kg) or 5 mg/kg famotidine dissolved in saline was administered intravenously to rats 30 min before reperfusion. Because the antiserotory activity of famotidine was about 5 times stronger than that of ranitidine, these doses of H2 receptor antagonists could reduce gastric mucosal injury by inhibiting gastric acid secretion to the same extent in rats (Scarpiogato et al., 1987). Saline solution was used in the control experiment.
Results of Bile Flow. Hepatic excretory function is very sensitive to ischemic injury, thus a reduction in bile flow indicates ischemia/reperfusion-induced hepatic dysfunction (Bowers et al., 1987). Bile flow was measured after the hepatic ischemia/reperfusion as described previously (Kushimoto et al., 1996). Bile production was expressed as microliters per minute per gram of wet liver weight.

Measurement of Serum Liver Enzymes. Blood samples were taken 12 h after reperfusion to measure the level of serum alanine aminotransferase and aspartate aminotransferase, as described previously (Kushimoto et al., 1996). These blood samples were collected into test tubes from the anesthetized animals via withdrawal from the abdominal aorta using a 22-gauge needle. Alanine aminotransferase and aspartate aminotransferase levels were measured by standard clinical automated analysis and the results were expressed in international units per liter.

Measurement of Hepatic Tissue Blood Flow. Hepatic tissue blood flow was measured by laser-Doppler flowmeter (ALF21N; Advance, Tokyo, Japan) during 3 h after reperfusion, as described previously (Harada et al., 1999b). After anesthesia with 100 mg/kg i.p. ketamine hydrochloride, the right jugular vein of these animals was cannulated with a polyethylene-10 catheter for continuous infusion of normal saline or test drugs. The Doppler flowmeter probe was placed on the medial hepatic lobe. Hepatic tissue blood flow was measured from 30 min before ischemia until 3 h after reperfusion. The results are expressed as percentage of preischemia levels.

Measurement of Hepatic Levels of TNF-α. Hepatic levels of TNF-α were determined by a method described previously with some modifications (Harada et al., 1999a). At the indicated times after reperfusion, the animals were anesthetized with an intraperitoneal injection of 100 mg/kg ketamine hydrochloride and exsanguinated via the abdominal aorta to separate circulating TNF-α from that in hepatic tissue. In brief, the medial hepatic lobe was weighed and then homogenized in 5 ml of 0.1 M phosphate buffer, pH 7.4, containing 0.05% w/v sodium azide at 5°C. The homogenate was first centrifuged at 2000g for 10 min to remove minute amounts of solid tissue debris. The supernatant was assayed using a rat TNF-α enzyme-linked immunosorbent assay system (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK). This enzyme-linked immunosorbent assay detects 31 to 2500 pg/ml TNF-α. The results are expressed as picograms of TNF-α per gram of tissue.

Determination of Hepatic Levels of Cytokine-Induced Neutrophil Chemoattractant (CINC), CINC, which was measured in the liver in the present study, is also known as growth-regulated gene product/cytokine-induced neutrophil chemoattractant-1, and it was originally purified from an epithelioid clone derived from normal rat kidney cells that had been treated with interleukin-1β (Shito et al., 1997). CINC is produced by both leukocytes and endothelial cells (Watanabe et al., 1989). Hepatic levels of CINC were determined as described previously (Harada et al., 1999b).

Determination of Hepatic Myeloperoxidase Activity. After the indicated period of reperfusion, the livers were quickly removed, and the accumulation of leukocytes was assessed by measuring myeloperoxidase activity, which reflects the tissue accumulation of neutrophils according to a method described previously (Harada et al., 1999a).

Histological Examination

After 6 h of reperfusion, liver specimens were fixed in 10% buffered formalin and then embedded in paraffin. These samples were used to assess the infiltration of polymorphonuclear leukocytes (PMNs). Tissue sections (4 μm) were stained using the naphthol AS-D chloroacetate esterase technique to investigate the accumulation of PMNs in the liver (Moloney et al., 1960). PMNs were identified by positive staining and morphology and counted under a ×100 high-power field of a light microscope.

Statistical Analysis

Data are expressed as the mean ± S.D. The results were compared using either analysis of variance followed by Scheffe’s post hoc test or an unpaired t test. A level of p < 0.05 was considered statistically significant.

Effects of Ranitidine and Famotidine on Neutrophil Activation in Vitro. To determine whether the H2 receptor blockade induced by ranitidine is important for the inhibition of neutrophil activation in vitro, the effect of famotidine, another H2 receptor antagonist, on the release of neutrophil elastase, the production of O2-, the cell-surface expression of CD11b/CD18, and the changes in the intracellular concentration of calcium in neutrophils were examined and compared with those of ranitidine (Figs. 1 and 2). Although ranitidine, at a concentration of 100 μM significantly inhibited these events induced by stimulation of neutrophils with fMLP (Figs. 1, A, C, E, and F, and 2A), famotidine significantly enhanced them (Figs. 1, B, D, G, and H, and 2B).

Effects of Ranitidine and Famotidine on Production of TNF-α by Lipopolysaccharide-Stimulated Monocytes in Vitro. To determine whether ranitidine and famotidine inhibit the monocytic production of TNF-α, the effects of these H2 receptor antagonists on the production of TNF-α by lipopolysaccharide-stimulated monocytes were examined in vitro. Ranitidine at a concentration of 100 μM significantly inhibited the production of TNF-α by lipopolysaccharide-stimulated monocytes (Fig. 3). However, famotidine did not inhibit the production of TNF-α (Fig. 3).

Effects of Intravenously Administered Ranitidine and Famotidine on Hepatic Ischemia/Reperfusion-Induced Changes in Hepatic Tissue Levels of TNF-α, CINC, and Myeloperoxidase in Rats. To examine whether ranitidine and famotidine affect the hepatic ischemia/reperfusion-induced leukocyte activation in vivo, we analyzed the effects of these H2 receptor antagonists on the ischemia/reperfusion-induced increases in hepatic tissue levels of TNF-α, CINC, and myeloperoxidase. Hepatic tissue levels of TNF-α, CINC, and myeloperoxidase were significantly increased after reperfusion compared with their levels in sham-operated animals, peaking 1, 2, and 6 h after reperfusion, respectively (Harada et al., 1999b). Intravenously administered 30 mg/kg ranitidine significantly inhibited these increases at each of the above-mentioned time points (Fig. 4). In contrast, 5 mg/kg i.v. famotidine significantly enhanced these increases (Fig. 4).

Effects of Intravenously Administered Ranitidine and Famotidine on Changes in Hepatic Tissue Blood Flow in Rats Subjected to Hepatic Ischemia/Reperfusion. During hepatic ischemia, the hepatic tissue blood flow decreased to approximately 30% of the preischemia level and then increased to 50% of the preischemia level 3 h after reperfusion (Harada et al., 1999b). Intravenously administered 30 mg/kg ranitidine significantly increased the hepatic tissue blood flow 1 to 3 h after reperfusion (Fig. 5A), whereas 5 mg/kg famotidine significantly decreased the hepatic tissue blood flow compared with that of control animals (Fig. 5B).
that of the sham-operated rats and it recovered to a level that was 55% of the level seen in the sham-operated animals 3 h after reperfusion (Kushimoto et al., 1996). Bile flow increased significantly in animals receiving 30 mg/kg i.v. ranitidine 1 to 3 h after reperfusion compared with animals subjected to ischemia/reperfusion without ranitidine (Fig. 6). The reduced bile flow observed in the ranitidine-treated animals 0 to 1 h after reperfusion recovered to a value comparable with that seen in the sham-operated rats 2 h after reperfusion. However, intravenously administered 5 mg/kg famotidine signif-

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**Fig. 1.** Effects of ranitidine and famotidine on neutrophil elastase release (A and B), the release of oxygen free radical (C and D) from activated neutrophils, and the expression of CD11b and CD18 on neutrophils (E–H) in vitro. The release of neutrophil elastase in the presence of various concentrations of ranitidine (A) and famotidine (B) was determined. The release of oxygen free radicals in the presence of various concentrations of ranitidine (C) and famotidine (D) was determined. Phosphate-buffered saline was used instead of H₂ receptor antagonists in the control experiments. Data are expressed as the mean ± S.D. of experiments done in triplicate. **, p < 0.01 versus control group. The expression of CD11b (E and G) and CD18 (F and H) on neutrophils was analyzed by flow cytometry. Neutrophils at a cell density of 1 × 10⁶ cells/ml were preincubated with various concentrations of ranitidine (E and F) and famotidine (G and H) and subsequently incubated with phosphate-buffered saline or 0.1 μM fMLP. Data are expressed as the mean ± S.D. (percentage of unstimulated cells) of five experiments. **, p < 0.01 versus phosphate-buffered saline-treated group.
Evidently decreased the bile flow 1 to 3 h after reperfusion compared with that seen in the control animals (Fig. 6).

Effects of Intravenously Administered Ranitidine and Famotidine on Ischemia/Reperfusion-Induced Liver Injury. Serum levels of aspartate aminotransferase and alanine aminotransferase were significantly increased 1 h after reperfusion compared with levels in sham-operated animals, peaking 12 h after reperfusion (Kushimoto et al., 1996). Ranitidine (30 mg/kg i.v.) significantly inhibited the increase in serum aminotransferase levels seen 12 h after reperfusion (Fig. 7). In contrast, intravenously administered 5 mg/kg famotidine significantly enhanced the increase in serum levels of transaminases seen 12 h after reperfusion (Fig. 7).

Effects of Intravenously Administered Ranitidine and Famotidine on Ischemia/Reperfusion-Induced PMN Accumulation in Liver. The number of PMNs in the liver was significantly increased in animals subjected to hepatic ischemia/reperfusion compared with that of sham-operated animals (Table 1). Although intravenously administered ranitidine significantly inhibited the hepatic accumulation of PMNs, famotidine significantly enhanced it (Table 1).

Discussion

We reported previously that ranitidine, an H2 receptor antagonist, inhibited both neutrophil activation and the increase in [Ca2+]i in vitro (Okajima et al., 2000). Because the increase in [Ca2+]i plays an important role in neutrophil elastase release, production of O2·− and the expression of CD11b/CD18 in activated neutrophils (Rodeber and Babcock, 1996), ranitidine could inhibit neutrophil activation at least partly by inhibiting the increase in [Ca2+]i. In contrast, famotidine, another H2 receptor antagonist, did not inhibit the activation of neutrophils, but enhanced it mainly by increasing [Ca2+]i as shown in the present study. These observations strongly suggest that the blockade of H2 receptor induced by ranitidine might not be implicated in the inhibition of neutrophil activation.

Activated leukocytes play a pivotal role in ischemia/reperfusion-induced liver injury by releasing various inflammatory mediators that are capable of damaging endothelial cells (Colletti et al., 1990). We previously demonstrated that neutrophil elastase could be involved in the pathogenesis of ischemia/reperfusion-induced liver injury in this rat model (Kushimoto et al., 1996). Because neutrophil elastase increases endothelial permeability in vitro (Suzuki et al., 1994), it might play an important role in the reduction of hepatic tissue blood flow by increasing the microvascular permeability in animals subjected to hepatic ischemia/reperfusion. A neutrophil elastase-induced increase in microvascular permeability might lead to local hemoconcentration in the microcirculation at the site of endothelial damage, leading to tissue ischemia (Liu et al., 1998). Furthermore, neutrophil elastase inhibits the endothelial production of prostacyclin in vitro (Weksler et al., 1989) and in vivo (Harada et al., 2000). Because prostacyclin plays an important role in maintaining the proper microcirculation of the liver (Harada et al., 1999b), neutrophil elastase-induced decrease in the endothelial production of prostacyclin in the liver might contribute to the development of ischemia/reperfusion-induced
liver injury. In a preliminary study, we observed that neutrophil elastase was critically involved in the ischemia/reperfusion-induced decrease in hepatic tissue levels of 6-keto-prostaglandin F1α, a stable metabolite of prostacyclin, 3 h after reperfusion in this rat model and that ranitidine significantly inhibited this decrease. This might explain at least partly why ranitidine inhibited the ischemia/reperfusion-induced decrease in hepatic tissue blood flow.

Tumor necrosis factor-α plays a role in ischemia/reperfusion-induced liver injury by activating both neutrophils and endothelial cells (Colletti et al., 1990). Tumor necrosis factor-α increases the expression of E-selectin and intercellular adhesion molecule-1 in endothelial cells by activation of nuclear factor-κB (May and Ghosh, 1998). These endothelial leukocyte adhesion molecules participate in the development of activated neutrophil-induced endothelial cell injury and subsequent neutrophil infiltration (Mulligan et al., 1991). Consistent with this notion is our previous report showing that gabexate mesilate, a synthetic serine protease inhibitor, reduced ischemia/reperfusion-induced liver injury by inhibiting the production of TNF-α production by monocytes in this rat model (Harada et al., 1999a). Because ranitidine inhibited the production of TNF-α by monocytes stimulated with lipopolysaccharide in vitro, it is possible that ranitidine also reduces ischemia/reperfusion-induced liver injury by inhibiting the production of TNF-α by circulating monocytes and Kupffer cells in the sinusoidal space (Okuaki et al., 1996). Consistent with this hypothesis, intravenously administered ranitidine significantly inhibited both the increases in tissue levels of TNF-α and the subsequent increases in hepatic tissue levels of CINC and myeloperoxidase.

Intravenously administered ranitidine inhibited the ischemia/reperfusion-induced increases in myeloperoxidase as shown in the present study. Because the liver has some peroxidases (Komatsu et al., 1992), myeloperoxidase activity might reflect not only the number of neutrophils but also other hepatic peroxidases. However, this possibility seems less likely because PMN accumulation in animals subjected to hepatic ischemia/reperfusion was also inhibited by ranitidine.

The concentration of ranitidine required to inhibit neutrophil functions and TNF-α production in vitro is higher than 30 μM, as shown in our previous study (Okajima et al., 2000).
and in the present study. Because the plasma concentration of ranitidine in rats given 2 mg/kg i.v. is 12.6 μM (S.-Y. Liou, personal communication), the concentration in rats given 30 mg/kg i.v. ranitidine could be higher than 30 μM. Thus, we can expect ranitidine to inhibit neutrophil activation and TNF-α production in rats administered ranitidine at the dose of 30 mg/kg i.v. Furthermore, the plasma level of ranitidine in normal human subjects has been reported to be 5.1 μM min after a bolus intravenous injection of 1 mg/kg ranitidine (Ebihara et al., 1983). Ranitidine, at the concentration of 10 μM, inhibited both O₂ production by activated neutrophils and the increases in the expression of CD11b and CD18 in vitro. Based on these findings, although we may not simply compare the concentrations of ranitidine in vitro with those in patients suffering from ischemia/reperfusion-induced liver injury, we can expect ranitidine to inhibit neutrophil activation in the patients shortly after an intravenous injection of 1 mg/kg i.v. ranitidine. However, the plasma level of ranitidine in humans after an oral dose of 150 mg of ranitidine has been reported to be about 1.5 μM (Castaneda-Hernandez et al., 1996), suggesting that ranitidine might not inhibit neutrophil activation in patients taking 150 mg of ranitidine orally.

Although ranitidine reduced the ischemia/reperfusion-in-

**Fig. 6.** Effects of ranitidine and famotidine on bile flow in rats after ischemia/reperfusion (I/R). Animals were subjected to 60 min of hepatic ischemia followed by reperfusion as described under **Experimental Procedures**. Sham-operated animals (Sham) were prepared in a similar manner, except that blood flow to the left and median hepatic lobes was not obstructed. Ranitidine (30 mg/kg) or 5 mg/kg famotidine was intravenously administered to rats 30 min before reperfusion. Control animals (I/R) received saline instead of these agents. Bile flow was measured by collecting bile at the preischemic period until 3 h after reperfusion as described under **Experimental Procedures**. Bile flow determined 0 to 1 h (A), 1 to 2 h (B), and 2 to 3 h (C) after reperfusion is shown. Each point represents the mean ± S.D. from six animal experiments. **p < 0.01 versus sham operation; ††, p < 0.01 versus I/R.**

**Fig. 7.** Effects of ranitidine and famotidine on serum concentrations of aminotransferases in rats after ischemia/reperfusion (I/R). Animals were subjected to 60 min of hepatic ischemia followed by reperfusion as described under **Experimental Procedures**. Sham-operated animals (Sham) were prepared in a similar manner, except that blood flow to the left and median hepatic lobes was not obstructed. Ranitidine (30 mg/kg) or 5 mg/kg famotidine was intravenously administered to rats 30 min before reperfusion. Control animals (I/R) received saline instead of these agents. Serum levels of aspartate aminotransferase and alanine aminotransferase were determined 12 h after reperfusion. Each bar represents the mean ± S.D. **p < 0.01 versus sham operation; ††, p < 0.01 versus I/R.**

**TABLE 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia (n = 3)</td>
<td>7.7 ± 2.1</td>
</tr>
<tr>
<td>Sham (n = 5)</td>
<td>10.4 ± 6.2</td>
</tr>
<tr>
<td>I/R (n = 5)</td>
<td>17.4 ± 4.0[^a]</td>
</tr>
<tr>
<td>I/R + ranitidine (n = 5)</td>
<td>9.8 ± 3.5[^b]</td>
</tr>
<tr>
<td>I/R + famotidine (n = 5)</td>
<td>23.6 ± 4.5[^c]</td>
</tr>
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[^a] p < 0.05 versus preischemia group.
[^b] p < 0.05 versus sham-operated group.
[^c] p < 0.05 versus I/R group.

Sham, sham-operation; I/R, ischemia/reperfusion.
duced liver injury, famotidine, another H₂ receptor antagonist, exacerbated liver injury in the present study, suggesting that the therapeutic effects of ranitidine seen in this animal model could not be explained by its blockade of H₂ receptor, but by its biological properties, i.e., a regulatory effect on leukocyte activation. Although it is still unclear why there was such a discrepancy between the effects of these two H₂ receptor antagonists on leukocyte activation both in vitro and at tissue level in vivo, it is possible that the in vitro effects of these drugs on leukocyte activation may be related to their in vivo effects. In contrast to the present results, Mikawa et al. (1999) demonstrated that famotidine, but not ranitidine, inhibited the increases in both reactive oxygen production and elevation of [Ca²⁺], in human neutrophils stimulated with fMLP. In that study, they isolated human neutrophils from heparinized blood. Because heparin modulates reactive oxygen production by neutrophils (Itoh et al., 1995), heparin might have influenced the effects of these drugs on neutrophil functions. To avoid the effects of heparin, we used sodium citrate as the anticoagulant in the isolation of neutrophils in the present study.

Kaneko et al. (1998) have reported that histamine plays a critical role in the development of ischemia/reperfusion-induced renal injury in rats by activating leukocytes and platelets through an increase of the vascular permeability. They showed that the blockade of both H₁ and H₂ receptors by the combined use of diphenylhydramine and ranitidine markedly reduced renal injury. We demonstrated previously that neutrophils were critically involved in ischemia/reperfusion-induced renal injury in rats (Mizutani et al., 2000). Preliminary experiments using our rat model of ischemia/reperfusion-induced renal injury demonstrated that ranitidine, but not famotidine, significantly reduced the renal injury by inhibiting the renal accumulation of neutrophils. Thus, it is possible that ranitidine reduces ischemia/reperfusion-induced renal injury both by direct inhibition of leukocyte activation and by a blockade of H₂ receptor. In contrast, H₂ receptor blockade by famotidine might be antagonized by leukocyte activation induced by famotidine in vivo, thus explaining why famotidine did not reduce ischemia/reperfusion-induced tissue injury.

Although famotidine did not enhance the lipopolysaccharide-induced increase in monocyte production of TNF-α in vitro, it enhanced the ischemia/reperfusion-induced increase in the hepatic tissue level of TNF-α in vivo. Because activated neutrophils enhanced the release of TNF-α from THP-1 cells, a human monocyte cell line (Coeshott et al., 1999), famotidine might enhance the ischemia/reperfusion-induced increase in the hepatic tissue level of TNF-α by activating neutrophils in vivo.

The dosage of ranitidine (30 mg/kg) administered i.v. to rats subjected to hepatic ischemia/reperfusion in the present study was about 10 times higher than that used clinically for ulcer prophylaxis (Grant et al., 1989). However, this dosage of ranitidine has been reported to be an antisecretion dose in rats (Scarpignato et al., 1987).

Intravenously administered ranitidine delayed the early lipopolysaccharide-evoked pulmonary changes and reduced the TNF-α spike in a mongrel pig model of post-traumatic sepsis (Stewart et al., 1995). Nielsen et al. (1994) reported that the increase in the neutrophil chemiluminescence response to zymosan on postoperative day 1 in control patients undergoing major elective abdominal surgery was not seen in those treated with ranitidine, although there was no significant difference between the two groups. These observations suggest that ranitidine might inhibit the production of reactive oxygen species by neutrophils in patients undergoing elective abdominal surgery. These observations are consistent with the observations of the present study.

Because ranitidine is frequently used to prevent upper gastrointestinal bleeding in critically ill patients who are also susceptible to liver injury due to hepatic ischemia/reperfusion (Scnoll-Sussman and Kurtz, 2000), the inhibitory effects of ranitidine on leukocyte activation might attenuate inflammatory responses in such patients, contributing thereby to protect the stomach and various other organs. Further clinical studies are necessary to examine this possibility.

References


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Liu W, Okajima K, Harada N, Isobe H, and Irie T (1998) Role of...


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