Effects of Rabeprazole, a Gastric Proton Pump Inhibitor, on Biliary and Hepatic Lysosomal Enzymes in Rats

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ABSTRACT—The effects of rabeprazole (E3810), omeprazole and chloroquine on hepatic lysosomal function were studied. After chloroquine (50 mg/kg), rabeprazole (5 mg/kg) or omeprazole (5 mg/kg) was given intraperitoneally to rats for 6 days, the bile was collected via a bile duct cannula for 5 hr, and hepatic and biliary lysosomal enzyme (*N*-acetyl- β -glucosaminidase and β -galactosidase) activities were measured. The latency (an index for the hepatic lysosomal membrane integrity) was calculated from the *N*-acetyl- β -glucosaminidase activity. The biliary constituents and plasma concentrations of lipids were also measured. The administration of chloroquine significantly increased hepatic and biliary lysosomal enzyme activities, but did not affect the lysosomal enzyme latency, hepatic and biliary protein content or bile flow. It significantly decreased the bile acid level. On the other hand, the administration of rabeprazole and omeprazole did not alter the lysosomal enzyme activities, lysosomal enzyme latency, protein content in liver or liver weight. Furthermore, no significant differences were observed in biliary lysosomal enzyme activity, protein content, bile flow, biliary constituents or in the plasma concentrations of lipids between the drug groups (rabeprazole or omeprazole) and the control group. The results of the present study indicate that rabeprazole, like omeprazole, does not influence hepatic lysosomal function.

Keywords: Rabeprazole, H^+ , K^+ -ATPase inhibitor, Chloroquine, Lysosomal enzyme, Biliary secretion

Rabeprazole (1–3) and omeprazole (4, 5) are potent gastric H^+ , K^+ -ATPase inhibitors that are activated in the acidic lumen of the gastric parietal cell. The acid-activated rabeprazole specifically binds to the Cys³²² residue of the α -subunit of gastric H^+ , K^+ -ATPase, resulting in inhibitions of K^+ -stimulated ATPase activity and proton transport across the apical membrane of the parietal cell or the gastric vesicle membrane (6).

The lysosome is a major intracellular organelle (7, 8), and the intra-lysosomal pH is 4–5 (9). The acidic pH within the interior of lysosomes is generated and maintained by an ATPase-dependent proton pump called vacuole-type H^+ -ATPase (10–13). It is known that chloroquine, an anti-malarial drug, accumulates in lysosomes (14, 15), resulting in an increase in intra-lysosomal pH (9), disruption of lysosomal functions such as degradation of protein and cholesterol (16–18), and morphological changes as determined from electron micrographs (16, 19, 20).

If H^+ , K^+ -ATPase inhibitors are activated and/or accumulate in lysosomes, there is a possibility that they might affect lysosomal function as has been shown for

chloroquine. However, experimental facts show that biliary and hepatic lysosomal enzyme activities were not affected after intraperitoneal administration of omeprazole to rats for 6 days (21). In previous *in vitro* studies, the rate constant of rabeprazole for activation in 0.1 N HCl was larger than that of omeprazole, and the inhibitory effect of rabeprazole on H^+ , K^+ -ATPase activity was 7 or 10 times more potent than that of omeprazole (1, 2). In fistula dogs, intraduodenal administration of rabeprazole was more potent than that of omeprazole in inhibiting histamine-stimulated gastric acid secretion (ED_{50} , 0.062 vs 0.098 mg/kg) (3). In pylorus-ligated rats, intraduodenal administration of these drugs induced similar extents of inhibitory effects on gastric acid secretion (22). Since it has not been reported for a vacuole-type H^+ -ATPase whether rabeprazole interacts with hepatic lysosomal H^+ -ATPase and thereby alters lysosomal function, it is important to examine whether rabeprazole affects the function of liver lysosomes. In the present study, we investigated the effect of rabeprazole on rat hepatic lysosomal function, comparing it with that of omeprazole.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (6.5-week-old at the start of the experiments) were obtained from Charles River Japan, Inc. (Kanagawa). The rats were housed in stainless steel cages (width: 330 mm, length: 385 mm, height: 210 mm) in an animal room controlled at $23 \pm 1^\circ\text{C}$ and a relative humidity of $55 \pm 10\%$ with a 12-hr light / 12-hr dark cycle (light from 7:00 to 19:00). Food as a commercially available formula (MF; Oriental Yeast Co., Ltd., Tokyo) and water were supplied *ad libitum*.

Grouping of animals and drug dosage

Two separate experiments were carried out.

Experiment 1: Twenty-six rats were separated into a control group and a chloroquine-treated group; each of the groups was comprised of 13 rats, ensuring that the mean body weight of each group was nearly equal. The dose and route of administration of chloroquine were set at 50 mg/kg, *i.p.* according to the findings reported in the literature on the effects of chloroquine on lysosomal enzymes (16). Saline solution was administered to the control group.

Experiment 2: Four groups of 13 rats each, that is, a rabeprazole, omeprazole, control-1 and control-2 group, were employed. One day before the start of the experiments, the animals were divided into blocks of four rats consisting of 1 animal from each group in order of decreasing body weight. Dosing was carried out in a ran-

domized manner in each block. The dose and route of administration of rabeprazole and omeprazole were set at 5 mg/kg, *i.p.*, according to the findings reported in the literature on the effect of omeprazole on lysosomal enzymes (21). In a preliminary study, it was shown that both rabeprazole and omeprazole produced a 90% or higher inhibitory effect on gastric acid secretion induced by histamine when they were given intraperitoneally to rats at a dose of 5 mg/kg. Since the saline solution of rabeprazole has a pH of approximately 10, the control-2 group received a saline solution whose pH had been adjusted to approximately 10. In the control-1 group, normal saline solution was used.

The dosing solution (0.2 ml per 100 g of body weight) was given once a day (in the morning) for 6 days. The animals were permitted free access to food and water until the morning of the day of surgery.

The experimental scheme is shown in Fig. 1.

Bile duct cannulation and bile collection

One day after the final administration, bile duct cannulation was performed as follows: Under pentobarbital (40 mg/kg, *i.p.*) anesthesia, the jugular vein was exposed, and a type C Surflo indwelling needle 22 G (Terumo Corporation, Tokyo) filled with a 5 U/ml heparin-containing saline solution was inserted. An abdominal midline incision approximately 2 cm in length was made extending from the xiphoid process and the duodenum was pulled out and exposed on the abdominal muscle. The common bile duct was ligated at its origin. An incision was made in

Fig. 1. Outline of the experiment.

the common bile duct on the side proximal to the liver and the common bile duct was cannulated (polyethylene tube, PE-10, Intramedic®; Becton Dickinson, Parsippany, NJ, USA). After checking that bile was being successfully collected, the cannula was ligated, and the exposed duodenum was restored to its original position. The open end of the bile duct cannula was placed into a preweighed tube and bile was collected (on ice) at 1-hr intervals for 5 hr. The rectal temperature was measured periodically to maintain the body temperature at approximately 37°C by adjusting the intensity of the lamp light. To maintain anesthesia, 0.1 ml (volume adjusted as needed) of a twofold dilution of the pentobarbital solution with saline was injected into the vein via the venous cannula as needed. The volume of bile collected was determined from the weight change of the tube, with 1.0 g/ml taken as the density of bile (16). The rats for which the bile duct cannulation had been unsuccessful were recorded in work sheets as being omitted from the study.

Liver homogenate preparation

Liver homogenate was prepared following the method described by de Duve et al. (23) with minor modifications. That is, after the 5-hr bile collection period, the animals were exsanguinated by collecting blood via the abdominal aorta into a heparinized container. The liver was immediately removed and immersed in ice-cold buffer (250 mM sucrose, 3 mM imidazole, and 0.1% ethanol solution at pH 7.4). After the liver was weighed, approximately 5 g of liver tissue was removed and weighed. Approximately 15 ml of buffer was added and the tissue was cut into slices with scissors. The tissue was homogenized in a Teflon homogenizer (3 cycles at about 1300 rpm, on ice), then centrifuged at 4°C at 1000 × *g* for 10 min, and the supernatant was collected. The tissue sediment was homogenized again and centrifuged. The resultant supernatant was combined with the previous supernatant and the total volume was adjusted to approximately 50 ml (about 10 ml/g of liver) by the addition of buffer (E-fraction). The tissue sediment was resuspended in approximately 25 ml of buffer (about 5 ml/g of liver) (N-fraction). The exact volumes of the two fractions were measured in a graduated cylinder and recorded.

Measurement of lysosomal enzyme activities

Two lysosomal glycosidases, β -galactosidase (EC 3.2.1.23) and *N*-acetyl- β -glucosaminidase (EC 3.2.1.30), were assayed fluorometrically using 4-methylumbelliferyl substrates (24). The assay conditions employed for liver and bile were as previously described (25). The compositions of the substrate mixtures used for each of the assays were as follows: β -galactosidase: 0.5 mM 4-methylumbelliferyl- β -D-galactoside, 20 mM MgCl₂, 0.2% Triton X-

100, and 100 mM formate buffer (pH 3.8); *N*-acetyl- β -glucosaminidase: 0.5 mM 4-methylumbelliferyl *N*-acetyl- β -D-glucosaminide, 0.2% Triton X-100, and 100 mM citrate buffer (pH 4.5).

Each 0.1 ml portion of bile and E- and N-fractions of liver tissue, suitably diluted in 250 mM sucrose, was incubated with 0.1 ml of a buffered substrate mixture at 37°C for 10, 15 or 30 min. To stop the reaction, 2 ml of 50 mM NaOH-glycine buffer (pH 10.4) containing 5 mM EDTA was added. The liberated 4-methylumbelliferone was measured using a fluorescence spectrophotometer (excitation wavelength, 355 nm; emission wavelength, 460 nm; Titer-Tech Fluoroscan II; Dainippon Pharmaceutical Co., Ltd., Osaka). Enzyme and substrate blanks were carried out for all assays. Quantitation of fluorescence was performed by mixing 0.2 ml of known concentrations of 4-methylumbelliferone with 2 ml of 50 mM NaOH-glycine buffer containing 5 mM EDTA and by measuring the amount of fluorescence as described above. One unit (hereafter abbreviated as U) of activity for these enzymes corresponds to the hydrolysis of 1 μ mol substrate/min. All assays were performed in duplicate. Values of enzyme activities for total liver were calculated as the sum of the activities found in the E- and N-fractions.

The latent hydrolase activity in liver E-fraction was used as an index of lysosomal membrane integrity (23) and was determined by assaying *N*-acetyl- β -glucosaminidase activity in the presence and absence of 0.1% Triton X-100. The latent hydrolase activity was defined as follows:

Latent hydrolase activity

$$= (\text{total activity} - \text{free activity}) \times 100 / \text{total activity}$$

total activity: *N*-acetyl- β -glucosaminidase activity
in the presence of 0.1% Triton X-100

free activity: *N*-acetyl- β -glucosaminidase activity
in the absence of 0.1% Triton X-100

Protein assay

Protein was determined by the method of Lowry et al. (26) using bovine serum albumin as the standard according to the spectrophotometric procedure (Spectra MAX 250 microplate spectrophotometer; Molecular Devices Co., Sunnyvale, CA, USA; the software used was SOFTmax™).

Analysis of biliary constituents

Biliary cholesterol, phospholipid and bile acid were measured at Koto Biseibutsu Kenkyusho (Biken Chuo Kenkyusho, Tsukuba, Ibaraki) by the methods of cholesterol oxydase-DAOS, cholineoxydase-DAOS and 3- α -hydroxysteroid dehydrogenase, respectively [DAOS: *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyani-

line sodium salt].

Analysis of plasma lipids

Heparinized blood was centrifuged at 4°C at 3000 rpm for 10 min, and the plasma was analyzed for total cholesterol, triglyceride and phospholipid with a model AU600 Olympus automatic analyzer using Santest® L-TC (cholesteroxidase method), Santest® L-TG (glycerol digestive enzyme colorimetric method), and Santest® L-PL (enzymic method) (Sanko Pure Chemical Inc., Tokyo), respectively.

Drugs

Chloroquine (diphosphate) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in saline solution (Otsuka Pharmaceutical Co., Ltd., Tokyo) immediately before use at a concentration of 50 mg/2 ml (50 mg/kg). Rabeprazole and omeprazole were synthesized by Eisai Chemical Co., Ltd. (Ibaraki). Rabeprazole was dissolved in saline solution immediately before use (approximately pH 10) at a concentration of 5 mg/2 ml (5 mg/kg). Omeprazole was dissolved immediately before use in an equivalent molar concentration of 1 N NaOH and then diluted to a specified volume with saline solution in order to obtain a concentration of 5 mg/2 ml (5 mg/kg) (approximately pH 10).

Reagents

Pentobarbital was purchased from Dainabot Co., Ltd. (Osaka). 4-Methylumbelliferone, 4-methylumbelliferyl-*N*-acetyl- β -D-glucosaminide, 4-methylumbelliferyl- β -D-galactoside, ethylenediaminetetraacetic acid (EDTA) disodium dihydrate, bovine serum albumin, and Folin-Ciocalteu's Reagent (phenol reagent) were purchased from Sigma Chemical Co. Sucrose, imidazole, citric acid monohydrate, glycine, formic acid and Triton X-100 were purchased from Wako Pure Chemical Industries, Co., Ltd. (Osaka).

Statistical analyses

Results are expressed as the mean $\pm$ standard error (S.E.). Comparisons between the control and chloroquine groups were performed by Student's *t*-test or Welch's *t*-test in experiment 1. Comparisons between the control-2 and drug groups (rabeprazole or omeprazole) were performed by analysis of variance followed by Dunnett's multiple comparison test, and comparisons between the control-1 and control-2 groups were analyzed by Student's *t*-test or Welch's *t*-test in experiment 2. For all evaluations, *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Effect of chloroquine on hepatic lysosomal enzyme activities in rats

After repeated administration, body weight (mean $\pm$ S.E.) of rats in the chloroquine group (276.9 ± 2.8 g, *n* = 13) became significantly less (*P* < 0.05) than that in the control group (294.8 ± 2.3 g, *n* = 13), although the initial mean body weights of the two groups were similar (control, 239.1 ± 1.9 g and chloroquine-treated, 237.6 ± 1.8 g). The following evaluations were made between the control group of 11 rats (two animals were omitted because the bile duct cannulation surgery and pentobarbital anesthesia maintenance had failed), and the chloroquine group of 12 rats (one was omitted because of failed surgery of bile duct cannulation) (Table 1). The liver weights were 12.2 ± 0.4 g for the control group and 10.1 ± 0.2 g for the chloroquine group, indicating a significant decrease in the chloroquine group (*P* < 0.05). The hepatic *N*-acetyl- β -glucosaminidase activities were 0.77 ± 0.03 U/g of liver for the control group and 1.10 ± 0.05 U/g of liver for the chloroquine group. Hepatic β -galactosidase activities were 0.36 ± 0.01 and 0.47 ± 0.01 U/g of liver for the control and chloroquine groups, respectively. These two enzyme activities were significantly increased after chloroquine administration (*P* < 0.05). The latencies calculated

Table 1. Effects of chloroquine on hepatic lysosomal enzyme activities and protein content in rats

Group	Dose (mg/kg)	No. of animals	<i>N</i> -Acetyl- β -glucosaminidase (U/g of liver)	β -Galactosidase (U/g of liver)	Protein content (mg/g of liver)
Control	—	11	0.77 ± 0.03	0.36 ± 0.01	235 ± 2
Chloroquine	50	12	$1.10 \pm 0.05^*$	$0.47 \pm 0.01^*$	236 ± 2

Chloroquine (50 mg/kg) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution. Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). At the time of necropsy, blood was collected and the liver removed to determine hepatic lysosomal enzyme activities (*N*-acetyl- β -glucosaminidase and β -galactosidase) and the protein content of homogenized liver tissue. The results are presented as the mean $\pm$ S.E. Comparisons between the control and chloroquine groups were performed by Student's *t*-test or Welch's *t*-test (*: significantly different from the control group, *P* < 0.05).

Fig. 2. Patterns of biliary excretion of *N*-acetyl- β -glucosaminidase (A), β -galactosidase (B), protein (C) and bile flow (D) in chloroquine-treated rats and control rats. Chloroquine (50 mg/kg, $\blacklozenge$) was given i.p. to rats once a day for 6 days. Controls ($\diamond$) were treated with saline solution. Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). The results calculated as per gram of liver are presented as mean $\pm$ S.E. ($n=11$ for control group, $n=12$ for chloroquine group).

from the *N*-acetyl- β -glucosaminidase activities were $78.3 \pm 1.1\%$ for the control group and $77.9 \pm 1.1\%$ for the chloroquine group (no difference between the two groups). There was no difference in the protein content of the liver between the control and chloroquine groups.

Effect of chloroquine on biliary lysosomal enzyme activities in rats

The patterns of excretion into bile of lysosomal enzymes (*N*-acetyl- β -glucosaminidase and β -galactosidase), protein, and the volume of the bile expressed on a per gram of liver basis are shown in Fig. 2. The biliary *N*-acetyl- β -glucosaminidase and β -galactosidase activities

were higher in the chloroquine group than in the control group, although the protein content and bile volume was similar in each group. The cumulative 5-hr outputs expressed per gram of liver are shown in Table 2. Both enzyme activities increased significantly after chloroquine administration ($P < 0.05$). There were no significant differences in biliary protein content or bile volume between the two groups.

The total *N*-acetyl- β -glucosaminidase activities in the bile collected for 5 hr were 60.42 ± 8.73 and 91.03 ± 12.76 mU/5-hr bile for the control and chloroquine groups, respectively. The corresponding values of biliary β -galactosidase activities were 4.89 ± 0.55 and 10.37 ± 1.96 mU

Table 2. Effects of chloroquine on lysosomal enzyme activities, protein content and volume of bile in rats

Group	Dose (mg/kg)	No. of animals	<i>N</i> -Acetyl- β -glucosaminidase (mU/5-hr bile/g of liver)	β -Galactosidase (mU/5-hr bile/g of liver)	Protein content (mg/5-hr bile/g of liver)	Biliary volume (ml/5 hr/g of liver)
Control	—	11	4.92 ± 0.67	0.40 ± 0.04	1.43 ± 0.10	0.51 ± 0.03
Chloroquine	50	12	$8.93 \pm 1.21^*$	$1.01 \pm 0.19^*$	1.44 ± 0.10	0.50 ± 0.03

Chloroquine (50 mg/kg) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution. Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). Biliary lysosomal enzyme activities (*N*-acetyl- β -glucosaminidase and β -galactosidase), protein content and the volume of bile were measured. The 5-hr cumulative outputs calculated as per gram of liver are presented as the mean $\pm$ S.E. Comparisons between the control and chloroquine groups were performed by Student's *t*-test or Welch's *t*-test (*: significantly different from the control group, $P < 0.05$).

Table 3. Effects of chloroquine on biliary constituents in rats

Group	Dose (mg/kg)	No. of animals	Total cholesterol ($\mu\text{g}/5\text{-hr bile/g of liver}$)	Phospholipid ($\mu\text{g}/5\text{-hr bile/g of liver}$)	Bile acid ($\mu\text{mol}/5\text{-hr bile/g of liver}$)
Control	—	11	97 $\pm$ 10	1491 $\pm$ 133	15.0 $\pm$ 0.9
Chloroquine	50	12	103 $\pm$ 9	1395 $\pm$ 111	11.3 $\pm$ 1.1*

Chloroquine (50 mg/kg) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution. Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). The constituents (total cholesterol, phospholipid and bile acid) of the bile were determined. The 5-hr cumulative outputs calculated as per gram of liver are presented as the mean $\pm$ S.E. Comparisons between the control and chloroquine groups were performed by Student's *t*-test or Welch's *t*-test (*: significantly different from the control group, $P < 0.05$).

/5-hr bile, indicating a significant increase after chloroquine administration ($P < 0.05$), irrespective of whether the outputs had been corrected for liver weight. The total biliary protein contents were 17.3 ± 1.2 and 14.5 ± 1.0 mg/5-hr bile for the control and chloroquine groups, respectively. The corresponding values of biliary volume were 6.16 ± 0.38 and 4.95 ± 0.30 ml/5-hr, indicating a significant decrease after chloroquine administration ($P < 0.05$).

Effect of chloroquine on biliary constituents in rats

The biliary levels of total cholesterol, phospholipid and bile acid were measured. The 5-hr cumulative outputs into bile of cholesterol, phospholipid and bile acid (each calculated as per gram of liver) are shown in Table 3. Bile acid was significantly decreased after chloroquine administration ($P < 0.05$).

Effect of chloroquine on plasma lipids

The values of total cholesterol, phospholipid and triglyceride for the control group were 62 ± 3 , 123 ± 6 and 101 ± 10 mg/dl, respectively. The corresponding values for the chloroquine group were 58 ± 2 , 119 ± 4 and 93 ± 10 mg/dl. There were no differences in these parameters

between the two groups.

Effect of rabeprazole and omeprazole on hepatic lysosomal enzyme activities in rats

During the treatment period with rabeprazole or omeprazole, the gains in body weight were similar for all groups including the 2 control groups and no abnormalities in the general condition of animals were noted. The following evaluations were made for 12, 11, 11 and 12 animals in the control-1, control-2, rabeprazole and omeprazole groups, respectively, because the bile duct cannulation procedure of one, two, two and one animal in each group, respectively, had failed (Table 4). The liver weights were 11.4 ± 0.3 , 11.5 ± 0.2 , 11.7 ± 0.2 and 11.6 ± 0.3 g for the control-1, control-2, rabeprazole and omeprazole groups, respectively (no significant differences). There were no significant differences in the hepatic *N*-acetyl- β -glucosaminidase and β -galactosidase activities when the pH-adjusted saline solution group (control-2) was compared to the rabeprazole or omeprazole group. Similarly, no significant differences were found in both enzyme activities between the control-1 and control-2 groups. The latencies calculated from the *N*-acetyl- β -glucosaminidase activities were $82.4 \pm 1.2\%$, $81.1 \pm 1.2\%$,

Table 4. Effects of rabeprazole and omeprazole on hepatic lysosomal enzyme activities and protein content in rats

Group	Dose (mg/kg)	No. of animals	<i>N</i> -Acetyl- β -glucosaminidase (U/g of liver)	β -Galactosidase (U/g of liver)	Protein content (mg/g of liver)
Control-1	—	12	0.81 $\pm$ 0.03	0.32 $\pm$ 0.01	204 $\pm$ 3
Control-2	—	11	0.81 $\pm$ 0.03	0.33 $\pm$ 0.01	207 $\pm$ 5
Rabeprazole	5	11	0.82 $\pm$ 0.03	0.34 $\pm$ 0.01	204 $\pm$ 3
Omeprazole	5	12	0.81 $\pm$ 0.03	0.32 $\pm$ 0.01	201 $\pm$ 4

Rabeprazole or omeprazole (5 mg/kg) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution (control-1) or saline solution adjusted to a pH of approximately 10 (control-2). Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). At the time of necropsy, blood was collected and the liver was removed to determine hepatic lysosomal enzyme activities (*N*-acetyl- β -glucosaminidase and β -galactosidase) and the protein content of homogenized liver tissue. The results are presented as the mean $\pm$ S.E. Comparisons between the control-2 and drug groups (rabeprazole or omeprazole) were performed by analysis of variance followed by Dunnett's multiple comparison test, and comparisons between the control-1 and control-2 groups were analyzed by Student's *t*-test or Welch's *t*-test. There were no significant differences between the groups.

Fig. 3. Patterns of biliary excretion of *N*-acetyl- β -glucosaminidase (A), β -galactosidase (B), protein (C) and bile flow (D) in rabeprazole, omeprazole-treated rats and control rats. Rabeprazole (5 mg/kg, ●) or omeprazole (5 mg/kg, ▲) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution (control-1, □) or saline solution adjusted to a pH of approximately 10 (control-2, ○). Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). The results calculated as per gram of liver are presented as mean $\pm$ S.E. The number of rats was 12 for the control-1 and omeprazole groups and 11 for the control-2 and rabeprazole groups.

83.0 $\pm$ 0.9% and 82.0 $\pm$ 1.1% for the control-1, control-2, rabeprazole and omeprazole groups, respectively. No significant differences were observed in the latency between the control-2 group and the drug groups and between the control-1 and control-2 groups. There were no significant differences in the protein content of the liver between the control-2 group and the drug groups and between the control-1 and control-2 groups.

Effect of rabeprazole and omeprazole on biliary lysosomal enzyme activities in rats

The patterns of excretion into bile of lysosomal enzymes (*N*-acetyl- β -glucosaminidase and β -galactosidase), protein, and the volume of the bile (calculated as per gram of liver) are shown in Fig. 3. Table 5 shows the lysosomal enzyme activities, protein content and volume of the bile secreted for 5 hr (per gram of liver). Statistical

Table 5. Effects of rabeprazole and omeprazole on lysosomal enzyme activities, protein content and volume of bile in rats

Group	Dose (mg/kg)	No. of animals	<i>N</i> -Acetyl- β -glucosaminidase (mU/5-hr bile/g of liver)	β -Galactosidase (mU/5-hr bile/g of liver)	Protein content (mg/5-hr bile/g of liver)	Biliary volume (ml/5 hr/g of liver)
Control-1	—	12	9.30 $\pm$ 1.36	0.95 $\pm$ 0.14	1.67 $\pm$ 0.07	0.53 $\pm$ 0.02
Control-2	—	11	7.49 $\pm$ 1.17	0.75 $\pm$ 0.16	1.91 $\pm$ 0.24	0.53 $\pm$ 0.03
Rabeprazole	5	11	7.98 $\pm$ 1.28	0.70 $\pm$ 0.08	1.72 $\pm$ 0.07	0.53 $\pm$ 0.02
Omeprazole	5	12	6.53 $\pm$ 1.03	0.49 $\pm$ 0.11	1.43 $\pm$ 0.17	0.47 $\pm$ 0.05

Rabeprazole or omeprazole (5 mg/kg) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution (control-1) or saline solution adjusted to a pH of approximately 10 (control-2). Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). Biliary lysosomal enzyme activities (*N*-acetyl- β -glucosaminidase and β -galactosidase), protein content, and the volume of bile were measured. The 5-hr cumulative outputs calculated as per gram of liver are presented as the mean $\pm$ S.E. Comparisons between the control-2 and drug groups (rabeprazole or omeprazole) were performed by analysis of variance followed by Dunnett's multiple comparison test, and comparisons between the control-1 and control-2 groups were analyzed by Student's *t*-test or Welch's *t*-test. No significant differences were found.

Table 6. Effects of rabeprazole and omeprazole on biliary constituents in rats

Group	Dose (mg/kg)	No. of animals	Total cholesterol ($\mu\text{g}/5\text{-hr bile/g of liver}$)	Phospholipid ($\mu\text{g}/5\text{-hr bile/g of liver}$)	Bile acid ($\mu\text{mol}/5\text{-hr bile/g of liver}$)
Control-1	—	12	111 $\pm$ 6	1490 $\pm$ 70	14.2 $\pm$ 0.7
Control-2	—	11	123 $\pm$ 8	1629 $\pm$ 150	15.5 $\pm$ 0.7
Rabeprazole	5	11	127 $\pm$ 9	1598 $\pm$ 109	15.8 $\pm$ 1.0
Omeprazole	5	12	102 $\pm$ 17	1325 $\pm$ 143	13.9 $\pm$ 0.8

Rabeprazole or omeprazole (5 mg/kg) was given i.p. to rats once a day for 6 days. Control rats were treated with saline solution (control-1) or saline solution adjusted to a pH of approximately 10 (control-2). Rats were fitted with a cannula inserted into the bile duct under pentobarbital anesthesia, and bile was collected at 1-hr intervals (for 5 hr). The constituents (total cholesterol, phospholipid and bile acid) of the bile were determined. The 5-hr cumulative outputs calculated as per gram of liver are presented as the mean $\pm$ S.E. Comparisons between the control-2 and drug groups (rabeprazole or omeprazole) were performed by analysis of variance followed by Dunnett's multiple comparison test, and comparisons between the control-1 and control-2 groups were analyzed by Student's *t*-test or Welch's *t*-test. There were no significant differences.

analyses were carried out for the cumulative values of the bile secreted for 5 hr. No significant differences in the two enzyme activities (*N*-acetyl- β -glucosaminidase and β -galactosidase) were observed when the control-2 group was compared with the rabeprazole or omeprazole group and when the control-1 group was compared with the control-2 group. No significant differences in the biliary protein content or bile volume were observed between the control-2 and drug groups and between the control-1 and control-2 groups.

Effect of rabeprazole and omeprazole on biliary constituents in rats

No significant differences in biliary cholesterol, phospholipid or bile acid were observed between the control-2 and drug groups (rabeprazole or omeprazole) and between the control-1 and control-2 groups (Table 6).

Effect of rabeprazole and omeprazole on plasma lipids

The values of total cholesterol, phospholipid and triglyceride for the control-1 group were 67 $\pm$ 2, 125 $\pm$ 3 and 86 $\pm$ 7 mg/dl, respectively; and those for the control-2 group were 64 $\pm$ 3, 123 $\pm$ 5 and 92 $\pm$ 14 mg/dl, respectively. For the rabeprazole group, the values were 63 $\pm$ 3, 127 $\pm$ 5 and 114 $\pm$ 12 mg/dl, respectively; and for the omeprazole group the values were 71 $\pm$ 3, 135 $\pm$ 7 and 96 $\pm$ 8 mg/dl, respectively. There were no significant differences in the plasma levels of total cholesterol, phospholipid or triglyceride between the control-2 group and the drug groups and between the control-1 and control-2 groups.

DISCUSSION

It is known that chloroquine accumulates in lysosomes (14, 15), resulting in an increase in intra-lysosomal pH (9) without direct inhibition of vacuole-type H^+ -ATPase

(27), and that lysosomal degradation of protein and cholesterol are affected by chloroquine (16–18). Sewell et al. (16) found that 5-day administration of chloroquine at a dose of 50 mg/kg caused marked increases in hepatic lysosomal enzyme activities and biliary lysosomal enzyme secretion and a decrease in the secretion of cholesterol into bile, but that other enzymes of non-lysosomal organelle origin remained unchanged, indicating that the effects of chloroquine are specific to lysosomes. Based on these findings, it is considered that chloroquine is a suitable positive reference in the evaluation of the effect of other drugs on biliary and hepatic lysosomal enzymes. In the present study, chloroquine (50 mg/kg) was given intraperitoneally to rats for 6 days to evaluate its effect on biliary and hepatic lysosomal enzymes. The results showed that chloroquine caused a significant increase in biliary and hepatic lysosomal enzyme activities, as previously reported by Sewell et al. (16). This finding suggests that the present method is appropriate for the evaluation of the effects of drugs on biliary and hepatic lysosomal enzymes.

Rabeprazole dissolved in saline solution is alkaline (approximately pH 10). Therefore, a group receiving saline solution (control-1) and a group receiving saline solution adjusted to pH 10 (control-2) were used as solution control groups to determine the effect of the pH of the dosing solution on biliary and hepatic lysosomal enzymes in experiment 2. It was found that the parameters studied were not affected by the pH of the solution, since there were no significant differences between the control-1 and control-2 groups.

Rabeprazole (1–3) and omeprazole (4, 5) are potent gastric H^+ , K^+ -ATPase inhibitors that are activated in the acidic lumen of the gastric parietal cell, resulting in inhibition of K^+ -stimulated ATPase activity and proton transport across the apical membrane of the parietal cell or gastric vesicle membrane (6). Intra-lysosomal pH is

4–5 (9). If H^+, K^+ -ATPase inhibitors accumulate and are activated in lysosomes, they may affect lysosomal function in the same manner as chloroquine. It is not known whether rabeprazole interacts with the hepatic lysosomal H^+ -ATPase and thereby alters lysosomal function. It is therefore important to examine whether rabeprazole, which is a more potent H^+, K^+ -ATPase inhibitor than omeprazole, affects the function of liver lysosomes. In the present study we have investigated the effects of rabeprazole on hepatic lysosomal function in rats and compared the results with those of omeprazole.

Rabeprazole and omeprazole (5 mg/kg) were given intraperitoneally to rats for 6 days. In the preliminary study, both rabeprazole and omeprazole showed a 90% or higher inhibition of histamine-induced gastric acid secretion when given intraperitoneally to rats at a dose of 5 mg/kg (data not shown). The results of the present study showed that rabeprazole did not influence biliary or hepatic lysosomal enzyme activities. It also had no effect on the latency of *N*-acetyl- β -glucosaminidase in the liver E-fraction. Moreover, rabeprazole had no effect on liver weight, liver protein content or biliary protein content. The reference drug, omeprazole, also had no effect on these lysosomal enzymes. The results with omeprazole agree with those in a previous report (21). The results of this study suggest that rabeprazole does not affect hepatic lysosomal enzyme activities, the integrity of the lysosomal membrane, and lysosomal enzyme secretion into bile.

These results also suggest that rabeprazole does not affect the lysosomal pH. If rabeprazole had inhibited the vacuole-type H^+ -ATPase, the lysosomal functions might have also been changed. In fact, bafilomycin A_1 , a specific inhibitor of the vacuole-type H^+ -ATPase, induces both increase in lysosomal pH and secretion of lysosomal enzyme at similar concentrations in macrophages (28). It has been reported that the inhibitory effect of omeprazole on vacuolar H^+ -ATPase (bovine kidney and long bone of hen (29) and adrenal chromaffin granules (30)) was weak, in contrast to its inhibitory effect on gastric H^+, K^+ -ATPase. The inhibitory effect of omeprazole at high concentrations on vacuolar H^+ -ATPase was reversed with exogenous glutathione which can not pass through the membrane, showing that omeprazole modifies the SH-residue of this enzyme present on the cytoplasmic surface of the vesicle membrane (29, 30), but not on the luminal surface. Since intracellular concentrations of reduced glutathione are high in liver tissues (31), it is speculated that the SH-residue of this enzyme present on the cytoplasmic surface is protected against omeprazole by endogenous glutathione. Based on these findings and our present results, it is considered that proton pump inhibitors such as omeprazole and rabeprazole do not affect

lysosomal H^+ -ATPase activity in vivo.

It has been reported that chloroquine modifies the secretion of lipid into bile (16). In this study, the output of bile acid was decreased but that of cholesterol was unaffected after the administration of chloroquine. These results do not agree with the findings of Sewell et al., in which biliary secretion of cholesterol was decreased by chloroquine treatment (16). One of reasons for this discrepancy may be the difference in the conditions of the animals after chloroquine administration. The gain of body weight after repeated chloroquine administration in this experiment was approximately 40 g, but that in the report of Sewell et al. was only about 5 g. Another reason may be the differences in the period of repeated chloroquine administration. It was reported that the influence of chloroquine on biliary secretion of cholesterol differ depending on the administration method and the period of administration; that is, Rahman and Coleman showed that chloroquine caused a substantial output of cholesterol into bile, when introduced into isolated perfused rat livers (32). Furthermore, Sewell et al. showed that autophagy induced by a single dose of chloroquine produced a dramatic decrease in the biliary excretion of lysosomal protein and no change in biliary lipid secretion in biliary fistula rats (33). Although the results of this study were somewhat different from the results in previous studies on the effect of chloroquine on the secretion of lipid into bile, rabeprazole and omeprazole had no effect on the biliary secretion of lipid into bile.

From the results of this study, it is concluded that rabeprazole, like omeprazole, has no significant influence on hepatic lysosomal function.

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