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# Anti-ulcer effects of antioxidants: effect of probucol

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**Abstract**

We investigated the effect of probucol, a lipid-lowering agent with antioxidant properties, on HCl plus ethanol-induced gastric mucosal injury and on the healing of acetic acid-induced gastric ulcers in rats. When the free radical-scavenging activity of probucol was measured by an electron spin resonance technique, the agent ($10^{-5}$–$10^{-3}$ M) scavenged both superoxide anions and hydroxyl radicals. Oral administration of probucol (250–1000 mg/kg) dose dependently prevented the HCl plus ethanol-induced gastric mucosal injury and the increase in thiobarbituric acid-reactive substances, an index of lipid peroxidation, in the injured mucosa. Repeated oral administration of probucol (250–1000 mg/kg twice daily) dose dependently accelerated the healing of acetic acid-induced gastric ulcers. In addition, probucol already inhibited the increase in the content of thiobarbituric acid-reactive substances in the ulcerated region before the ulcer-healing effect of this agent was recognized. These results suggest that probucol may partly protect gastric mucosa from acute gastric mucosal injury and promote the healing of chronic gastric ulcers by its antioxidant activity. © 1998 Elsevier Science B.V. All rights reserved.

*Keywords:* Probucol; Antioxidant; Gastric cytoprotection; Gastric ulcer; Free radical

## 1. Introduction

Probucol has been widely used clinically for the prevention of the progression of atherosclerosis, because this agent acts as a potent antioxidant (Parthasarathy et al., 1986; Regnstrom et al., 1990; Baumstark et al., 1992) in addition to having a lipid-lowering action (Yamamoto et al., 1986; Schwartz, 1988). Recently, oxygen-derived free radicals have been postulated to play an important role in the pathogenesis of acute gastric mucosal injuries such as ischemia-reperfusion (Pery et al., 1986)-, stress (Cochran et al., 1982)-, ethanol (Pihan et al., 1987)- and anti-inflammatory drug (Del Soldato et al., 1985; Pihan et al., 1987)-induced gastric mucosal injuries in rats. Furthermore, it has been suggested that free radicals generated by neutrophils may be important factors in delaying the healing of acetic acid-induced chronic gastric ulcers in rats (Shii et al., 1992). We have already demonstrated that polaprezinc (Z-103) (Ito et al., 1990, 1992) and zinc-cimetidine complex (Ito et al., 1995) possess potent antioxidant actions and are effective in healing experimentally induced chronic gastric ulcers. From these findings, probucol is expected to be effective in preventing ulcer formation and in ulcer healing.

In the present study, we confirmed in vitro that probucol scavenges superoxide anions ($O_2^-$) and hydroxyl radicals ($\cdot OH$) by using an electron spin resonance technique. In addition, we examined the effects of cimetidine and omeprazole on these free radicals. We then examined the effects of probucol in comparison to cimetidine and omeprazole on HCl plus ethanol-induced acute gastric mucosal injury and the healing of acetic acid-induced chronic gastric ulcers in rats. In addition, in order to clarify whether or not probucol exerts an anti-ulcer action by means of its antioxidant activity, we examined the effect of this drug on the content of thiobarbituric acid-reactive substances, an index of lipid peroxidation, in the injured or ulcerated region in both acute and chronic experimental models.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley strain SPF rats (Nippon SLC, Shizuoka, Japan), weighing 210–230 g, were used in the

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experiment. The animals were housed in an air-conditioned room at 23 ± 1°C.

### 2.2. Drugs

The drugs used were probucol (Sinlestal R, Daiichi Pharmaceutical, Tokyo, Japan), cimetidine (Sigma Chemical, St. Louis, MO, USA) and omeprazole (Fujisawa Astra, Osaka, Japan). These drugs were suspended in 1% gum arabic for in vivo experiments.

### 2.3. $O_2^-$ and ·OH-scavenging activities of probucol, cimetidine and omeprazole in vitro

The $O_2^-$ and ·OH-scavenging activities of probucol, cimetidine and omeprazole were measured by an electron spin resonance (ESR)-spin trapping technique (Buettner, 1987). 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) (Sigma Chemical) was used as a spin trapping agent for $O_2^-$ and ·OH. Hypoxanthine and xanthine oxidase in Tris–HCl buffer (pH 7.4) were used as a $O_2^-$-generating system. To generate ·OH, the Fenton reaction ($H_2O_2 + Fe^{2+}$) (Cohen and Sinet, 1982) was used. Diethylenetriaminepentaacetic acid (DTPA) (Sigma Chemical) was used as a metal chelator. The ESR signals of the adducts of DMPO-$O_2$H and DMPO-OH in the presence and absence (control) of probucol, cimetidine or omeprazole were recorded on a JES-RE2X ESR Spectrometer (JEOL, Tokyo, Japan). The amount of $O_2^-$ and ·OH is expressed as the peak area of DMPO-$O_2$H/the peak area of $Mn^{2+}$ and the peak area of DMPO-OH/the peak area of $Mn^{2+}$, respectively.

### 2.4. Effects of probucol and cimetidine on HCl plus ethanol-induced gastric mucosal injury and on the content of thiobarbituric acid-reactive substances in the injured mucosa

After a 24-h fast, HCl plus ethanol (150 mM HCl in 60% ethanol) was instilled in a volume of 1 ml per 100 g of body weight into the stomach of rats. Probucol (250, 500 and 1000 mg/kg) was given orally in a volume of 1 ml per 100 g of body weight 18 h prior to HCl plus ethanol instillation, because plasma probucol levels reach a maximum 18 h after oral administration of the agent (Kondo et al., 1983). Cimetidine (100 mg/kg, 1 ml per 100 g of body weight) was given orally at 1 h prior to treatment with HCl plus ethanol. Furthermore, as control, vehicle (1% gum arabic) was given instead of test drugs. In addition, to compare the content of thiobarbituric acid-reactive substances in the injured mucosa with that in the mucosa of normal rats, distilled water was given intragastrically to rats instead of HCl plus ethanol. At 1 h after treatment with the necrotizing agent, the animals were killed under ether anesthesia, and the stomach was removed and then opened along the greater curvature. In order to evaluate the degree of gastric mucosal injury, the length (mm) and width (mm) of hemorrhagic erosions in the gastric mucosa were measured under observation with a stereoscopic microscope, and the area of the erosions ($mm^2$) was calculated. The sum of the area of each erosion is expressed in terms of a lesion index. After the lesion index was measured, the mucosa of the glandular part of the stomach was removed by scraping. The thiobarbituric acid-reactive substances in the mucosa were determined by the method of Ohkawa et al. (1979). The content of thiobarbituric acid-reactive substances is expressed as nanomoles of malondialdehyde per milligram protein. Thiobarbituric acid (Kanto Chemicals, Tokyo, Japan) and 1,1,3,3-tetramethoxypropane (Tokyo Kasei, Tokyo, Japan) were used for the thiobarbituric acid assay.

### 2.5. Effects of probucol and cimetidine on the ulcer index and the content of thiobarbituric acid-reactive substances in the ulcerated region of acetic acid-induced gastric ulcers

The rats were allowed daily access to commercial food pellets between 9:00–10:00 a.m. and 5:00–6:00 p.m. throughout the experimental period from 3 days prior to ulcer induction (Ito et al., 1990, 1994). However, tap water was always supplied ad libitum. Gastric ulcers were induced in these rats by the injection of 20% acetic acid (v/v) in a volume of 0.05 ml into the submucosal layer at the junction of the fundus and antrum in accordance with the method described by Takagi et al. (1969). The rats with gastric ulcers were divided into six groups. The animals of five groups were given probucol at 250, 500 and 1000 mg/kg and cimetidine at 50 and 100 mg/kg, respectively, orally, twice daily (10:30 a.m. and 6:30 p.m.) for 14 consecutive days from the day (the 1st day) after acetic acid injection. The animals of the remaining group were given the vehicle instead of test drugs as control.

On the 15th day, all animals were killed with an overdose of ether. The stomach was cut open along the greater curvature. Immediately after the ulcer size was measured for the ulcer index, the mucosa was collected from the ulcerated and unulcerated regions. The thiobarbituric acid-reactive substances in the mucosa were determined.

### 2.6. Time course-related effects of probucol and cimetidine on the ulcer index and the content of thiobarbituric acid-reactive substances in the ulcerated region of acetic acid-induced gastric ulcers

Rats with gastric ulcers were divided into three groups and were given probucol (1000 mg/kg), cimetidine (100 mg/kg) and vehicle (control), respectively, orally, twice daily from the day after acetic acid injection. On the 3rd, 7th, 10th and 15th days, the animals were killed with an overdose of ether. The effects of probucol and cimetidine on ulcer healing and the content of thiobarbituric acid-reactive substances in ulcerated region were evaluated.

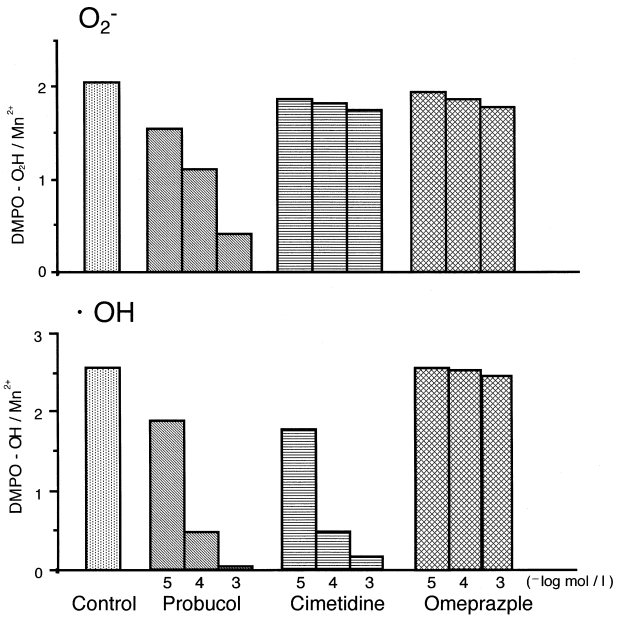


Fig. 1. Superoxide anion ($O_2^-$)- and hydroxyl radical (·OH)-scavenging activities of probucol, cimetidine and omeprazole in vitro. Each column denotes the mean for two measurements.

*2.7. Effects of omeprazole on HCl plus ethanol-induced gastric mucosal injury and the healing of acetic acid-induced gastric ulcers and on the content of thiobarbituric acid-reactive substances in the injured and ulcerated regions*

In the experiment involving HCl plus ethanol-induced gastric mucosal injury, omeprazole (50 mg/kg) was given orally to the fasted rats at 1 h prior to the administration of HCl plus ethanol. The effects of test drug on gastric mucosal lesions and the content of thiobarbituric acid-reactive substances in the injured mucosa were evaluated at 1 h after treatment with the necrotizing agent. In the experiment involving acetic acid-induced gastric ulcers, the rats with ulcers were given omeprazole (50 mg/kg) orally, twice daily from the day after the acid injection. The effects of test drug on the ulcer healing (ulcer index) and the content of thiobarbituric acid-reactive substances in the ulcerated region were evaluated on the 10th and 15th days.

*2.8. Effects of probucol, cimetidine and omeprazole on basal gastric secretion*

After a 24-h fast, the pylorus of each rat was ligated under ether anesthesia to determine gastric secretion. Probucol was given orally at 16 h prior to pylorus ligation. Cimetidine or omeprazole was given orally at 1 h prior to pylorus ligation. Control animals were given orally the vehicle instead of test drugs. The gastric contents were collected for 5 h after ligation. The volume of gastric juice was measured, the acidity was determined with an automatic titrator (ABT-101, Tohadenpa, Tokyo, Japan) and total acid output during the 1-h period was calculated.

*2.9. Statistical analysis*

The results obtained are expressed as the means ± S.E. The data were analyzed by one-way analysis of variance, and the statistical significance among groups was determined by Duncan's multiple-range test.

## 3. Results

*3.1. $O_2^-$- and ·OH-scavenging activities of probucol, cimetidine and omeprazole in vitro*

When the free radical-scavenging activity of probucol was measured with an ESR-spin trapping technique, this agent at $10^{-5}$–$10^{-3}$ M scavenged both $O_2^-$- and ·OH in a concentration-dependent manner (Fig. 1). Cimetidine

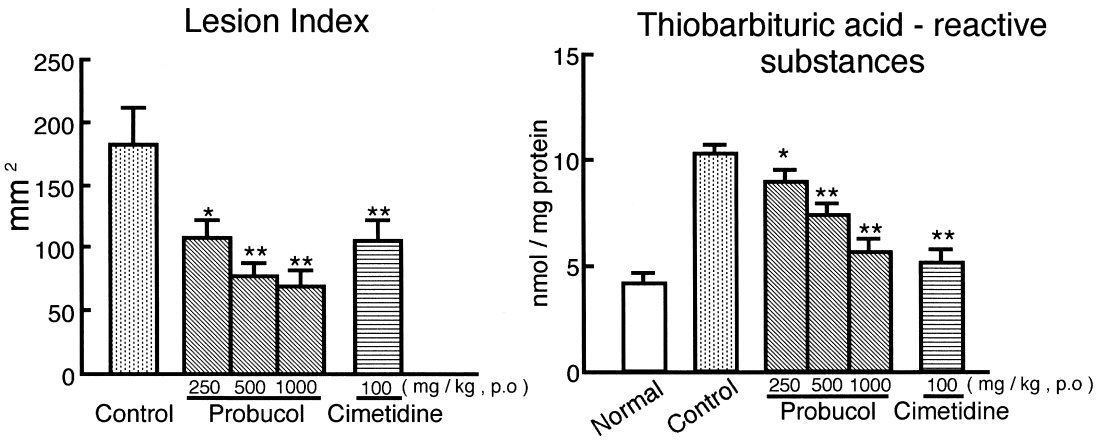


Fig. 2. Effects of probucol and cimetidine on HCl plus ethanol-induced gastric mucosal injury and the content of thiobarbituric acid-reactive substances in the injured mucosa in rats. Probucol and cimetidine were given orally at 18 and 1 h, respectively, prior to intragastric instillation of HCl plus ethanol. At 1 h after HCl plus ethanol instillation, the effects of test drugs on gastric mucosal injury were evaluated and the content of thiobarbituric acid-reactive substances in the injured mucosa was determined. Each column denotes the mean ± S.E. for 7 to 9 rats. Significantly different from respective control, $* P < 0.05$, $** P < 0.01$.

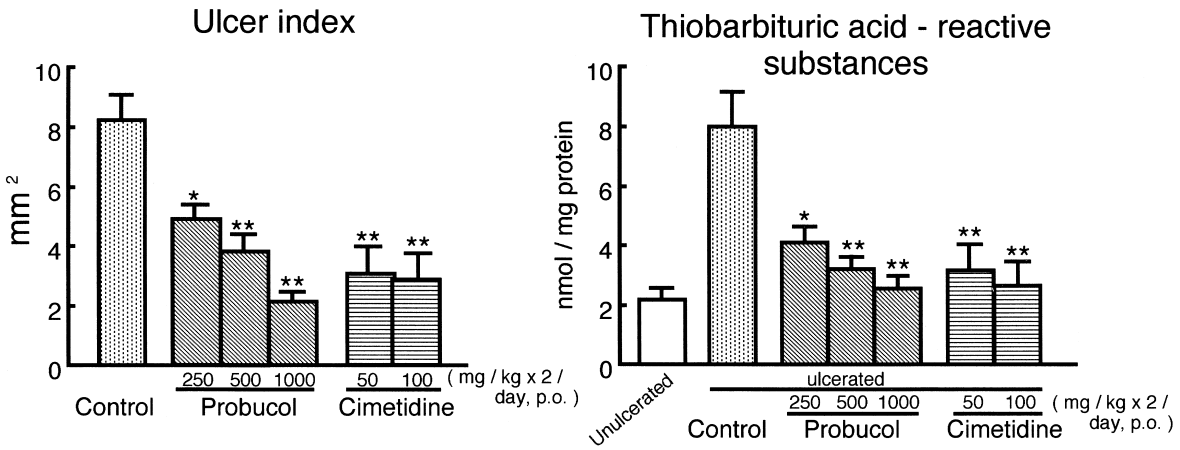


Fig. 3. Effects of probucol and cimetidine on ulcer index and the content of thiobarbituric acid-reactive substances in the ulcerated region of acetic acid-induced gastric ulcers in rats. Probucol or cimetidine was given twice daily for 14 consecutive days, beginning the first day after acetic acid injection. On the 15th day, the effects of both drugs on ulcer healing were evaluated and the content of thiobarbituric acid-reactive substances in the ulcerated mucosa was then determined. Each column denotes the mean ± S.E. for 8 to 10 rats. Significantly different from respective control, $* P < 0.05$, $** P < 0.01$.

($10^{-5}$–$10^{-3}$ M) was effective in scavenging ·OH only. However, omeprazole ($10^{-5}$–$10^{-3}$ M) failed to scavenge either free radical.

### *3.2. Effects of probucol and cimetidine on HCl plus ethanol-induced gastric mucosal injury and on the content of thiobarbituric acid-reactive substances in the injured mucosa*

Intragastric administration of HCl plus ethanol to control rats produced large hemorrhagic erosions in the glandular stomach. Probucol at oral doses of 250, 500 and 1000 mg/kg prevented the gastric mucosal injury by 41%, 58% and 62%, respectively (Fig. 2, left). Cimetidine at an oral dose of 100 mg/kg also prevented the mucosal injury by 44%. The content of thiobarbituric acid-reactive substances in the injured mucosa was 2.5 times higher than that in the mucosa of normal rats (Fig. 2, right). Probucol (250, 500 and 1000 mg/kg) inhibited the increase in the content of thiobarbituric acid-reactive substances in a dose-dependent manner (percent decrease, compared to control: 13%, 29% and 46%, respectively). Cimetidine

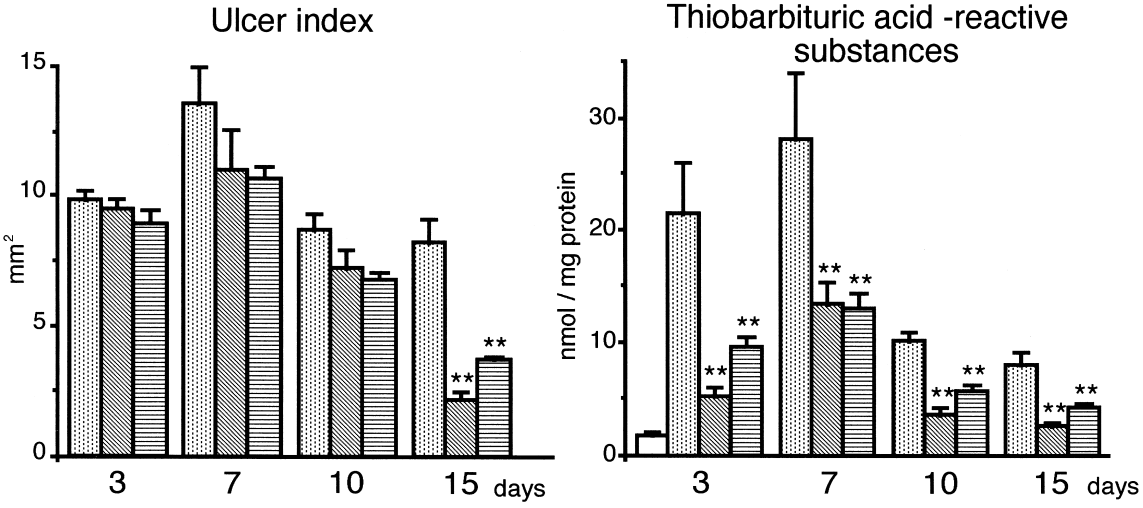


Fig. 4. Time course-related effects of probucol and cimetidine on ulcer index and the content of thiobarbituric acid-reactive substances in the ulcerated region of acetic acid-induced gastric ulcers in rats. Probucol or cimetidine was given daily from the first day after acetic acid injection. On the 3rd, 7th, 10th and 15th days, the effects of test drugs on the ulcer healing were evaluated and the content of thiobarbituric acid-reactive substances in the ulcerated region was then determined (□: Unulcerated, (square with dots): Control, (square with diagonal lines): Probucol 1000 mg/kg twice daily, p.o., (square with horizontal lines): Cimetidine 100 mg/kg twice daily, p.o.). Each column denotes the mean ± S.E. for 7 to 9 rats. Significantly different from respective control, $** P < 0.01$.

(100 mg/kg) also inhibited it significantly (percent decrease, compared to control: 57%).

### 3.3. Effects of probucol and cimetidine on ulcer index and the content of thiobarbituric acid-reactive substances in the ulcerated region of acetic acid-induced gastric ulcers

Repeated oral administration of probucol and cimetidine for 14 consecutive days accelerated the healing of gastric ulcers (Fig. 3, left). Probucol given in doses of 250, 500 and 1000 mg/kg twice daily decreased the ulcer index by 39%, 53% and 74%, respectively. Cimetidine given in doses of 50 and 100 mg/kg twice daily decreased the ulcer index by 62% and 65%, respectively. The content of thiobarbituric acid-reactive substances in the ulcerated region was 3.7 times higher than that in the unulcerated region (Fig. 3, right). Probucol given in doses of 250, 500 and 1000 mg/kg twice daily decreased the content of thiobarbituric acid-reactive substances in the ulcerated region by 48%, 60% and 69%, respectively, as compared to the control. Cimetidine given in doses of 50 and 100 mg/kg, twice daily decreased the content by 60% and 67%, respectively.

### 3.4. Time course-related effects of probucol and cimetidine on ulcer index and the content of thiobarbituric acid-reactive substances in the ulcerated region of acetic acid-induced gastric ulcers

Probucol (1000 mg/kg twice daily, p.o.) decreased the content of thiobarbituric acid-reactive substances in the ulcerated region by 76%, 53%, 68% and 68%, as com-

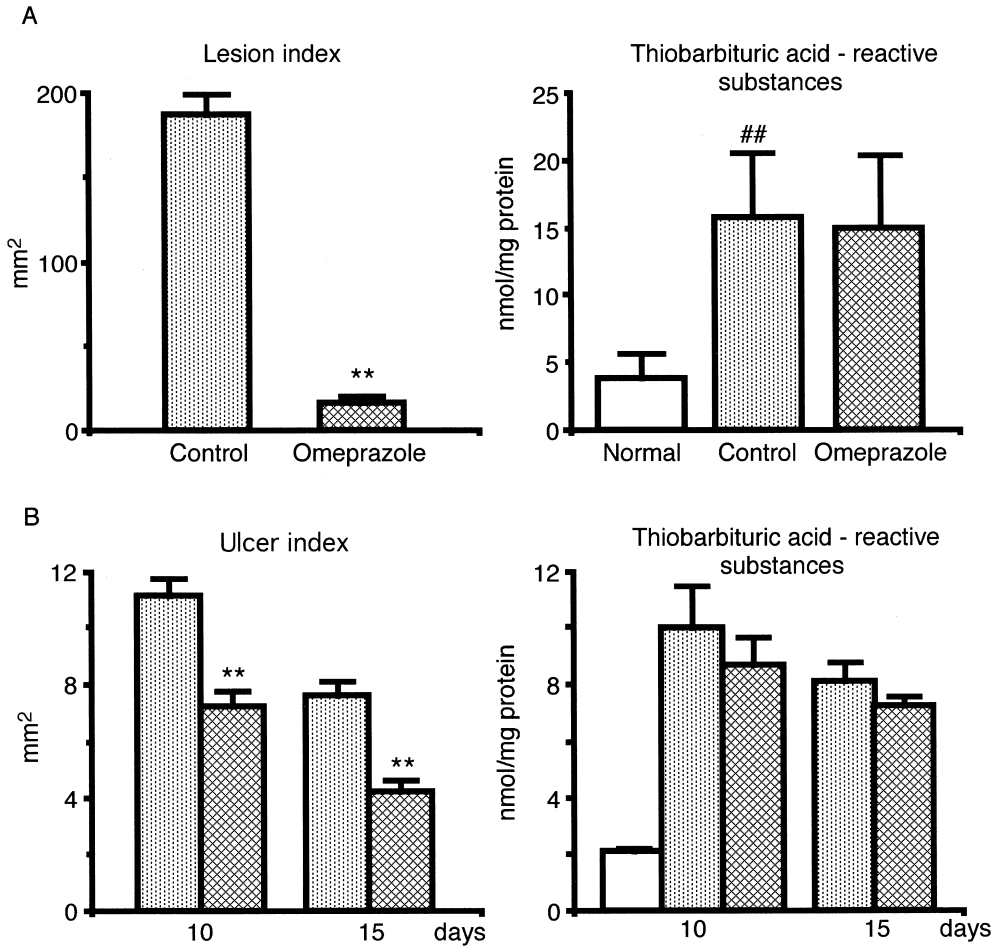


Fig. 5. Effects of omeprazole on HCl plus ethanol-induced gastric mucosal injury (A) and the healing of acetic acid-induced gastric ulcers (B) and on the content of thiobarbituric acid-reactive substances in the injured or ulcerated region. In A experiment, omeprazole was given at 1 h prior to intragastric instillation of HCl plus ethanol. At 1 h after HCl plus ethanol instillation, the effect of omeprazole on gastric mucosal injury was evaluated and the content of thiobarbituric acid-reactive substances in the injured mucosa was then determined (open bar, Normal; dotted bar, Control; cross-hatched bar, Omeprazole 50 mg/kg, p.o.). In B experiment, omeprazole was given daily from the first day after acetic acid injection. On the 10th and 15th days, the effect of omeprazole on ulcer healing was evaluated and the content of thiobarbituric acid-reactive substances in the ulcerated region was then determined (open bar, Unulcerated; dotted bar, Control; cross-hatched bar, Omeprazole 50 mg/kg per day, p.o.). Each column denotes the mean ± S.E. for 7 to 9 rats. Significantly different from respective control, $** P < 0.01$.

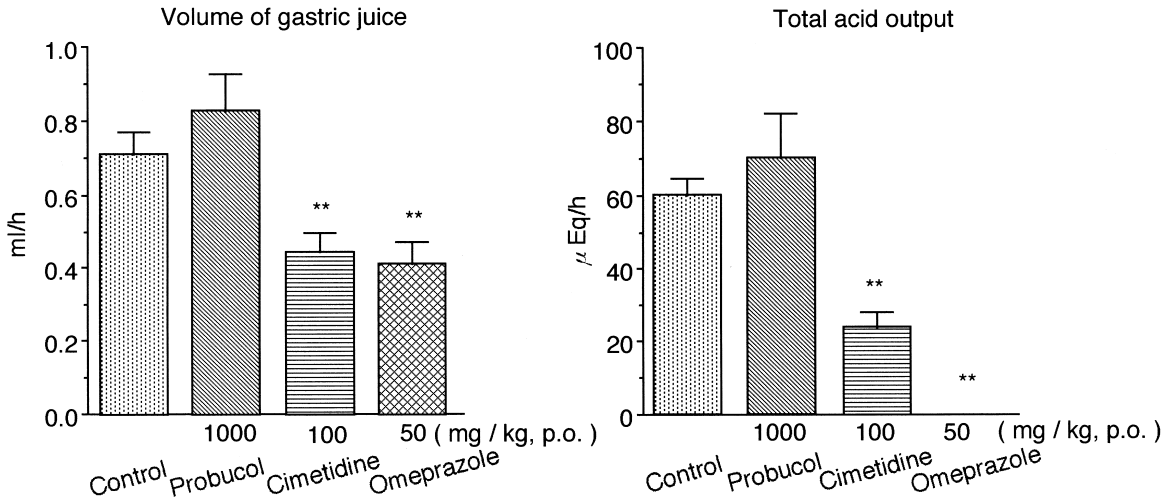


Fig. 6. Effects of probucol, cimetidine and omeprazole on basal gastric acid secretion in normal rats. To determine gastric secretion, each animal received a 5-h pylorus ligation from 16 h after the administration of probucol and 1 h after the administration of cimetidine or omeprazole. Each column denotes the mean ± S.E. for 6 rats.

pared to the corresponding control on the 3rd, 7th, 10th and 15th days after acetic acid injection, respectively (Fig. 4, right). The ulcer-healing effect of probucol was recognized on the 15th day but not before (Fig. 4, left). Cimetidine (100 mg/kg twice daily, p.o.) already inhibited the increase in the content of thiobarbituric acid-reactive substances in the ulcerated region before the 15th day when the ulcer-healing effect of the agent was recognized.

### *3.5. Effects of omeprazole on HCl plus ethanol-induced gastric mucosal injury and the healing of acetic acid-induced gastric ulcers and on the content of thiobarbituric acid-reactive substances in the injured or ulcerated regions*

Omeprazole at 50 mg/kg p.o. prevented the HCl plus ethanol-induced gastric mucosal injury by 91%, but did not inhibit the increase in the content of thiobarbituric acid-reactive substances in the injured mucosa (Fig. 5A). Omeprazole at 50 mg/kg once daily p.o. significantly decreased the ulcer index by 35% and 45%, on the 10th and 15th days after acetic acid injection, respectively (Fig. 5B). However, this agent failed to inhibit the increase in the content of thiobarbituric acid-reactive substances in the ulcerated region.

### *3.6. Effects of probucol, cimetidine and omeprazole on basal gastric secretion*

A single oral administration of probucol (1000 mg/kg) to normal rats failed to alter to basal gastric secretion (the volume of gastric juice and total acid output). In contrast, cimetidine (100 mg/kg, p.o.) and omeprazole (50 mg/kg, p.o.) markedly inhibited the volume of gastric juice and total acid output (Fig. 6).

## 4. Discussion

The present study demonstrates that probucol exhibits both gastroprotective and ulcer-healing properties, probably as a result of the antioxidant action of the drug.

In the first experiment, probucol protected rat gastric mucosa against HCl plus ethanol-induced damage. It has been demonstrated that oxygen-derived free radicals are involved in the pathogenesis of ethanol-induced gastric mucosal damage. However, the exact mechanism for the formation of oxygen-derived free radicals in the stomach after administration of ethanol is still unknown. Allopurinol and oxypurinol, inhibitors of xanthine oxidase, have been shown to prevent ethanol-induced gastric mucosal damage in rats (Mizui et al., 1987; Mutoh et al., 1990). Furthermore, instillation of 50% ethanol in the rat stomach has been shown to cause neutrophil infiltration into the gastric mucosa (Tepperman and Soper, 1990). Therefore, xanthine oxidase and neutrophils are considered main sources of free radicals in ethanol-induced damage. As mentioned in the introduction, probucol is a lipid-lowering agent with antioxidant properties. In the present study, we also confirmed in vitro that probucol at $10^{-5}$–$10^{-3}$ M concentration dependently scavenged both $O_2^-$ and ·OH. It has been shown that probucol inhibits the $Cu^{2+}$-catalyzed oxidative modification of low-density lipoprotein particles by cells (Parthasarathy et al., 1986; Regnstrom et al., 1990; Baumstark et al., 1992). Recently, Ricardo et al. (1994) demonstrated in in vitro studies that probucol added to kidney slice cultures containing puromycin aminonucleoside scavenged reactive oxygen species and inhibited

changes in glomerular epithelial cells. In the present experiment, probucol also inhibited the increase in the content of thiobarbituric acid-reactive substances (lipid peroxidation) in mucosa injured by HCl plus ethanol. This observation suggests that the protective action of probucol against HCl plus ethanol-induced mucosal damage may be due in part to the scavenging of free radicals produced in the injured mucosa. In this experiment, cimetidine, a histamine $H_2$ receptor antagonist, also prevented the formation of HCl plus ethanol-induced mucosal lesions and lipid peroxidaion in the injured mucosa. In this experiment, we used HCl plus ethanol as a necrotizing agent instead of absolute ethanol. The results obtained with probucol and cimetidine indicate that both drugs have gastric cytoprotective effect, independent of their actions on gastric acid secretion. Cimetidine has been shown to be a powerful ·OH scavenger (Uchida and Kawakishi, 1990). We also confirmed in vitro that cimetidine ($10^{-5}$–$10^{-3}$ M) was effective in scavenging ·OH, although it was ineffective against $O_2^-$. Accordingly, the ·OH-scavenging property of cimetidine may be related to its gastric cytoprotective action. However, the inhibition of lipid peroxidation in the injured mucosa by probucol or cimetidine may be secondary to the gastric cytoprotective actions of both agents. Omeprazole, a proton pump inhibitor, was ineffective in scavenging both $O_2^-$ and ·OH in the in vitro experiment. Omeprazole (50 mg/kg) markedly prevented the HCl plus ethanol-induced gastric injury without inhibiting lipid peroxidation in the injured mucosa. The results obtained with omeprazole suggest that the gastric cytoprotective actions of probucol and cimetidine may be due, at least in part, to the inhibition of lipid peroxidation. However, the gastric cytoprotective properties of histamine $H_2$ receptor antagonists including cimetidine remain controversial, since it has been reported that these drugs are protective (Konturek et al., 1981; Miyata et al., 1991) or not protective (Puurunen, 1980) against ethanol or aspirin-induced gastric mucosal injury. Miyata et al. (1991) reported that cimetidine (10 to 100 mg/kg, p.o.) as well as pirenzepine (10 to 100 mg/kg, p.o.) and cetraxate (100 to 300 mg/kg, p.o.) prevented acidified (150 mM HCl) ethanol (60%)-induced gastric mucosal injury in a dose-dependent manner. Thus, the results obtained with cimetidine in this study are in agreement with their results. It has been reported that cimetidine and famotidine do not prevent the gastric lesions induced by severe conditions such as 600 mM HCl and absolute ethanol (Robert et al., 1979; Takagi et al., 1983). Therefore, the discrepant results obtained with histamine $H_2$ receptor antagonists regarding gastric cytoprotection may be partly attributable to the difference in the concentrations of ethanol or HCl plus ethanol used as a necrotizing agent. Ethanol-induced gastric mucosal injury has been also indicated to be due to impairments in defensive factors such as mucus (Kuwata et al., 1985) and mucosal microcirculation (Trier et al., 1987) in addition to neutrophil-generated free radicals as mentioned above. Therefore, probucol may also protect the mucosa against HCl plus ethanol-induced damage by activating mucosal defensive factors. Furthermore, it is not excluded that probucol protects the mucosa by enhancing mucosal repair. Therefore, in order to clarify the mechanisms for the gastric cytoprotective action of probucol, further experiments are necessary to examine actions other than the free radical-scavenging action of this drug.

In the next experiment, we studied acetic acid-induced gastric ulcers in rats with a limited food intake time to assess the effect of probucol on the healing of chronic gastric ulcers, because cimetidine and omeprazole were more effective in promoting ulcer healing in this model than in the model of rats with an unrestricted access to food (Ito et al., 1994). We have already reported that neutrophils and thiobarbituric acid-reactive substances are markedly increased in the ulcerated region after ulcer induction in this model and then decreased gradually as the day went on (Shii et al., 1992). In addition, more recently we have demonstrated that the activity of superoxide dismutase, a $O_2^-$-scavenging enzyme, in the ulcerated region is decreased after induction of gastric ulcers and that the time course of the evolution of enzyme activity shows a negative correlation with that for the ulcer index and lipid peroxidation (unpublished data, Ito et al.). In general, it is thought that the activity of free radical-scavenging enzymes is elevated to scavenge free radicals generated in injured inflamed tissues. However, it is unlikely that superoxide dismutase activity in the ulcerated region is secondarily decreased as a result of the decrease in the generation of free radicals, because lipid peroxidation was markedly increased in the ulcerated region. It seems reasonable to consider that the activity of free radical-scavenging enzymes is decreased when the generation of free radicals exceeds the ability of free radical-scavenging enzymes to scavenge free radicals. Furthermore, we have already demonstrated that the scavenging of free radicals by human Cu, Zn-superoxide dismutase and compounds with antioxidant activity is effective in promoting ulcer healing (Ito et al., 1990, 1995). These findings suggest that neutrophil-generated free radicals are important factors that delay ulcer healing in this model. Probucol markedly enhanced the healing of chronic gastric ulcers. The ulcer-healing effect of probucol (1000 mg/kg, twice daily, p.o.) was as potent as that of cimetidine. In addition, probucol, like cimetidine, already inhibited the increase in lipid peroxidation in the ulcerated region before its ulcer-healing effect was observed. Omeprazole, which is without an antioxidant action, was ineffective in inhibiting the increase in lipid peroxidation. This result indicates that the antiulcer effect of probucol is, at least in part, due to the potent antioxidant action of this drug. It is generally believed that histamine $H_2$ receptor antagonists and proton pump inhibitors accelerate the healing of gastric ulcers by potent and long-lasting antisecretory actions. Therefore, the inhibition of gastric acid secretion is considered an

important factor to accelerate the healing of acetic acid-induced gastric ulcers. However, in the present study, probucol (1000 mg/kg, p.o.) was ineffective in inhibiting gastric acid secretion.

In summary, the present study indicated that probucol exhibits, at least in part, gastric cytoprotective and ulcer-healing actions as a result of its antioxidant activity. However, further studies are needed to clarify the mechanisms of the anti-ulcer action of probucol.

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