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Inhibitory Effect of Quercetin and Desferrioxamine in Rat Reflux Esophagitis

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This study was aimed to evaluate the effects of quercetin and desferrioxamine on the development of the reflux esophagitis induced surgically, on gastric secretion and on lipid peroxidation which is a marker of oxidative stress. Omeprazole was used as a positive control drug. Omeprazole significantly and dose-dependently prevented the development of reflux esophagitis, but quercetin or desferrioxamine prevented only at high dose. Omeprazole significantly and dose-dependently inhibited the gastric acid secretion (gastric volume, pH and acid output), but quercetin or desferrioxamine did not inhibit. Malonyldialdehyde content, the end product of lipid peroxidation, increased significantly after the induction of reflux esophagitis. Omeprazole prevented lipid peroxidation. Quercetin and desferrioxamine inhibited the lipid peroxidation independent of their actions on gastric secretion. This result indicates that omeprazole confirmed preventing effect of rat reflux esophagitis, but quercetin and desferrioxamine inhibited esophagitis by reduction of lipid peroxidation irrespective of gastric acid secretion.

Key Words: Quercetin, Desferrioxamine, Esophagitis

Abbreviation: Lower esophageal sphinctor; LES, Malonyldiadehyde; MAD, Thiobarbituric acid reactive substance; TBARS

INTRODUCTION

Esophageal reflux is a common condition that affects children and 1 of 10 adults, and if untreated, may result in chronic esophagitis, aspiration pneumonia, esophageal strictures and Barrett's esophagus, a premalignant condition (Biancani et al, 1997). Reflux esophagitis is a multifactorial disease that may depend on transient lower esophageal sphincter (LES) relaxation, speed of esophageal clearance, mucosal resistance and other factors, and is often associated with low LES pressure (Bell et al, 1992b).

Therapeutic medicines for reflux esophagitis are H_2-receptor antagonists, prokinetic agents and proton pump inhibitors. H_2-receptor antagonists and prokinetic agents promote symptom relief and esophageal healing in mild esophagitis, but are less effective in the treatment of moderate to severe esophagitis. For patients from moderate to severe esophagitis, rapid symptom relief and esophageal healing have been achieved with proton pump inhibitors through their profound and long-lasting antisecretory activities (Ljubicic et al, 1998).

Meanwhile, oxygen-derived free radicals are known to be mediators of acute gastric mucosal injury caused by ischemia/reperfusion (Stein et al, 1990), ethanol, NSAIDs (Pihan et al, 1987) and Helicobacter pylori (Davies et al, 1994). Chronic free radical damage may also produce a carcinogenic effect by modulating the DNA information (Haegele et al, 1994). In the recent studies, it has been shown that reflux esophagitis in rats is mediated by oxygen-derived free radicals and superoxide anions are the main source of free-radical damage in reflux esophagitis of rats (Wetscher et al, 1995b).

Quercetin, a flavonoid, has been shown to scavenge

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superoxide anions (Martin et al, 1998; Robak et al, 1988) or to chelate iron ions (Kostyuk et al, 1996). It has also been reported that quercetin prevented the gastric mucosal lesions produced by ethanol (Alarcon de la Lastra et al, 1994) and cold-restraint stress (Martin et al, 1993). Desferrioxamine, a nontoxic transition metal ion chelator (Halliwell et al, 1986), protected the gastric mucosa against stress-ulceration and prevented the increase of lipid peroxidation (Das et al, 1997).

This study was aimed to evaluate the effects of quercetin and desferrioxamine, on development of the reflux esophagitis (inflammation index, gastric acid secretion and lipid peroxidation).

METHODS

Animals

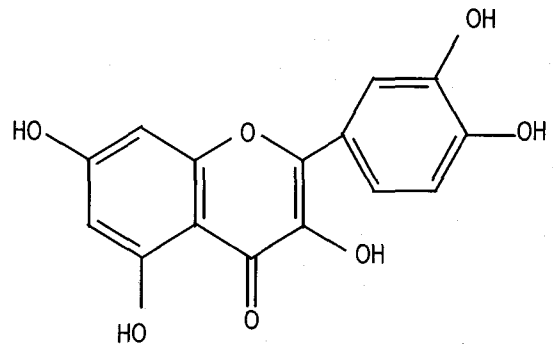
Male Sprague-Dawley rats, weighing 200~250 g, were fasted for 24 hr but allowed free access to water prior to the experiment. All animals were kept in raised mesh-bottom cages to prevent coprophagy. Five to seven rats were included in each group.

Esophagitis induction

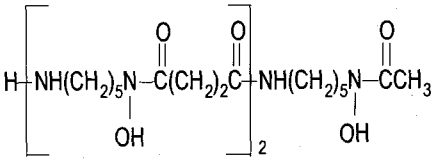
The rats were anesthetized with the optimal inhalation of ether. The abdomen was incised along the midline and then limiting ridge (transitional region between the forestomach and corpus) was ligated very carefully, and continuously the pylorus portion was also performed. A longitudinal cardiomyotomy of about 1cm length across the gastroesophageal junction was performed to enhance reflux from the stomach contents in esophageal body. Immediately the incised regions were sutured and the animals were returned to their home cages. 6 hr later, the animals were sacrificed by cervical dislocation and then the esophagus was harvested (Kil et al, 2001).

The total area (mm^2) of the lesions that had developed in the esophagus was determined under a dissecting microscope ($\times 10$) and graded as follows: 0, no visible lesions; 1, a few erosions; 2, total area of lesions ≤ 30 mm^2; 3, total area of lesions ≥ 30 mm^2; 4, perforation

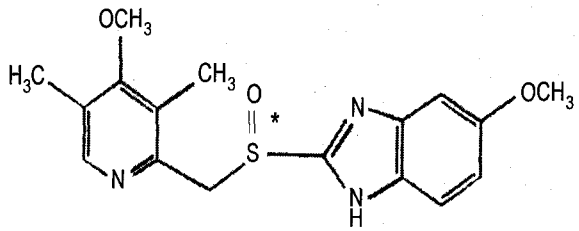
Quercetin, desferrioxamine, omeprazole (Fig. 1) was administered (p.o.) in a volume of 0.3 ml/100 g of body weight at 2 hr prior to surgery. The drugs were prepared freshly each time by the dilution from the high each stock solution.



Quercetin (MW 302.2)



Desferrioxamine (MW 560.7)



Omeprazole (MW 345.4)

Fig. 1. Structure of quercetin, desferrioxamine and omeprazole.

Gastric secretory study

The gastric contents were collected. After centrifugation, the supernatant was measured for volume (ml/rat), pH (Toledo 320, Mettler, Swiss) and acidity (mEq/l). Total acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0. Acid output was expressed as μEq/hr (Okabe et al, 1995).

TBARS assay

Lipid peroxidation, which is a marker of oxidative stress, was determined according to the method of

Buege and Aust measuring spectro-photometrically the formation of thiobarbituric acid-reactive substances (TBARS) (Buege et al, 1972).

Esophageal mucosa was harvested, sonicated in 1 ml of Tris-HCl buffer (pH 7.0). After centrifugation at 600×g for 10 min at 4°C (Micro17TR, Hanil, Korea), 0.9 ml of trichloroacetic acid (8%) was added to 0.3 ml of supernatant. After centrifugation at 10,000×g for 5 min at 4°C, 0.25 ml of TBA (1%) was added to 1 ml of supernatant and the resulting solution was heated at 100°C for 20 min. The tubes were cooled, 2 ml of n-butanol was added and each tubes was vortexed for 90s. After centrifugation at 3,000×g for 5 min at 4°C, 1 ml of butanol phase was utilized for TBARS assay at 532 nm (UV-160A, Shimadzu, Japan) against malonaldehyde bis (dimethyl acetal) standards. Results were expressed as pmol/mg protein. Protein assay was determined according to the Bradford method.

Drugs

Quercetin, desferrioxamine, omeprazole, thiobarbituric acid, trichloroacetic acid, malonaldehyde bis (dimethyl acetal) and bovine serum albumin were purchased from Sigma (St. Louis, MO, USA). Protein assay kits were purchased from BioRad (Richmond, CA, USA).

Analysis of data

Data were presented as mean±SEM (standard error of mean). Student's *t*-test was used to determine the statistical significance of the data.

RESULTS

The comparative effects of omerpazole, quercetin and desferrioxamine on the reflux esophagitis induced surgically in rats

We have previously shown that reflux esophagitis in rats developed with the surgical procedure 4 hr after ligation used in this study (Kil et al, 2001). However, both the severity and incidence of the esophagitis were increased as ligation time of each esophagus passed 6 hr over which can be considered optimal period. Omeprazole dose dependently prevented the development of reflux esophagitis (Fig. 2). Quercetin (100, mg/kg, p.o.) or desferrioxamine (300 mg/kg, p.o) administered at 2 hr prior to surgery prevented ($P<0.05$ in Fig. 2) the development of reflux esophagitis, but other lower dose did not.

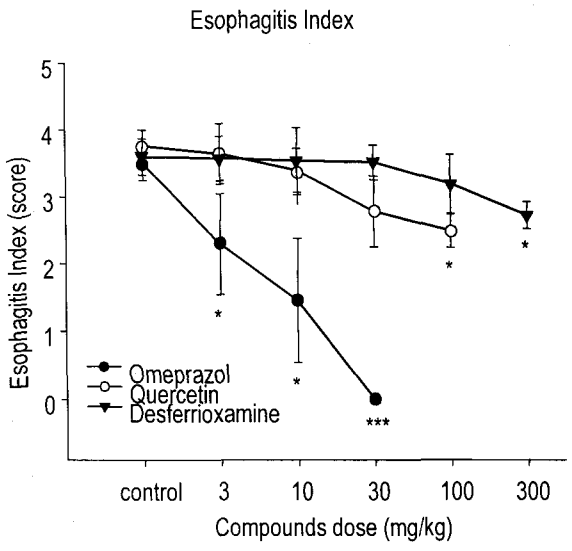


Fig. 2. The effects of quercetin, desferrioxamine and omeprazole on the reflux esophagitis induced surgically in rats. Data are means±SEM. *: $P<0.05$, ***: $P<0.001$ vs. the control.

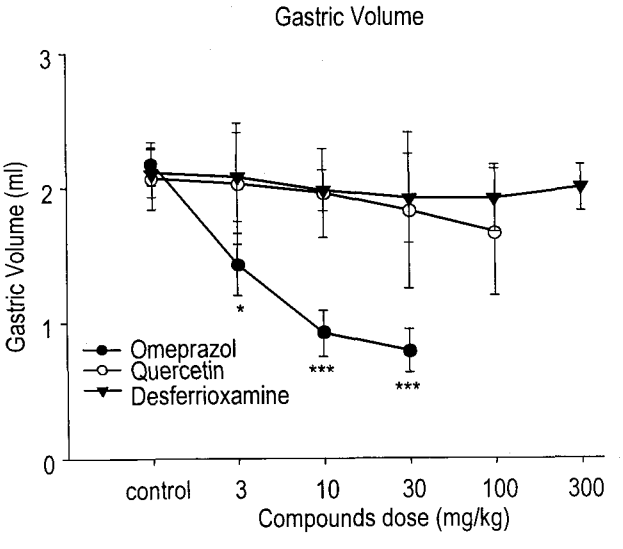


Fig. 3. The effects of quercetin, desferrioxamine and omeprazole on gastric volume in esophagitis rats. Omeprazole (3 or 10 or 30 mg/kg) dose-dependently decreased the gastric volume. Data are means±SEM. *: $P<0.05$, ***: $P<0.001$ vs. the control.

The effects of omeprazole, quercetin and desferrioxamine on gastric secretion in reflux esophagitis

Gastric volume, pH, and acid output were compared the influence on gastric acid secretion after esophagitis (Fig. 3, 4, 5). Omeporazole dose dependently decreased (3, 10, 30 mg/kg) gastric volumewhen compared to control after esophagitis, but quercetin or desferrioxamine did not have inhibitory action even at high dose (Fig. 3). Omeporazole dose dependently increased (10, 30 mg/kg) gastric pH when compared to control after esophagitis, but quercetin or desferrioxamine did not increase action even at high dose (Fig. 4). Omeporazole dose dependently decreased (10, 30 mg/kg) acid output gastric pH when compared to control after esophagitis, but quercetin or desferrioxamine did not change even at high dose (Fig. 5).

The effects of omerpazole, quercetin and desferrioxamine on lipid peroxidation

We measured lipid peroxidation, which is a marker of oxidative stress (Fig. 6). It was determined according to the method of Buege & Aust (1972) measuring the formation of TBARS. Omeporazole dose dependently decreased (10, 30 mg/kg) acid output gastric pH when compared to control after esophagitis. Quercetin or desferrioxamine at high

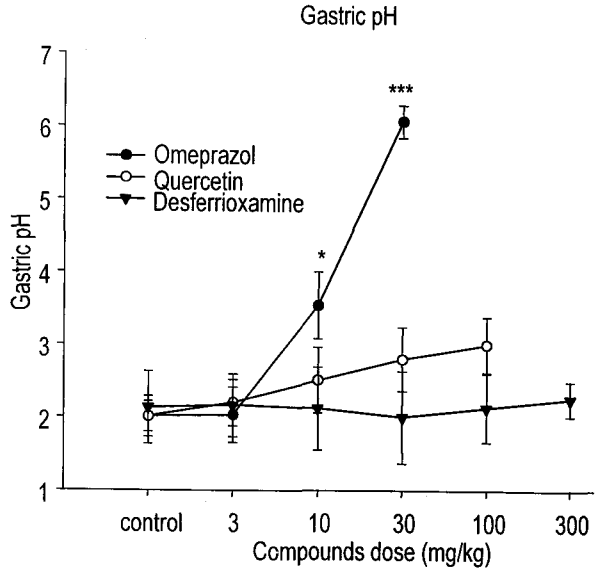


Fig. 4. The effects of quercetin, desferrioxamine and omeprazole on gastric pH in esophagitis rats. Omeprazole (10 or 30 mg/kg) dose-dependently increased the pH of the gastric content. Data are means ± SEM. *: $P<0.05$: ***: $P<0.001$ vs. the control.

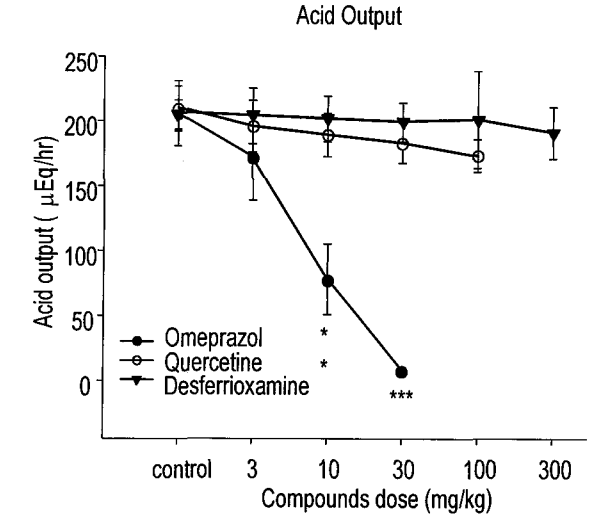


Fig. 5. The effects of quercetin, desferrioxamine and omeprazole on acid output in esophagitis rats. Omeprazole (10 or 30 mg/kg) dose-dependently decreased acid output. Data are means ± SEM. **: $P<0.01$, ***: $P<0.001$ vs. the control.

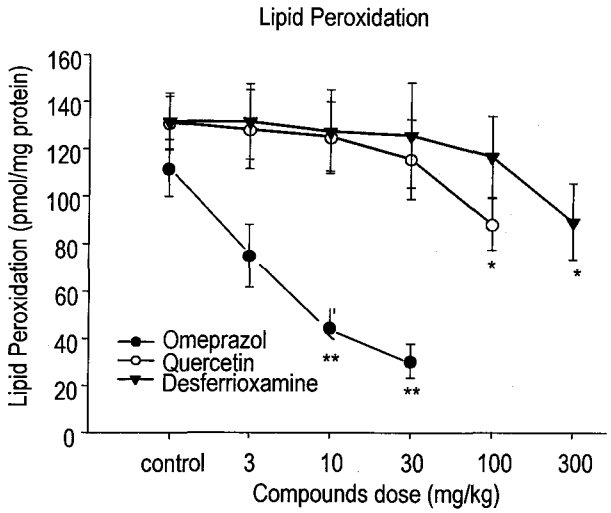


Fig. 6. The effects of quercetin, desferrioxamine and omeprazole on lipid peroxidation. Malonyldialdehyde content, the end product of lipid peroxidation, increased significantly in the esophageal mucosa after the induction of reflux esophagitis. Omeprazole (10 or 30 mg/kg) prevented lipid peroxidation. Data are means ± SEM. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$ vs. control of reflux esophagitis.

dose significantly prevented the increase of lipid peroxidation.

DISCUSSION

Gastro-esophageal reflux disease is a common condition with a complex pathophysiology. Despite the spectrum of abnormalities, gastric acid has a central role in mucosal damage, and the mainstream of medical treatment is suppression of gastric acid secretion (Bell et al, 1992a). There is increasing evidence that gastric acid pump inhibitors exert much more favorable effects on reflux esophagitis than H_2-blockers through their profound and long-lasting antisecretory activities (Thomson, 1992).

Omeprazole inhibited the gastric acid output over 70~80%, and prevented the development of reflux esophagitis, but quercetin and desferrioxamine did weakly. Our finding suggests that quercetin and desferrioxamine is less effective in the inhibition of esophagitis unlike omeprazole. It has been reported that over 40% of acid output inhibition is enough to prevent the development of esophagitis (Okabe et al, 1995) and gastric acid is considered essential to esophageal mucosal damage (Bell et al, 1991). This finding suggests that quercetin and desferrioxamine may not inhibit the reflux esophagitis by acting differently when compared to omeprazole. We furthermore assayed malonyldialdehyde content, an end product of lipid peroxidation, to compare the mechanism of oxygen-derived free radicals on the oxidative stress of them.

It is known that oxygen-derived free radicals are known to be mediators of acute gastric mucosal injury caused by ischemia/reperfusion (Stein et al, 1990), ethanol, NSAIDs (Pihan et al, 1987) and Helicobacter pylori (Davies et al, 1994). Chronic free radical damage may produce a carcinogenic effect by modulating the DNA information (Haegele et al, 1994). It has been shown that reflux esophagitis in rats mediated by oxygen-derived free radicals or superoxide anions are the main source of free radical damage in reflux esophagitis of rats (Wetscher et al, 1995). Superoxide anions produced by inflammatory cells (neutrophils, macrophages, and monocytes) play an important part in the pathogenesis of acid and pepsin induced esophagitis in rabbits (Naya et al, 1997). In recent study, the production of free radical and lipid peroxidation increased with the degree of esophagitis and was the highest in patients with Barrett's esophagus, a premalignant condition (Wetscher et al, 1995c).

It will be possible interest in the present study if we examined the preventing effects of quercetin and desferrioxamine on the lipid peroxidation in esophagitis rat. In this study, malonyldialdehyde content, the end product of lipid peroxidation, significantly increased about 4~5 folds in the esophageal mucosa after the induction of reflux esophagitis. This is constent with the result reported previously (Wetscher et al, 1995a; 1995c; Kil et al, 2001).

As expected, like omeprazole, quercetin and desferrioxamine prevented the development of reflux esophagitis and inhibited the lipid peroxidation independent of their actions on gastric secretion.

Quercetin significantly prevented the development of reflux esophagitis by 32% and inhibited TBARS production by 33%. Quercetin is a natural flavone derivative with anti-inflammatory (Mascolo et al, 1987), antithrombotic (Landoffi et al, 1984), antibacterial (Waage et al, 1984) and antitumoral (Scambia et al, 1991) effects. It has been reported to prevent gastric mucosal lesions produced by ethanol (Alarcon de la Lastra et al, 1994), cold-restraint stress (Martin et al, 1993), and the HCI plus ethanol (Suzuki et al, 1998). It has been reported that quercetin exerts a cytoprotective activity through a complex mechanism involving stimulation of prostaglandin and inhibition of leukotriene production, via mucus secretion and through its antioxidant properties; namely scavenging reactive oxygen species (ROS) and iron ions chelation (Alarcon de la Lastra et al, 1994). It has also been reported that acid-induced esophagitis in cats is not prevented synthetic prostaglandin E and prostaglandins, particularly of the E class, have been shown to have protective effects on gastric mucosa in many animal species but the effect of prostaglandins on esophageal mucosa is not clear (Katz et al, 1988). These reports can answer why quercetin didn't markedly prevent the development of reflux esophagitis.

Desferrioxamine (800 mg/kg) administered i.p. significantly prevented the development of reflux esophagitis by 28% and inhibited TBARS production by 32% independent of their actions on gastric secretion. It has been reported that hydrogen peroxide can react with ferrous iron by Fenton reaction to produce the very reactive hydroxyl radical and hydroxyl radical mediated cell injury is inhibition by iron chelation with desferrioxamine (Olson, 1988; Das et

al, 1997). In the recent study, it has also been shown that superoxide anions are the main source of free-radical damage in reflux esophagitis of rats (Wetscher et al, 1995b). Therefore desferrioxamine didn't markedly prevent the development of reflux esophagitis. However, the role of hydroxyl radical in esophagitis cannot be ruled out.

This result suggests that quercetin and desferrioxamine may be inhibited on the reflux esophagitis irrespective of gastric acid secretion.

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