



## Pulmonary, Gastrointestinal and Urogenital Pharmacology

Influence of aging on gastric ulcer healing activities of the antioxidants  $\alpha$ -tocopherol and probucol

Masashi Ishihara, Ryoji Kojima, Mikio Ito \*

Laboratory of Analytical Pharmacology, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468-8503, Japan

## ARTICLE INFO

## Article history:

Received 14 April 2008

Received in revised form 8 October 2008

Accepted 9 October 2008

Available online 21 October 2008

## Keywords:

Gastric ulcer

Aging

 $\alpha$ -Tocopherol

Probuco

Antioxidant

## ABSTRACT

In the present study, we compared the effects of  $\alpha$ -tocopherol and probucol, antioxidants, on the healing of acetic acid-induced gastric ulcers in 8-, 48- and 96-week-old rats. The repeated oral administration of  $\alpha$ -tocopherol (16 mg/kg twice daily) and probucol (1000 mg/kg twice daily) for 14 consecutive days markedly accelerated the gastric ulcer healing in 48- and 96-week-old rats as well as 8-week-old ones. The ulcer healing effects of both drugs were not significantly different among the rats at three different ages. The superoxide dismutase (SOD) activity in the ulcerated region of 8-, 48- and 96-week-old rats was markedly lower than that in the un ulcerated region. In contrast, the thiobarbituric acid (TBA)-reactive substance content, an index of lipid peroxidation, in the ulcerated region of rats at three different ages markedly increased, as compared to that in the un ulcerated region. The SOD activity tended to decrease with aging, while the TBA-reactive substance content gradually increased. The repeated administration of  $\alpha$ -tocopherol and probucol accelerated the ulcer healing and inhibited the increase in the TBA-reactive substance content in the ulcerated region. These results suggest that  $\alpha$ -tocopherol and probucol promote the ulcer healing by their potent antioxidant activities in 48- and 96-week-old rats as well as 8-week-old rats.

© 2008 Elsevier B.V. All rights reserved.

## 1. Introduction

Aging causes gastrointestinal functional changes. Aggressive factors such as gastric acid secretion (Khalil et al., 1988; Majumdar et al., 1988; Ohno et al., 1988) and pepsin activity (Ohno et al., 1988) have been shown to decrease with aging in humans and animals. Defensive factors such as gastric mucosal blood flow (Masuda et al., 1991), gastric mucosal prostaglandin (PG) content (Cryer et al., 1992; Goto et al., 1992; Lee and Feldman, 1994), and bicarbonate secretion (Kim et al., 1990) have been reported to show an age-related decrease in humans and animals.

Cimetidine and omeprazole are thought to cause hypergastrinemia through the elevation of intragastric pH due to antisecretory action (Peters et al., 1983; Larsson et al., 1986). In addition to stimulating gastric acid secretion, gastrin has been shown to possess a trophic action; for example, it stimulates the proliferation of gastric mucosal cells (Willems et al., 1972; Hansen et al., 1976). We have already reported that cimetidine, a histamine H<sub>2</sub> receptor antagonist, and omeprazole, a proton pump inhibitor, accelerate the healing of gastric ulcers by the trophic action of gastrin via the increase in gastrin secretion rather than by an antisecretory action (Ito et al., 1994a,b). Furthermore, we have shown that cimetidine and omeprazole have potent gastric ulcer healing actions in 8- and 48-week-old rats, mainly

through potent serum gastrin-elevating actions, but both drugs are ineffective in 96-week-old rats which have lost their gastrin-elevating actions (Ishihara and Ito, 2002). Oxygen-derived free radicals have been postulated to play an important role in the pathogenesis of acute gastric mucosal injuries (Pery et al., 1986; Cochran et al., 1982; Pihan et al., 1987; Del Soldato et al., 1985) or in the delay of chronic gastric ulcer healing (Shii et al., 1992). In the previous study, we have reported that the following drugs possess a potent antioxidant action and are effective in acute gastric mucosal injury and/or in promoting the healing of chronic gastric ulcers: polaprezinc, an anti-ulcer drug (Ito et al., 1992); probucol, a lipid-lowering drug (Ito et al., 1998); quercetin, a flavonoid;  $\alpha$ -tocopherol, a vitamin; nifedipine, a Ca<sup>2+</sup>-channel blocker; and tetracycline, an antibiotic (Suzuki et al., 1998). Little is known about the influence of aging on the gastric ulcer healing-promoting actions of the antioxidants mentioned above. Therefore, in the present study, we investigated the influence of aging on the gastric ulcer healing activities of  $\alpha$ -tocopherol and probucol, antioxidants, using 8-, 48- and 96-week-old rats.

## 2. Materials and methods

## 2.1. Animals

Three different age (8, 48 and 96 weeks of age) male Wistar rats (Clea Japan Inc., Tokyo, Japan) were used in the experiments. The animals were housed in an air-conditioned room at 23±1 °C. All experimental

\* Corresponding author. Tel.: +81 52 839 2692; fax: +81 52 834 8090.  
E-mail address: [mitoh@ccmfs.meijo-u.ac.jp](mailto:mitoh@ccmfs.meijo-u.ac.jp) (M. Ito).

procedures described were approved by the Experimental Animal Research Committee of Faculty of Pharmacy, Meijo University.

## 2.2. Drugs

Test compounds used were  $\alpha$ -tocopherol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and probucol (Sinlestal<sup>®</sup>, Daiichi Pharmaceutical Co. Ltd., Tokyo, Japan). Both drugs were suspended in 1% gum arabic.

## 2.3. Influence of aging on gastric ulcer healing activities of test compounds

The rats of three different ages were allowed daily access to commercial food pellets from 9:00 to 10:00 a.m. and 5:00 to 6:00 p.m. throughout the experimental period from 3 days prior to ulcer induction (Ito et al., 1994a). Tap water was always available ad libitum. Gastric ulcers were induced in the rats of three different ages by exposing the serosal surface of the corpus wall to 100  $\mu$ l of 100% acetic acid for 60 s in accordance with the method of Okabe et al. (1977). Test compound ( $\alpha$ -tocopherol or probucol) was given orally twice daily (8:30 a.m. and 4:30 p.m.) to each rat of the three different ages at 0.5 ml per 100 g of body weight for 14 consecutive days from the first day after serosal application of acetic acid. Control animals were given the vehicle (0.5% gum arabic) orally twice daily instead of test compound. On the 15th day, the animals were killed with an overdose of ether. The stomachs were removed, filled with 10% formalin, and allowed to stand for 5 min. Stomachs were then cut open along the greater curvature. The longitudinal and abscissal lengths of the upper, opened part of the ulcer were measured with a micrometer, which was mounted on a stereoscopic microscope, and the product of both lengths ( $\text{mm}^2$ ) was expressed in terms of the ulcer index. After the ulcer size was measured, the stomach tissue was again immersed in 10% formalin for 24 h. The formalin-fixed tissue was then cut so that a little of the normal tissue surrounding the ulcer remained. Thereafter, the central part of the ulcer was cut vertically against the serosa along the long diameters. These tissues, cut in half, were embedded in paraffin and cut into 2- and 3- $\mu$ m-thick sections, which were then

stained with hematoxylin and eosin (HE). Micrographic histological measurements of the stained preparations were performed as shown in Fig. 1. These histological measurements were carried out under a light microscopy of H.E.-stained preparations by Toyobo Image Analyzer (Osaka, Japan). The ulcer size and histological measurements were carried out by two independent observers. The healing effect of each test compound was evaluated by comparing the ulcer index, the defective area in the ulcerated region, the index for the decrease in the exposed floor, and the index for the mucosal regeneration of each test compound with the indexes of the respective control.

## 2.4. Changes in ulcer index, superoxide dismutase (SOD) activity and thiobarbituric acid (TBA)-reactive substance content in ulcerated region during the process of gastric ulcer healing

In order to clarify changes in ulcer index, SOD activity and TBA-reactive substance content in the ulcerated region during the process of gastric ulcer healing, gastric ulcers were induced in 8-week-old rats as mentioned above. On the 3rd, 7th, 10th and 15th days after serosal application of acetic acid, the animals were killed with an overdose of ether. After the ulcer size was measured as the ulcer index, gastric mucosa was collected from the ulcerated and un ulcerated regions by scraping. The SOD activity and TBA-reactive substance content in the ulcerated and un ulcerated regions were determined by the methods of Ohyanagi (1984) and Ohkawa et al. (1986), respectively. The SOD activity is expressed as nitrate units per mg protein. On the other hand, TBA-reactive substance content is expressed as nmol of malondialdehyde per mg protein. TBA (Kanto Chemicals, Tokyo, Japan) and 1,1,3,3-tetra-methoxypropane (Tokyo Kasei, Tokyo, Japan) were used for the TBA assay. Furthermore, the SOD activity and TBA-reactive substance content in ulcerated and un ulcerated regions were determined on the 7th day after application of acetic acid to 8-, 48- and 96-week-old rats.

## 2.5. Effects of test compounds on ulcer index and TBA-reactive substance content in ulcerated region

Gastric ulcers were induced in rats of three different ages. Test compound ( $\alpha$ -tocopherol or probucol) was given orally, twice (8:30 a.m.

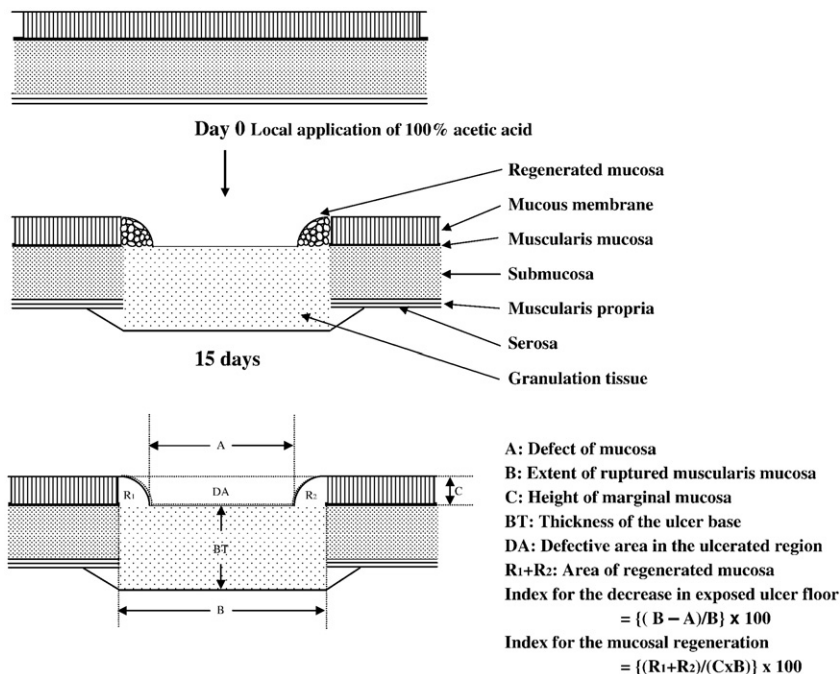


Fig. 1. Method used for histological measurements. Schematic drawings of the vertical section in the ulcerated region on the 15th day after local application of acetic acid.

Download English Version:

<https://daneshyari.com/en/article/2534704>

Download Persian Version:

<https://daneshyari.com/article/2534704>

[Daneshyari.com](https://daneshyari.com)