Original Article

Comparative effects of a tocotrienol-rich fraction and tocopherol in aspirin-induced gastric lesions in rats

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This study examined the effects of a tocotrienol-rich fraction (TRF) obtained from palm oil on the healing of aspirin-induced gastric mucosal lesions. Thirty-six male Sprague–Dawley rats (200–250 g) were randomly divided into three groups. Group I was fed a vitamin E-deficient diet (control), Group II was fed a vitamin E-deficient diet supplemented with tocopherol (300 mg/kg food) and Group III was fed a vitamin E-deficient diet supplemented with TRF (300 mg/kg food). After eight weeks, the control and treated groups received a single intragastric dose of 400 mg/kg body weight aspirin. The rats were killed 24 h after exposure to aspirin. Assessment of gastric lesions showed a lower gastric lesion index in the TRF ($P = 0.0005$) and tocopherol groups ($P = 0.0008$) compared to the control. The gastric malondialdehyde (MDA) content was also lower in the TRF ($P = 0.025$) and tocopherol groups ($P = 0.025$) compared to control. There were, however, no significant differences in the gastric lesion index and gastric MDA content between the TRF and tocopherol-fed groups. There were no significant differences in the adherent gastric mucous concentration and gastric acid concentration among all groups. We conclude that the TRF and tocopherol are equally effective in preventing aspirin-induced gastric lesions. The most probable mechanism is through their ability to limit lipid peroxidation, which is involved in aspirin-induced gastric lesions.

Key words: aspirin, gastric lesions, tocopherol, tocotrienol.

Introduction

Many structurally unrelated chemicals, such as strong acids, alcohol or drugs, damage the gastric mucosa and induce lesions in human and experimental animals.[1] Over 30 million people worldwide use non-steroidal anti-inflammatory drugs (NSAID) daily. Numerous human studies have shown that the use of NSAID is associated with various gastroduodenal mucosal lesions.[2,3] The mechanism by which aspirin and other NSAID produce acute and chronic gastroduodenal mucosal injury are incompletely understood.[4] It has been suggested that the mechanism of aspirin-induced gastric lesion is mediated through lipid peroxidation.[5]

Alpha-tocopherol (vitamin E) is a naturally occurring antioxidant in biological systems and is present in the cell membrane of various tissues, including the intestine and stomach.[6] Vitamin E prevents free radical-induced injury by blocking the free radical chain reaction. The formation of experimental gastric lesions may be achieved through decreasing free radicals and minimizing lipid peroxidation.[5] Previous studies have shown that deficiencies in vitamin E have resulted in peptic ulcerations[7] and that vitamin E supplementation to the diet has protective effects on the gastric mucosa.[8,9]

Serbinova and Packer[10] have shown tocotrienol to be a more potent antioxidant than α-tocopherol. Palmvitee is a vitamin E concentrate from palm oil that contains approximately 80% tocotrienols and 20% tocopherols.[11] This study examines the effectiveness of palmvitee compared to pure α-tocopherol in preventing aspirin-induced gastric lesions in rats.

Materials and methods

Thirty-six male Sprague–Dawley rats (200–250 g) were randomly divided into three groups. The rats were fed either a vitamin E-deficient diet (control), a vitamin E-deficient diet supplemented with tocopherol (300 mg/kg food) or a vitamin E-deficient diet supplemented with TRF (300 mg/kg food). The compositions of the vitamin E-deficient diet are shown in Table 1. Diets supplemented with tocopherol or TRF were prepared by dissolving either 300 mg of tocopherol (equivalent to 360 IU) or TRF in a sufficient amount of acetone, pouring it over 1 kg of diet and allowing the acetone to evaporate. The TRF contains 80% tocotrienol (equivalent to 72 IU) and 20% tocopherol (equivalent to

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Accepted 18 January 2002

72 IU). The control diet was treated with acetone only. After eight weeks of feeding, the control and treated groups received a single intragastric dose of 400 mg/kg body weight aspirin suspended in propylene glycol. Animals were fasted and housed in cages with wide mesh-wire bottoms to prevent coprophagy. The rats were killed 24 h after exposure to aspirin. The variables measured were gastric lesion index ($n = 12$), gastric acid concentration ($n = 6$), gastric mucous concentration ($n = 6$) and gastric malondialdehyde (MDA) content ($n = 6$).

The lower end of the oesophagus and pylorus were clamped and the stomach removed. Samples of gastric juice were collected and centrifuged at 1500 *g* for 10 min. Aliquots of each sample were titrated with 0.01 mol/L NaOH to pH 7.0. The hydrogen ion concentration was calculated as described in 1954 by Shay *et al.*[12] The gastric mucous was then exposed by cutting the stomach along the greater curvature, washed with saline and laid on a flat wooden board. The severity of gastric mucosal lesions, expressed as the ulcer index, was determined semiquantitatively as described in 1988 by Berry *et al.*[13] and graded as follows: 5 = multiple ulcers following almost the entire length of gastric fold; 4 = lesions that followed approximately 80% of the folds; 3 = ulcer 1–4 mm in length on 80% of the folds; 2 = at least two ulcers approximately 2 mm in length; 1 = the presence of one ulcer and generalised erythema; and 0 = no visible damage. The gastric tissue MDA content was measured using the method described in 1986 by Ledwozyw *et al.*[14] Gastric adherent mucous was quantitatively measured by the Alcian blue dye binding method as described in 1974 by Corne *et al.*[15]

This study was approved by the Ethics Committee for animal studies, Faculty of Medicine, University of Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

Table 1. Composition of vitamin E-deficient diet

Ingredient	Concentration
Vitamin-free casein	20.0%
Glucose	66.0%
Corn oil tocopherol stripped	10.0%
Salt mixture	4.0%
Sodium chloride	11.88%
Potassium phosphate dibasic	8.5%
Potassium carbonate	8.75%
Calcium phosphate dibasic	39.11%
Calcium carbonate	18.5524%
Magnesium carbonate	5.89%
Ferric citrate (16–17% Fe)	1.7555%
Manganese sulphate.H_2O	0.41%
Zinc carbonate	0.1%
Copper sulphate.H_2O	0.06%
Sodium selenite	0.000055%
Potassium iodate	0.0021%
Chromium potassium sulphate.$12H_2O$	0.01%
Vitamin A acetate (500 000 IU/g)	1.8 g/kg
Vitamin D_2 (850 000 IU/g)	0.125 g/kg
Ascorbic acid	45.0 g/kg
Inositol	5.0 g/kg
Choline chloride	75.0 g/kg
Menadione	2.25 g/kg
P-Aminobenzoic acid	5.0 g/kg
Niacin	4.25 g/kg
Riboflavin	1.0 g/kg
Pyridoxine hydrochloride	1.0 g/kg
Thiamine hydrochloride	1.0 g/kg
Calcium phentothenate	3.0 g/kg
Biotin	0.02 g/kg
Folic acid	0.09 g/kg
Vitamin B_{12}	0.00135 g/kg

Statistics

Data are expressed as mean ± SEM. Statistical significance ($P < 0.05$) was determined by the Kruskal–Wallis and Mann–Whitney *U*-tests.

Results

Effects of TRF and tocopherol on gastric ulcer index after aspirin administration

The gastric ulcer index was significantly lower in the TRF- and tocopherol-fed groups as compared to control (2.917 ± 0.273 compared with 4.417 ± 0.320 mm, $P = 0.005$ and 3.000 ± 0.323 compared with 4.417 ± 0.323 mm, $P = 0.008$, respectively). However, there was no significant difference in ulcer index between the groups fed TRF compared with those fed tocopherol (Fig. 1).

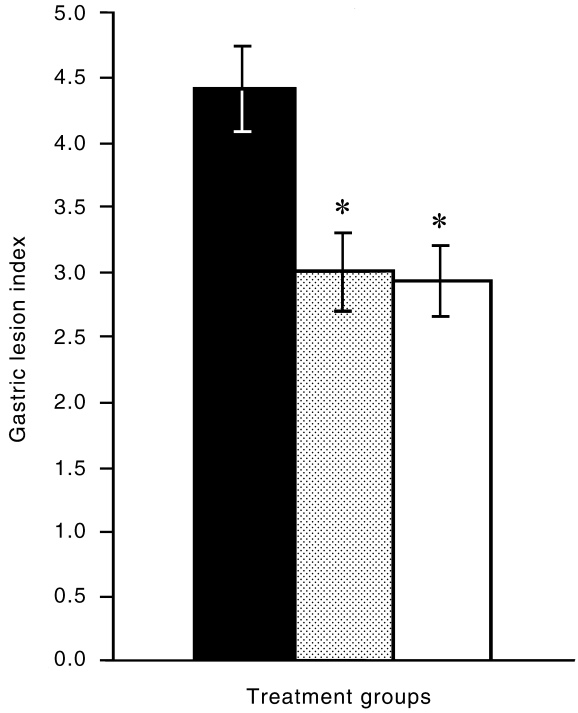

Figure 1. Effect of the tocotrienol-rich fraction (TRF) and tocopherol on gastric ulcer index after 24 h of aspirin exposure (*$P < 0.05$ compared to control). (■), Control animals; (▩), tocopherol-fed group; (□), palmvitee diet group.

Effects of TRF and tocopherol on gastric MDA after aspirin administration

The gastric tissue content of MDA was significantly lower in both the TRF and tocopherol groups compared to the control group (0.105 ± 0.022 compared with 0.314 ± 0.074 nmol/g protein, $P = 0.025$ and 0.110 ± 0.022 compared with 0.314 ± 0.074 nmol/g protein, $P = 0.025$, respectively). However, there was no significant difference in gastric MDA content between the TRF and tocopherol groups (Fig. 2).

Effects of TRF and tocopherol on adherent mucous concentration after aspirin administration

There was no significant difference in the concentration of adherent mucous in the TRF and tocopherol groups compared to the control group after 8 weeks of study. The adherent mucous concentration was 160.856 ± 18.66 compared with 189.893 ± 22.303, $P = 0.34$ and 149.554 ± 23.344 compared with 189.893 ± 22.303, $P = 0.26$, respectively (Fig. 3).

Effects of TRF and tocopherol on gastric acidity concentration after aspirin administration

The gastric acid concentration was numerically lower in the TRF and tocopherol groups compared to the control group at the end of the 8-week feeding period. The gastric acid concentration was 3.346 ± 0.318 compared with 4.319 ± 0.592 /mL, $P = 0.2556$ and 3.468 ± 0.469 compared with 4.319 ± 0.592 /mL, $P = 0.2504$, respectively. However, the difference was not significant (Fig. 4).

Discussion

Previous studies have shown that vitamin E can improve healing of gastric ulcers and reduce inflammation caused by ethanol, NSAID and ischemic reperfusion. The pathogenesis of aspirin-induced gastritis is complex and remains poorly understood. Pihan *et al.* reported that there exists a similarity between aspirin- and ethanol-induced gastric lesion in rats.[9] There is substantial evidence to support the claim that reactive oxygen species are involved in gastric injury caused by ethanol and aspirin exposure. Our earlier work, for instance, has shown that TRF in a dose of 150 mg/kg food given for three weeks to rats was unable to prevent ethanol-induced lesion but did accelerate the healing.[16]

In the present study, we found that the vitamin E groups did not have any significant effect on gastric acid concentration, although there was an initial decrease in gastric acid concentration in the vitamin E-treated groups compared to the control group. This confirms our previous findings in which the gastric acid concentration measured after treatment with TRF administered at 150 mg/kg diet for three weeks did not affect gastric acid concentration.[17]

We also found that vitamin E-supplemented groups did not have an increase in the adherent mucous concentration compared to the control group. It has generally been accepted that gastric adherent mucous confers mucoprotective effects,

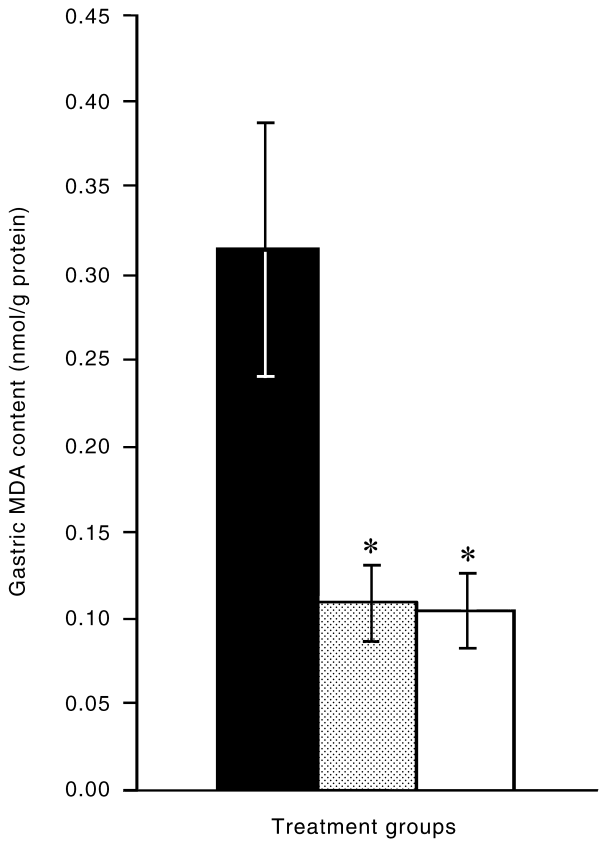

Figure 2. Effect of the tocotrienol-rich fraction (TRF) and tocopherol on gastric malondialdehyde content after 24 h of aspirin exposure (*$P < 0.05$ compared to control). (■), Control animals; (▩), tocopherol-fed group; (□), palmvitee diet group.

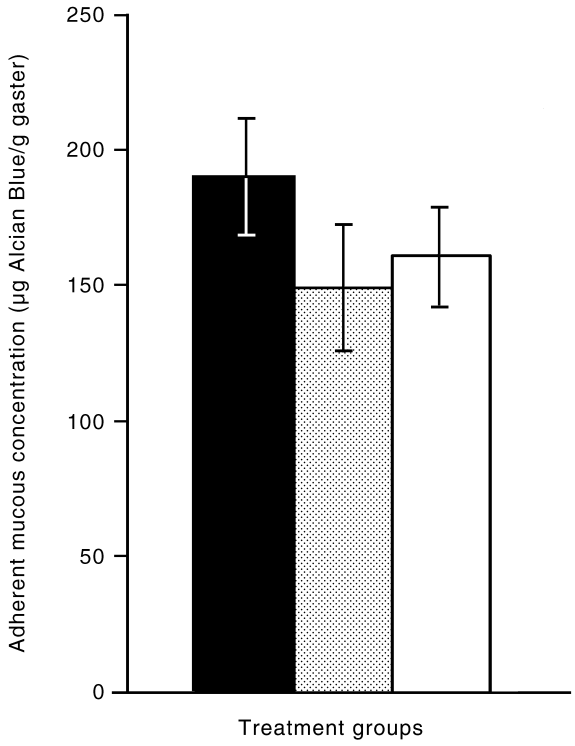

Figure 3. Effect of the tocotrienol-rich fraction (TRF) and tocopherol on adherent mucous concentration after 24 h of aspirin exposure. (■), Control animals; (▩), tocopherol-fed group; (□), palmvitee diet group.

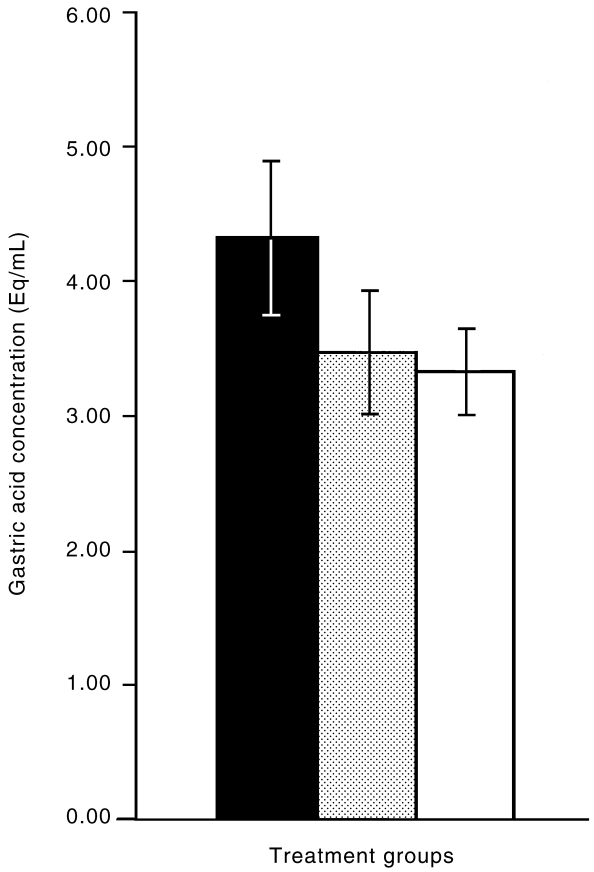

Figure 4. Effect of the tocotrienol-rich fraction (TRF) and tocopherol on gastric acid concentration after 24 h of aspirin exposure. (■), Control animals; (▩), tocopherol-fed group; (□), palmvitee diet group.

functioning as a physical barrier toward an ulcerogen. Although vitamin E does not act as a physical barrier toward an ulcerogen, it is possible that it confers gastroprotective effects through its antioxidant properties. As such, it is possible that the continuous presence of TRF has led to an adaptation of the gastric microenvironment, which recognises that it does not need to produce more gastroprotective agents like the gastric mucous.

In the current study, the gastric tissue content of MDA was significantly reduced in the vitamin E groups compared with the control group. There was, however, no difference between the MDA content in the gastric tissue of the TRF and the tocopherol groups. This indicates that a similar dose of palmvitee confers an equivalent antilipid peroxidation effect on the gastric tissue. In the present study, we found that the mean ulcer index was significantly lower in the groups fed with 300 mg/kg food containing TRF or tocopherol for eight weeks compared with the control group. However, there was no significant difference in the mean ulcer index between the TRF and tocopherol groups. These findings suggest that both palmvitee and tocopherol, at a dose of 300 mg/kg food given for eight weeks before the induction of gastric lesion, are equally effective in preventing aspirin-induced gastric lesions. The findings of the present study do not seem to be in agreement with our previous study, which reported that TRF in a dose of 150 mg/kg food administered over three weeks did not have any protective effects against ethanol-induced gastric lesions. It was, however, reported that this small dose was able to accelerate the healing of the ethanol-induced gastric lesions.[17] The protective effects appear to be dependent on the dose as well as the duration of feeding with a vitamin E-supplemented diet. It therefore appears that a higher dose of vitamin E over a longer period of time (300 mg/kg food given for eight weeks) is required to obtain the preventive effect. In contrast, the healing effect of palm vitamin E can occur at a smaller dose over a shorter duration of time (150 mg/kg food given for three weeks).

The reasons for these discrepancies are not clear. The lower mean ulcer index occurred in the absence of an effect on gastric acid concentration as well as adherent mucous. This indicated that the preventive effect of TRF and tocopherol were not mediated by a reduction in gastric acid secretion nor by interfering with mucous production. The most likely mechanism by which TRF and tocopherol prevented the aspirin-induced gastric lesion was by reducing the lipid peroxidation process as reflected by a significant reduction in gastric MDA content. In conclusion, TRF and tocopherol given at a dose of 300 mg/kg diet for eight weeks were equally effective in the prevention of aspirin-induced gastric lesions.

Acknowledgement. This study was funded by the Government of Malaysia through the Ministry of Science, Technology and Environment (grant code IRPA 06-02-02-0137).

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