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Effect of Tocotrienol on Lipid Peroxidation in Experimental Gastritis Induced by Restraint Stress

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Abstract: Damage to gastric mucosa caused by restraint stress has been attributed to impaired blood flow that resulted in ischemia followed by reperfusion, a process that generates free radicals. Therefore oxygen radicals may mediate the lesions produced in restraint stress. To test this hypothesis, we studied the effect of free radical scavengers on restraint-induced lesions in rats. Forty rats were divided in to four groups of 10 rats. Two control groups was fed with a normal rat diet and two treatment groups fed with a vitamin E deficient diet with either tocopherol or tocotrienol, which serves as free radical scavengers. The two forms of vitamin E were administered orally at 60mg/kg body weight for 28 days. After 28 days, rats from one control group and the two treated groups were subjected to restraint stress 2 hours daily for 4 consecutive days. The rats were killed after the fourth exposure, their stomach isolated and examined for lesions, and gastric malondialdehyde (MOA) content and the gastric reduced glutathione level were measured as an index to reflect the scavenging abilities of tocopherol and tocotrienol. Both the regimes significantly attenuated the total lesion area in the stomach compared to the control. The MOA content was also significantly lower in the rats given tocopherol and tocotrienol supplementation compared to the control and the reduced glutathione levels were preserved in rats supplemented with both tocopherol and tocotrienol. We conclude that it is indeed probable that oxygen radical is involved in the pathogenesis of restraint stress-induced lesions thus supplementation with antioxidant such as vitamin E may be able to reduce or inhibit the formation of these lesions.

Key words: Tocotrienol, tocopherol, restraint-stress, peroxidation, gastric lesions

Introduction

One of the common factors causing gastrointestinal injury is oxygen-derived free radical, which overwhelm the exogenous antioxidant system (Nafeeza *et al.,* 1999; Naito *et al.,* 1993). Agents with ability to catalytically reduced free radical or act as antioxidant had been shown to protect the gastric mucosa against a variety of noxious stimuli (Izgut-uysal *et al.*, 2001, Hirota *et al.*, 1990).

Oxygen-derived free radicals are cytotoxic and mediate tissue damage by injuring cellular membranes and releasing intracellular components (Salim, 1990). Although it is widely accepted that the pathogenesis of gastric mucosal lesions involves oxygen-derived free radicals, the role of lipid peroxidation induced by stress remains uncertain. Among various stressor used in animals, one of the most reproducible results can be obtained by restraint stress (Hayase and Takeuchi, 1986; Ainsah *et al.*, 1999), which lead to the formation of gastric lesions.

Vitamin E is a naturally occurring antioxidant available in the biological system. It was postulated that tocotrienol are more mobile and less restricted in their interactions with lipids radicals in the membrane than tocopherol (Serbinova *et al.,* 1991). The biological activity of vitamin E is believed to be due to its antioxidant action to inhibit lipid peroxidation in biological membranes by scavenging the peroxyl chain reaction. Studies had shown that tocotrienol to be a more potent antioxidant compared to tocopherol (Serbinova and Packer, 1994).

The effect of tocotrienol and tocopherol on oxidative stress could account for the beneficial effect of this vitamin in model of stress injury. Vitamin E is known to have a scavenger effect on reactive oxygen species and a stabilizing effect on damaged cell membrane. To confirm the hypothesis of the involvement of lipid peroxidation and vitamin E in stress, rats were subjected to restraint stress and stomach was examined for lesions and oxidative damage.

Materials and Methods

Male *Sprague-Dawley* rats (n = 40) were divided into four equally sized groups. The two control groups was fed with normal rat diet (RC) while the treatment group received a vitamin E deficient (VED) diet with oral supplement of tocotrienol (TT) or alpha-tocopherol (TF) at 60mg/kg body weight for 28 days. At the end of the treatment period the rats from one control group (stress control) and both treated groups were exposed to restraint-stress. Stress-induced gastric lesions, gastric

malondialdehyde level, gastric glutathione level and gastric prostaglandin E_2 (PGE_2) content were measured in all rats. The measurement was done immediately after the rats were killed. Palm sources of tocotrienol, which was a combination of all isomers as present in palm oil, supplied by Carotech Sdn. Bhd. was used in this study. Alpha-tocopherol was supplied by Sigma.

All rats were kept on a regular night/day cycle, with natural light for a period of 10 hours (0700 to 1700 h). Throughout the feeding period all rats were habituated to handling to reduce their stress-related disturbances. The rats were housed in large cages with wide wire-mash bottoms to prevent coprophagy. Food and water were given ad libitum throughout the experiment. The Animal Care and Use Committee (ACUC) of the Faculty of Medicine, National University of Malaysia, had approved this study (*approval number*: FAR/2000/NAFEEZA/30-NOVEMBER/031).

Rats were restrained by placing them in individual plastic restrainer measuring approximately 17 x 5-cm,[7] for two hours daily for 4 consecutive days. Following the restraining procedure on the fourth day, the rats were killed. The stomach was opened along the greater curvature and examine for lesions.

The macroscopic assessment of stress-induced gastric lesions in the gastric mucosa was performed by two independent examiners who were blinded to the treatment that the rats received. The assessment of lesions were done according to a semi quantitative scale. The scale used was as followed 5 = generalized hemorrhage covering more than 90% of the gastric mucosa, 4 = hemorrhage covering 60-90% of the gastric mucosa, 3 = hemorrhage covering 30-60% of the gastric mucosa, 2 = hemorrhage covering 10-30% of the gastric mucosa, 1 = generalized erythema with present of hemorrhage, and 0 = no visible lesion.

The content of malondialdehyde (MDA) in the stomach was determined using method described by Ledwozyw *et al.*, 1986. The gastric tissue content of protein was determined by the Lowry *et al.* (1951) method and MDA was expressed in terms of gram protein. Measurements of gastric glutathione content were done following method by Griffith and Meister, 1979. Sample preparation for prostaglandin E_2 (PGE_2) assay was done following method previously described by Redfern *et al.*, 1987. Prostaglandin E_2 was measures using Enzyme Immuno Assay (EIA) kit (RPA 530, IBL Hamburg).

Results are expressed as mean ± SEM. Statistical significance ($P<0.05$) was determined by ANOVA or student's t-test for parametric analysis and Kruskal Wallis or Wilcoxon Signed Test for non-parametric analysis where appropriate.

Results

Rats exposed to restraint stress for 2 hours a day for 4 consecutive days shows presence of considerable ulcerogenicity in the form of hemorrhagic mucosal lesions confined to the corpus (glandular part of the stomach). As shown in Fig. 1, the gastric lesions index in the stressed control (SC) group was higher by 46% ($P=0.0068$) compared to the TT group and 31% ($P=0.049$) compared to TF group, this findings indicates that vitamin E both tocotrienol and tocopherol are able to reduce the formation of stress-induced gastric lesions. Rats killed after the 28 days feeding period and not exposed to stress had either no gastric mucosal lesion or just erythema in the gastric mucosa. The gastric lesion index in the control group is 8.4 fold higher compared to the non-stressed rats in the same group.

As shown in Fig. 2, treatment of rats with both TT ($P=0046$) and TF ($P=0.0042$) individually causes a significant reduction in gastric MDA level compared to stress control. Stress causes an increased in gastric Thiobarbituric Reactive Substance (TBARS) as indicated by the increased of gastric MDA content.

Reduced glutathione (GSH) is the major endogenous antioxidant in life organism (Inoeu *et al.* 1987). The results are expressed by the ratio of GSH to the oxidized form of glutathione (GSSG). Exposure to restraint stress for 2 hours a day for 4 consecutive days resulted in a significant reduction of gastric glutathione level by 26.3% ($P=0.027$) in the stressed control compared to the non-stress control (NSC) group as shown in Fig. 3. Rats treated with either tocotrienol or tocopherol showed no significant different in the gastric glutathione level compared to the non-stress control. The finding suggest that vitamin E can restore a normal gastric glutathione level which was altered by stress.

The mean gastric PGE_2 content in all group studied was not significantly different ($P>0.05$), as shown in Fig. 4. The findings suggests that stress does not alter the gastric PGE_2 content and supplementation with tocotrienol or tocopherol does not change the gastric PGE_2 content in stressed rats.

Discussion

The finding shows that rats exposed to restraint stress for 2 hours daily for 4 consecutive days developed gastric mucosal lesion at the glandular part of the stomach. The lesion is in the form of hemorrhage and generalized erythema. The results of this study demonstrates that pretreatment of rats with vitamin E either tocotrienol or tocopherol individually markedly reduce gastric mucosal damage induces by stress. However no different between this two agents was observed, showing equal effectiveness in preventing stress-induced gastric injury.

It is well known that gastric mucosa is continuously exposed to harmful factors. Destruction and protective capacity should be in balance to maintain functional integrity of the gastric mucosa. Among the various hazardous effects on biological system, oxidative

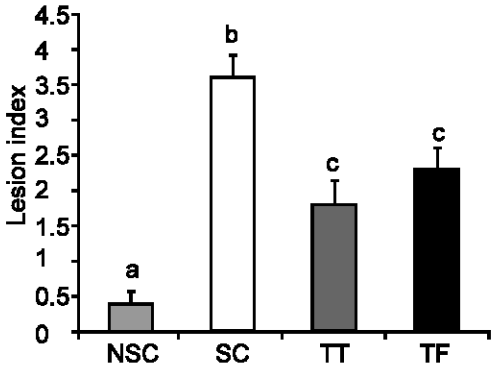

Fig. 1: Effects of TT and TF on lesion index in rats exposed to restraint-stress. Mean lesion index with or without exposure to restraint-stress in control rats and rats supplemented with TT or TF. Different letters between bars indicate significant difference (P<0.05).

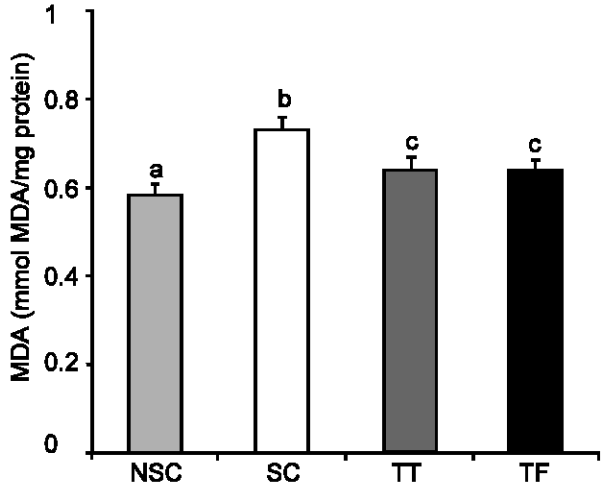

Fig. 2: Effects of TT and TF on gastric MDA level in rats exposed to restraint-stress. Mean MDA level with or without exposure to restraint-stress in control rats and rats supplemented with TT or TF. Different letters between bars indicate significant difference (P<0.05).

destruction of membrane polyunsaturated fatty acid or more commonly known as lipid peroxidation has been observed in numerous tissue (Izgut-uysal *et al.*, 2001). The failure of the endogenous antioxidant defense system was attributed to the stress-induced generation of free radicals. We observed a significant depletion of glutathione content in the gastric mucosa following exposure to stress. The treatment of rats with either tocotrienol or tocopherol significantly attenuated stress-induced depletion of gastric mucosal glutathione.

Studies had shown that reduced glutathione, a major endogenous non-protein sulfhydryl compound in the

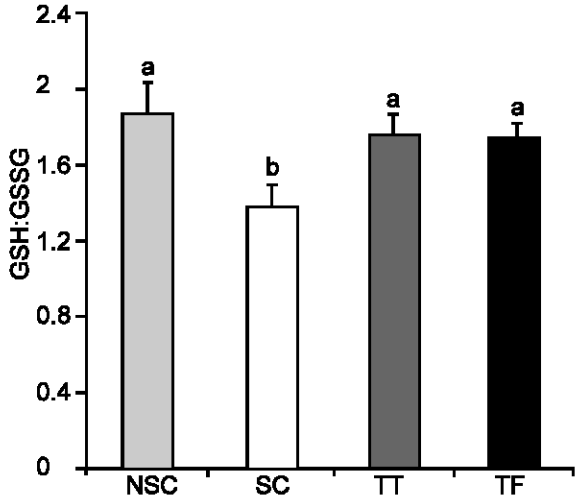

Fig. 3: Effects of TT and TF on gastric glutathione level in rats exposed to restraint-stress. Mean gastric glutathione level with or without exposure to restraint-stress in control rats and rats supplemented with TT or TF. Different letters between bars indicate significant difference (P<0.05).

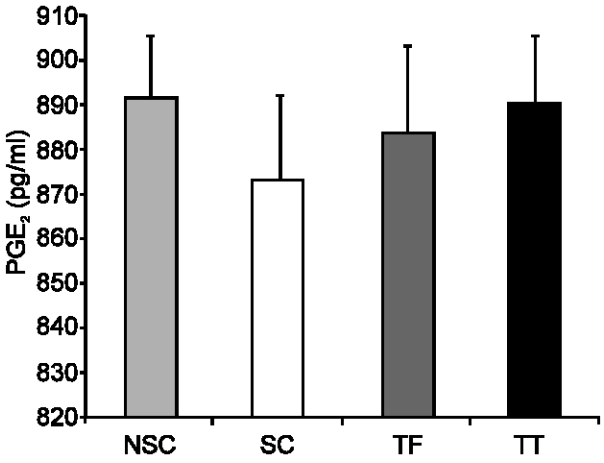

Fig. 4: Effects of TT and TF on gastric prostaglandin E_2 content in rats exposed to restraint-stress. Mean gastric prostaglandin E_2 content with or without exposure to restraint-stress in control rats and rats supplemented with TT or TF. No significant difference was observed.

stomach, plays an important role in the formation of gastrointestinal mucosa mucus, which protect the underlying gastric mucosa against acid secretion, pepsin and exogenous necrotizing agents (Stein *et al.*, 1990, Szabo *et al.*, 1987). Hirota *et al.* (1989) found that intraperitoneal injection of reduced glutathione significantly increases plasma level of glutathione and inhibit the occurrence of gastric injury induced by stress.

The decrease in gastric mucosal glutathione content with the development of stress-induced gastric mucosal lesions shows the important of free radicals in causing gastric injury in stress, thus prevention using exogenously administration of vitamin E seems to be a logical alternative to the prevention of such injuries.

In this study, the high gastric MDA content in the stressed control stomach support the hypothesis that stress-induced injury is mediated by lipid peroxidation process. This indicates that reactive oxygen species and lipid peroxidation is important in the pathogenesis of gastric mucosal injury induced by stress. We also showed that vitamin E decreased the breakdown of gastric mucosal barrier by reducing the product of lipid peroxidation (MDA). The reduced MDA levels accompanying the improved gastric lesions in these groups suggest that vitamin E probably reduced injury by retarding the lipid peroxidation process. The findings also showed no difference in the ability to reduced gastric MDA content or maintain the GSH level between tocotrienol and tocopherol, suggesting the a similar radicals scavenging ability.

Although the result showed an increased in the gastric PGE_2 content when rat are exposed to restraint stress, the findings was not significant. Both tocotrienol and tocopherol in the dose used in this study, did influence the gastric PGE_2 content towards the non-stress level but significant difference was not observed. The findings suggest that PGE_2 does not play a major role in the stress induced injuries, where this parameter was only slightly altered by stress.

In conclusion, our data suggest that vitamin E, both tocotrienol and tocopherol intake prevents the occurrence of gastric mucosal lesions by strengthening the gastric mucosal barrier against stress-induced elevation of lipid peroxidation. The protective effect of vitamin E is related to a decrease in lipid peroxidation and prevention of the reduction in gastric glutathione content produced by the harmful effects of stress.

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