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Tocotrienol and α-tocopherol reduce corticosterone and noradrenalin levels in rats exposed to restraint stress

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This study investigates the effects of tocotrienol (TT) or α-tocopherol (TF) supplementation on corticosterone level, noradrenalin level and gastric lesions in rats exposed to restraint stress. Twenty-four male Sprague Dawley rats were randomly assigned into 4 equally sized groups; two control groups were given olive oil, while the treated group was supplemented with either tocotrienol of tocopherol orally at a dose of 60 mg/kg body weight. After 28 days of treatment, one control group, TT group and TF group were subjected to restraint stress, 2 hours daily for 4 consecutive days. After the last exposure to stress, plasma samples were taken to determine the corticosterone and noradrenalin levels, after which the rats were sacrificed. The stomach was excised for the evaluation of gastric lesions. Our findings showed that TT and TF were able to maintain the corticosterone level to the prestress values, while only TT was able to maintain the noradrenalin level in rats exposed to stress. Tocotrienol was found to be better in preventing formation of gastric lesions compared to TF. As a conclusion, the protective effect of vitamin E was related to the ability to inhibit stress induced elevation of corticosterone and noradrenalin levels.

1. Introduction

Physical and emotional stress does not only cause behavioral changes but also stimulates the neuroendocrine system (Lombardi 1994; Child and Unabia 1990). Adrenocorticotropin hormones (ACTH) are secreted simultaneously from the pituitary in response to stress (Charmandari et al. 2005). The adrenocorticotropin hormone then stimulates the adrenal gland to secrete corticosteroid or cortisol, which in turn inhibits the ACTH secretion via negative feedback (Dronjak et al. 2004; Bhatia and Tandon 2005).

Various responses to stress exposure are important for human and animal survival. However repeated activation of responses to stress as well as sustained activation will cause an overexposure to stress hormones, thereby increasing the risk for various health problems (Lundberg 2005). It has been established that acute stress causes an increase in blood corticosteroid level and produces gastric ulceration in rats (Dai and Chan 1982; Ainsah et al. 1999). However, it is difficult to relate the increased corticosteroid secretion to stress induced lesions formation because adrenalectomy had been reported to either increase (Brodie and Hanson 1960) or inhibit (Sethbhadki et al. 1970) the production of stress-induced lesions. Re-evaluations of the role of endogenous glucocorticoids released during stress showed that it has a gastroprotective action rather than an ulcerogenic effect as was generally accepted (Filaretova et al. 1998).

In the presence of stress, activation of the hypothalamic-pituitary-adrenal axis (HPA) and sympatheto-adrenal-medullary (SAM) systems causes the release of not only cortisol but also catecholamines and endorphins. These also cause increase in fatty acids and glucose (Ainsah et al. 2001) which may result in free radicals formation (Cariello 2000). Oxidation of catecholamines during stress leads to the formation of quinones and semiquinone which are free radicals that have the abilities to generate more free radicals (Graham 1979).

Oxygen-derived free radicals are cytotoxic and mediate tissue damage by injuring cellular membranes and releasing intracellular components. 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 stressors used in animals, the most reproducible results can be obtained by restraint stress (Hirota et al. 1990; Salim 1990; Nur Azlina et al. 2007; Brzozowski et al. 2000) which lead to the formation of gastric lesions.

The effect of tocotrienol and tocopherol on oxidative stress could account for the beneficial effect of this vitamin in model of stress induced gastric injury. Vitamin E is known to have a scavenging effect on reactive oxygen species and a stabilizing effect on damaged cell membrane. To confirm the hypothesis of the involvement of lipid peroxidation and the role of corticosterone and catecholamine release in stress-induced gastric lesion, rats were subjected to restraint stress, blood was taken and the stomach was examined for lesions.

2. Investigations and results

Rats exposed to restraint stress for 2 h a day for 4 consecutive days showed considerable lesions in the form of

Table: Effects of TT and TF on lesion formation in rats exposed to restraint-stress

Groups	Non-stressed Control (NSC)	Stressed Control (SC)	Tocotrienol (TT)	Tocopherol (TF)
Lesions	0%	83%	0%	33%
Without Lesion	100%	17%	100%	67%

Percent of rats with lesion, with or without exposure to restraint-stress in control groups and rats supplemented with TT or TF

mucosal hemorrhage confined to the corpus (glandular part of the stomach). As shown in the Table, the percentage of rats with gastric lesions in the stressed control (SC) group was 100% as compared to 0% in the TT group and 33% in the TF group. These findings indicate that both tocotrienol and α-tocopherol are able to reduce the formation of stress-induced gastric lesions. Rats sacrificed after the 28 days feeding period and not exposed to stress had no gastric mucosal lesion.

Corticosterone levels as predicted was 18.6% higher ($p = 0.028$) in the stressed control rats compared to their pre-stress values, as shown in Fig. 1. In the tocotrienol and α-tocopherol treated groups, there was no significant difference in the pre- and post-stress values of the plasma corticosterone levels. The finding suggests that treatment with both forms of vitamin E can block stress induced increase in corticosterone levels.

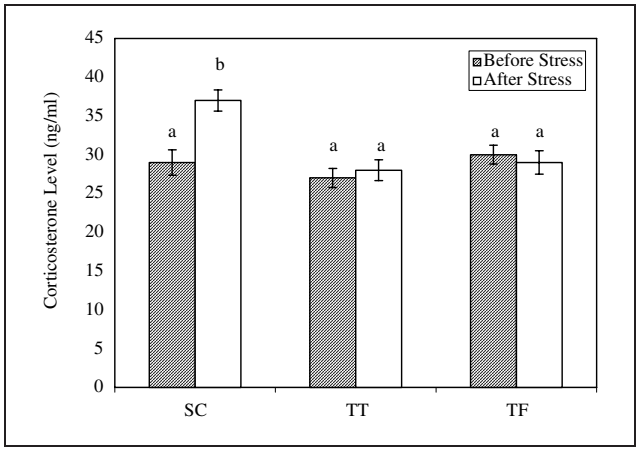

Fig. 1: Effects of TT and TF on plasma corticosterone levels in rats exposed to restraint-stress. Plasma corticosterone levels with exposure to restraint-stress in control rats (SC) and rats supplemented with TT or TF. Different letters between bars indicate significant difference ($P < 0.05$)

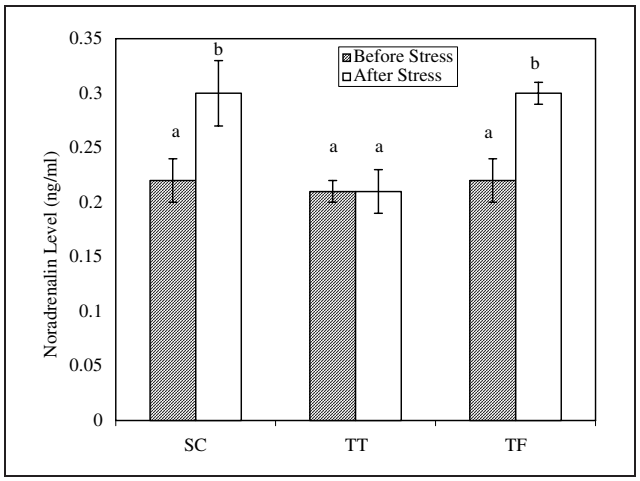

Fig. 2: Effects of TT and TF on plasma noradrenalin levels in rats exposed to restraint-stress. Plasma noradrenalin levels with exposure to restraint-stress in control rats (SC) and rats supplemented with TT or TF. Different letters between bars indicate significant difference ($P < 0.05$)

The noradrenalin levels were also increased due to stress. But unlike the findings with the corticosterone levels, only tocotrienol was able to inhibit the increased in the noradrenalin levels. The plasma noradrenalin levels in the α-tocopherol group were not significantly different from those in the stressed control group, as shown in Fig. 2.

3. Discussion

The results of this study demonstrates that pretreatment of rats with vitamin E either tocotrienol or α-tocopherol individually markedly reduce gastric mucosal damage induced by stress. The lesion was in the form of hemorrhage and generalized erythema. We also found that there was a difference between these two agents where a few rats in the α-tocopherol group developed lesions, while all the rats in the tocotrienol treatment group were completely protected against stress-induced gastric injury.

Gastric ulceration and corticosteroid had been discussed in many contexts. Studies had shown that high doses of glucocorticoids increase the incidence of gastric ulcers (Jacobs and Bijlsma 1993; Black 1988). These findings lead to the conclusion that the stress induced increase in corticosteroid levels is also an ulcerogenic factor (Murphy et al. 1979).

In the present study, we found that the increase in corticosterone levels was correlated to the increased incidence of gastric lesions as seen in the stressed control rats. We also observed that supplementation of both tocotrienol and α-tocopherol, resulted in corticosterone levels similar to the non-stressed values. This ability to maintain a normal corticosterone level in stress is associated with the ability of these agents to block the stress-induced formation of gastric lesions. Similarly, Ainsah et al. (1999) found that α-tocopherol supplementation was able to block the increase in corticosterone in rats exposed to repetitive restraint stress while their locomotor activities were normal compared to the untreated rats. These finding suggests that vitamin E does not only act as an antioxidant but may also have effects on stress hormones. Pretreatment of rats with naloxone, an opioid antagonist, reduces the plasma levels of corticosterone following stress (Welt et al. 2006), it is possible that vitamin E inhibits the release of ACTH and thereby reduce the levels of plasma corticosterone. Vitamin E may also reduce the response to stress by reducing the stress-induced radical formation from adrenal catecholamines resulting in less corticosterone released (Ainsah et al. 2001).

Immobilization stress has been shown to increase plasma catecholamine levels (Bodnar et al. 2004; Perveen et al. 2003). Catecholamines are significantly involved in the regulation of homeostasis of the organism at rest and especially during stressful situations. Stress induces the increase in both plasma adrenalin and noradrenalin levels. Hamada et al. (1993) found that rats exposed to stress produce gastric lesions associated with reduced brain noradrenalin content and increased plasma catecholamine and corticosterone levels. Similarly in our study, rats exposed to restraint stress had a higher level of plasma noradrenalin compared to the non-stressed rats. The increase in the noradrenalin level was blocked when the rats received toco-

trienol supplementation but not in rats receiving α-tocopherol. These findings suggest that tocotrienol but not α-tocopherol is potent in blocking the effects of stress. The ability of tocotrienol to inhibit stress induced increase in noradrenalin correlates with its ability to block the formation of lesions in rats exposed to stress. The inability of α-tocopherol supplementation to totally block the incidence of stress-induced gastric lesions could partly be due to its inability to block the effect of stress at the higher level.

In conclusion, our data suggests that supplementation with vitamin E, both tocotrienol and α-tocopherol, may be beneficial in preventing the occurrence of stress-induced gastric mucosal lesions. The protective effect of vitamin E was related to the ability to block stress induced increase in corticosterone levels. We also found a significant difference between the two forms of vitamin E, where tocotrienol was more effective in blocking the effect of stress by maintaining normal noradrenalin levels leading to their ability to totally block the formation of lesions in these rats. The study opens the door for further investigation on the mechanism of how tocotrienol and α-tocopherol reduce the corticosterone levels in rats exposed to stress.

4. Experimental

Male Sprague-Dawley rats ($n = 24$) were divided into four equally sized groups. Blood samples from the orbital sinus were taken from each rat for baseline measurement. Two control groups were fed with normal rat diet (RC) while the treatment groups received the same diet but with oral supplement of tocotrienol (TT) or α-tocopherol (TF) at 60 mg/kg body weight for 28 days. Tocotrienol used in this study contains a mixture of tocotrienol isomers which was obtained from Hovid Sdn. Bhd. The dose chosen was based on previous studies which had shown a protecting effect of tocotrienol and α-tocopherol on stress-induced gastric lesions (Nur Azlina et al. 2005a, 2005b). Tocotrienol and α-tocopherol were given in olive oil which acts as the vehicle and was administered by oral gavage using an 18G gavage needle. The control groups were sham administered with olive oil orally. At the end of this treatment period, the rats from one control group (stressed control) and both of the treated groups were exposed to restraint-stress. After the last exposure to stress, plasma samples were taken to determine corticosterone and noradrenalin levels, after which the rats were sacrificed. Plasma samples for the baseline and post stress exposure were taken at a similar time which was between 8 am and 9 am to avoid variability in the samples due to the fluctuating hormone levels. The dissected stomach was taken for evaluation of gastric lesions. The measurement was done immediately after the rats were sacrificed by an overdose of anesthesia.

All rats were kept on a regular night/day cycle, with natural light for a period of 10 h (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. Prior ethical approval was obtained from The Animal Care and Use Committee (UKMAEC) of Universiti Kebangsaan Malaysia (*approval number*: FAR/2006/AZLINA/12-JULY/129).

Rats were restrained by placing them in individual plastic restrainer measuring approximately 17×5-cm for 2 h daily for 4 consecutive days, as previously described by Ainsah et al. (1999). Following the restraining procedure on the fourth day, blood was collected, after which the rats were killed. The stomach was dissected 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. A qualitative assessment of lesions was performed. The data collected involved either presence of lesions or no visible lesion observed.

Measurement of plasma corticosterone level was performed using Enzyme-Link Immunosorbent Assay (ELISA) kit (UK 450 11, IBL Hamburg). While the plasma noradrenalin levels were measured using Enzyme-Link Immunosorbent Assay (ELISA) kit (RE59261, IBL Hamburg).

Statistical analysis was carried out using the SPSS statistical package version 12 (SPSS Inc. USA). Normal distribution of all variables was examined by Kolmogrov-Smirnov test. The results showed that all variables were normally distributed. The results are expressed as mean + SD. Statistical significance ($P < 0.05$) was determined by ANOVA and Tukey's post-hoc test.

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