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Protective Effects of Vitamin E Selenium and Allopurinol Against Stress-induced Ulcer Formation in Rats

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Abstract: This study was carried out in the Health Research Center (DÜSAM) laboratory of Dicle University. Vitamin E, Selenium and Allopurinol were given to counteract gastric damage caused by stress in rats. In the study 64 male Sprague dawley rats were used. They were divided into 8 groups, and protective agents were applied at different doses for 8 weeks. Then, performing cold-restraint stress stomach tissues were taken out and investigated macroscopically to determine mucosal damage.

Lipid peroxidation levels in the stomach specimens were determined by identifying Malondialdehyde (MDA) levels by the thiobarbituric acid method. These agents have shown protective effects against cold-restraint stress. Evaluating macroscopic examinations of stomach specimens and considering MDA levels determined this. The results obtained from the Vit-E (10 mg/kg/day), Allopurinol and Selenium groups were found to be statistically significant when compared to the control group ($p<0.05$). The most significant results were obtained from the Vit-E + Allopurinol group ($p<0.01$). In conclusion, to prevent stress-induced gastric mucosal damage and to reduce lipid peroxidation products, we are of the opinion that separate or combined administration of Vit-E, Selenium and Allopurinol as protective agents will be useful and that further studies are needed on this matter.

Key Words: Vit-E, Selenium, Allopurinol and Stress-induced ulcer

Introduction

Lipid peroxidation is a chemical process started by free radicals, resulting in polyunsaturated fatty acid oxidation (PUFA) in the membrane structure (1). It is known that lipid peroxidation leads to many pathologies caused by stress and chemical agents (2, 3). Lipid peroxidation formation by stress and chemical agents results in a reduction in the fluidity of the membrane, degradation of membrane functions, inactivation of membrane receptors and enzymes and an increase in non-specific biological membrane permeability to Ca^{2+} ions (1, 4. 5). In order to prevent damage caused by stress-induced lipid peroxidation, it has been proved useful to give enzymes such as superoxide dismutase, catalase, glutathione peroxidase as well as antioxidants such as Vit-E, Selenium and Allopurinol before stress (6, 7, 8).

In this study, our main purpose was to determine the effects of Vit-E, Selenium and Allopurinol as protective agents on Malondialdehyde (MDA) levels, which are known to be an indicator of gastric mucosal damage caused by cold-restraint stress.

Materials and Methods

This study was carried out in the Health Research Center (DÜSAM) laboratory of Dicle University. In the study, 64 male Sprague dawley rats, each weighing 200 ± 50 g, were used. They were divided into 8 groups, each consisting of 8 rats and the following processes were performed for 8 weeks.

CONTROL GROUP: No application was done in this group.

VITAMIN E GROUPS: Vit-E at doses of 3, 10 and 100 mg/kg/day as deep i.m. injections was given to the rats for 8 weeks. Ephynal Amp. was used as the source of Vit-E (so 3 different Vit-E groups were formed).

SELENIUM GROUP: Na_2SeO_3; $5H_2O$ at a dosage of 350 mg/kg/week was given to rats by i.m. injections for 8 weeks.

ALLOPURINOL GROUP: 50 mg/kg/day s.c. Allopurinol was administrated during the last 5 days of the experimental procedures (Allopurinol was dissolved in equimolar NaOH solution).

VIT-E + SELENIUM GROUP: In this group 350 mg/kg/week of Na_2SeO_3; 5H2O i.m. injections were added to 10 mg/kg/day Vit-E i.m. injections for 8 weeks.

VIT-E + ALLOPURINOL GROUP: In this group, during the last 5 days, 50 mg/kg/day Allopurinol s.c. injections were added to 10 mg/kg/day Vit-E i.m. injections for 8 weeks.

In order to prevent coprophagy 24 hours prior to the experiments, male Sprague dawley rats placed in a special cage and were given nothing but water. On the day of the experiment the rats were put in pocket-like restraint cages of wire mesh. The cages were reduced in a size to ensure that the rats could not move. Then stress was induced 3 hours at 4ºC. After that, the rats were taken out of the cages and decapitated under Ketamine anesthesia. To wash out blood from the tissues; perfusion was performed with 0.9% NaCl by aorta, and then the stomach was dissected out. The stomach was opened along the greater curvature and the tissue was examined macroscopically in order to determine mucosal damage. Each lesion was measured along its greatest diameter (as mm).

Each five petechiae were considered equivalent to a 1 mm long ulcer. Ulcer index was determined as the ratio of total lesion length to the number of rats in the groups (9). Stomach tissues were investigated by the thiobarbituric acid method to determine Malondialdeyde (MDA) levels, which are accepted as an indicator of lipid peroxidation (10, 11). Stomach specimens, each weighing 0,5 g, were put into plastic tubes, and 4.5 ml of 5.5% trichloroacetic acid was added and then they were homogenized in a homogenizer (20,000 rpm) to determine MDA levels by thiobarbituric acid method. Then, they were centrifuged at 4,000 rpm for 10 minutes, and 1 ml of 0.67% thiobarbituric acid (sigma T.5,500) was added to supernatant and heated at 100ºC for 10 minutes. The mixtures were left to cool and absorbent values were read via a spectrophotometer (Shimadzu UV-1201) at 532 nm. By using the following formula, the MDA concentration value in mol/l was calculated as nmol/g stomach tissue (10, 11):

$C=A/E$

Where

C = Concentration (mol/l)

A = Absorbent

$E = 1.56*10^5$ (Malondialdehyde Molar Extinction Coefficient)

For determining the meaning of differences between groups the Mann-Whitney-U test was used; when p was ≤ 0.5 the differences were considered statistically significant (12).

Results

MDA levels were found to be 33.54 nmol/g tissue in the control group, 13.55 nmol/g tissue in the Vit-E group (3 mg/kg/day), 10.03 nmol/g tissue in the Vit-E group (10 mg/kg/day), 20.89 nmol/g tissue in the Vit-E group (100 mg/kg/day), 14.41 nmol/g tissue in the Selenium group, 12.74 nmol/g tissue in the (10 mg/kg/day) Vit-E + Selenium group, 17.98 nmol/g tissue in the Allopurinol group and 9.36 nmol/g tissue in the (10 mg/kg/day) Vit-E + Allopurinol group.

The results of comparisons between the groups are presented in Table 1.

Table 1. Differences in stomach tissue MDA levels (nmol/g tissue) between cold-restraint stress applied control and experimental groups of rats.

Groups	Median		P
Control and Vit-E (3 mg/kg/day)	Control Vit-E	M=33.54 M=13.55	P<0.05
Control and Vit-E (10 mg/kg/day)	Control Vit-E	M=33.54 M=10.03	P<0.05
Control and Vit-E (100 mg/kg/day)	Control Vit-E	M=33.54 M=20.89	P<0.05
Control and SEL	Control SEL	M=33.54 M=14.41	P<0.05
Control and Vit-E+SEL (10 mg/kg/day)	Control Vit-E+SEL	M=33.54 M=12.74	P<0.05
Control and Allopurinol	Control Allopurinol	M=33.54 M=17.89	P<0.05
Control and (10 mg/kg/day) Vit-E+Allopurinol	Control Vit-E+Allo.	M=33.54 M= 9.36	P<0.01

When cold-restraint stress-induced gastric lesions were examined, it was seen that 8 week daily 3 mg and 100 mg i.m. doses of vitamin E before stress could not prevent gastric ulcers, whereas 8 week daily 10 mg i.m. Vitamin E application was found to be effective in preventing gastric ulcer when compared to the control group ($p<0.05$).

The Allopurinol, Selenium, Vit-E+Allopurinol and Vit-E+Selenium groups were also determined to be effective in preventing gastric lesions in comparison to the control group ($p<0.05$). The results are shown in Table 2. In this stage Studet's t test was used for statistical analyses.

Table 2 . The effects of Vit-E, Allopurinol, Selenium, Vit-E + Allopurinol and Vit-E + Selenium on gastric ulcer induced by cold-restraint stress in rats.

APPLICATION	ULCER INDEX (mm)
Control	Excessive Ulceration and Hemorrhage
Vit-E 100 mg/kg/day	Excessive Ulceration and Hemorrhage
Vit-E 3 mg/kg/day	Excessive Ulceration and Hemorrhage
Vit-E 10 mg/kg/day	21.72±3.18
Allopurinol	60.86±2.15
Selenium	1.26±0.35
Vit-E + Allopurinol	5.96±2.38
Vit-E + Selenium	5.53±2.74

Discussion

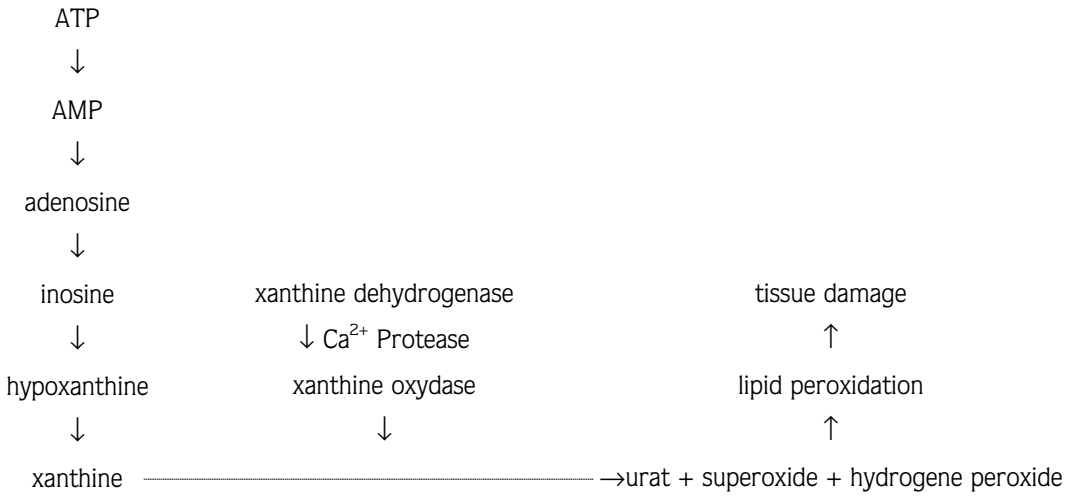
Free radicals affect all cells in the organism and lead to lipid peroxidation. This can be controlled, and its damage can be reduced by antioxidants in the plasma and tissues (15). MDA is a product of lipid peroxidation that is an indicator of free radical damage (1). MDA, as an indicator of lipid peroxidation, is also accepted as the basic indicator of tissue damage in the hearth, lungs, kidney, small intestine and stomach (1, 13, 14).

In this study, we used Allopurinol (which is a specific inhibitor of xanthine oxidase), Vit-E and Selenium, whether separately or in a combination, against gastric mucosal damage caused by free radicals from cold-restraint stress. Stress causes mucosal alterations and gastric motility (2). It may lead to the formation of lipid peroxidation, a decrease in membrane fluidity, inactivation of membrane receptors and enzymes, and an increase in nonspecific permeability against ions such as Ca^{2+} (2, 4, 5) and so degradation of membrane functions. Calcium is an important second messenger for the secretion of histamine in the mast cells of gastric mucosa (16). Histamine was shown to stimulate gastric acid secretion and to be an essential factor in the pathogenesis of stress-induced ulcers (17). It was also suggested that stress causes a rapid decrease in glutathione levels in gastric mucosa (18). And it is well known that reduced glutathione serves as an important radical scavenger in the organism.

In this study, we investigated the effects of Vit-E, given as an antioxidant, on gastric mucosa. We observed that the application of 10 mg/kg/day Vit-E produced more protective effect when compared with other dosages of it (3 mg and 100 mg/kg/day). The MDA levels, which are the last product of lipid peroxidation, decreased significantly in all groups with respect to the control group ($p<0.05$). Previously, Vitamin E has been shown to prevent from gastric mucosal damage and to have anti-ulcer activity (2). Stress inhibits prostaglandin synthesis, whereas Vitamin E stimulates it. Vitamin E carries out this by inhibiting lipooxigenases and activating phospholipase A_2 enzymes (19, 20). It was also reported that Vitamin E has an antioxidant effect by scavenging free radicals and preventing a decrease in reduced glutathione levels.

In the study, Vitamin E was given before cold-restraint stress, so possibly because of an increase in prostaglandin levels, a decrease in damage produced by free radicals and/or an increase in reduced glutathione levels, gastric cells maintained their integrity. Thus gastric mucosal damage was reduced.

Free oxygen radicals, such us superoxide anion and its active compounds, initiate lipid peroxidation. Under anaerobic conditions and during hypoxy, cell functions still continue (21). But in these conditions, because of shortage of oxygen and nutrient supplement, ATP formation decreases and consequently adenosine, inosine and hypoxantine levels increase. In that environment, xanthine dehydrogenase is transformed into the xanthine oxidase form of that enzyme. Xanthine oxidase is an enzyme responsible for the production of free oxygen radicals (22).



Allopurinol and its metabolite oxipurinol have a protective role against free radical-induced damage by inhibiting xanthine oxidase (23). Allopurinol decreases the formation of xanthine oxidase products, and thereby reduces both the cell necrosis and increased microvascular permeability. Allopurinol, by inhibiting xanthine oxidase, protects gastric mucosa from free oxygen radicals, and reduces lesions in gastric mucosa (24, 25). In this study, we examined the effects of Allupurinol, which was given before cold-restraint stress as an xanthine oxidase inhibitor, on gastric mucosal injury. When it was compared with the control group, we determined that it has a protective effect on gastric mucosa. In view of lipid peroxidation, it was observed that MDA levels decreased significantly ($p<0.05$).

Selenium is an important element for metabolic functions. It is found in glutathione peroxidase (GSH-PX), serum, plasma, erythrocytes, thrombocytes, plancenta, GIS and other tissues. It is also a co-factor of GSH-PX (26). Glutathione peroxidase is an enzyme that detoxifies hydroxyl radicals (27). So Selenium is related with GSH-PX activation (28), and activates GSH-PX to prevent damage from free radicals.

In this study, we investigated the effects of Selenium, which is a co-factor of GSH-PX, on gastric mucosal injury. When we compared it with the control group, we determined that it has a protective effect on gastric mucosa and in terms of lipid peroxidation-induced damage, a statistically significant decrease was observed in gastric tissue MDA levels ($p<0.05$).

When Allopurinol and Selenium applications were added to Vit-E groups (10 mg/kg/day), in the Vit-E+Allopurinol and Vit-E+Selenium groups there was a significant decrease in gastric mucosal damage and lipid peroxidation-induced MDA levels compared to the control group ($p<0.01$).

In conclusion, we are of the opinion that to prevent gastric mucosal damage induced by cold-restraint stress and to reduce lipid peroxidation that is induced by free radicals, exogenous agents such as moderate doses of Vit-E, Allopurinol and Selenium may play an important role.

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