Effect of Long Term Supplementation of Vitamin E Attwo Different Doses on Lipid Peroxidation and Antioxidative Enzymes Activity During Aging in Rats

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Abstract: Vitamin E a biological lipid antioxidant has been reported to influence the aging process while antioxidative enzymes activity have been postulated to be affected with age. The aim of our study is to look at the changes in the Malondialdehyde (MDA) levels (the product of lipid peroxidation) in plasma and activities of erythrocyte antioxidative enzymes such as Glutathione Peroxidase (GPx), Catalase (Cat) and Superoxide Dismutase (SOD) in rats supplemented with two different doses of vitamin E during aging. Twenty-four Wistar rats aged 6 months, weighing 250-300 grams were divided into three groups. Control group was fed with basal diet, while treated groups were supplemented with α-tocopherol at 60 and 120 mg kg⁻¹ diet. The MDA levels and erythrocyte enzyme activities were determined every 10 weeks for 70 weeks. The results showed that MDA levels increased progressively until the rats were 16 months old (week 40) where it reached its peak value of 5.79±0.54 nmol mL⁻¹. During subsequent 30 weeks, lower MDA levels were observed. This decrease in MDA levels was statistically significant compared to the peak value (p<0.05). In the treated groups, similar patterns were observed. However, the values attained were different. Rats supplemented with 60mg kg⁻¹ diet of vitamin E had a significant lower peak value (5.53±0.49 nmol mL⁻¹) compared to control. While those treated with 120 mg kg⁻¹ diet of vitamin E attained a significantly higher peak value (7.72±0.43 nmol mL⁻¹) as compared to control (p<0.05). GPx activity increased rapidly (p<0.05) until week 30 but subsequently the increase in activity was not as rapid. The group supplemented with 120-mg kg⁻¹ diet showed a higher activity as compared to the lower dose and control group. Similarly, cat activity was found to decrease after 30 weeks, but supplementation with vitamin E did not cause significant changes in Cat activity compared to control group. Activity of SOD showed a different pattern, where its activity in control group peaked at week 40. Its activity initially reduced with vitamin E supplementation but increased significantly (p<0.05) after 40 weeks treatment. In conclusion, the study had shown that higher dose of vitamin E supplementation (120 mg kg⁻¹ diet) generally increased the antioxidative enzymes as compared to the control however supplementation at lower dose (60mg kg⁻¹ diet) reduced lipid peroxidation during aging in rats.

Key words: Aging, Malondialdehyde, vitamin e, superoxide dismutase, catalase, glutathione peroxidase

INTRODUCTION

The free radical hypothesis of aging proposed that aging is due to the accumulation of unrepaired damage from free radical attack on cellular components (Harman, 1956). The accumulation of damage may be attributed to disease, environment, immune dysfunction and the intrinsic aging process (Holmes et al., 1992). Reactive Oxygen Species (ROS) are formed in the body as a consequence of aerobic metabolism in intracellular components such as nucleic acids, proteins and lipids (Sagar et al., 1992). MDA, one of the major products of lipid peroxidation has been widely used as an indicator of oxidative damage to unsaturated lipids (Janero, 1990). The endogenous production of MDA can be increased by accumulation of highly unsaturated fatty acids and deficiency in vitamin E or selenium (Chaudary, 1994).

A complex defence strategy arises to minimize the damaging effect of various oxidants such as vitamin E and antioxidative enzymes including Superoxide Dismutase (SOD), Catalase (Cat) and Glutathione Peroxidase (GPx) (Mulholland et al., 1999). SOD accelerates the dismutation of superoxide radical (O₂⁻) to Hydrogen peroxide (H₂O₂) and Oxygen (O₂). The selenium containing enzyme GPx detoxifies H₂O₂ by utilizing reduced glutathione (GSH) and H₂O₂ as substrates to yield H₂O and oxidized glutathione.

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(GSSG), while Cat removes H₂O₂ breaking it down directly to O₂ (Halliwell, 1994). However, disturbances of these defence systems can lead to age-related diseases such as atherosclerosis (Craig et al., 1999) and cancer (Choi et al., 1999). Administration of vitamin E has been shown to alter the activity of GPx in certain tissues of rats (Chow et al., 1973) and in human the activity of GPx in the red cells has been found to be linearly correlated to the concentration of plasma vitamin E (Emerson et al., 1972). On the other hand, SOD and Cat have been reported to exhibit variable response to vitamin E supplementation (Lammi et al., 1984). The aim of this study is to determine the effect of life-long supplementation of vitamin E at different doses (60 and 120 mg kg⁻¹ diet) in rats by measuring the levels of MDA and antioxidative enzymes at different stages of life.

MATERIALS AND METHODS

Twenty-four male Wistar rats weighing 250-300 g aged 6 months were obtained from the Animal House, Institute of Medical Research (IMR), Kuala Lumpur. Animals were individually caged in metabolic cages sized 37×25×18 cm. Standard rat chow and tap water was given ad libitum. The animals were kept at room temperature and controlled humidity. All experiments were carried out in accordance to the specification of the University’s Animal Ethics Committee (UKMAEC). The animals were divided into three groups. The control group A was given basal diet while groups B and C were supplemented with α-tocopherol 60 and 120 mg kg⁻¹ diet respectively. Blood was taken at every 10 weeks for 70 weeks from the orbital sinus vein.

Malondialdehyde in plasma was estimated by the method of Ledwozyw et al (1986). Its reaction with thiobarbituric acid was measured by spectrophotometer at EX 515 and EM 553. The MDA standard curve was obtained by using malondialdehyde standard (1,1,3,3-Tetraetoxypropane).

SOD activity was determined according to the method of Beyer and Fridovich (1987). It was assayed by the Nitro Blue Tetrazolium (NBT) method. In this assay, the sensitizing dye (riboflavin) is activated by a photon, yielding an excited state, which oxidizes some Electron Donor (EDTA). The dye is thereby reduced to a semiquinone, which reduces O₂ to O₂⁻ which in turn, reduces NBT to an insoluble purple formazon and its absorbance was read at 560 nm.

CAT activity was determined according to the method of Aebi (1984). In the ultraviolet range H₂O₂ shows a continual increase in absorption with decreasing wavelength. The decomposition of H₂O₂ can be followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time is a measure of the catalase activity.

Fig. 1: Levels of MDA with progressing age. There is a progressive increase in MDA level until week 40, where it reaches its peak in the control group. The supplemented group generally shows a higher MDA level at dose 120 mg kg⁻¹ diet as compared to 60 mg kg⁻¹ diet with a significant difference at the end of the study. significant difference between the two doses of vitamin E.

Gpx activity was determined according to the method of Paglia and Valentine (1967). Red blood cell glutathione peroxidase was accurately measured by a direct spectrophotometric procedure. The activity appears to be dependent upon active sulphhydryl groups and is unaffected by low concentrations of azide, cyanide or ferricyanide. The conversion of NADPH to NADP was followed at 340 nm.

Statistical analysis was carried out using ANOVA and LSD (Least Square Difference), taking p<0.05 as its significance value.
RESULTS

MDA levels in the control group were shown to increase progressively until the rats were 16 months old (40 weeks) where it reached its peak value of 5.79±0.54 nmol mL⁻¹ and this increase was significant compared to 0 week (Fig. 1). However, in the subsequent 30 weeks, MDA levels showed a progressive decrease in its level.

MDA levels were shown to increase significantly (p<0.05) in the groups treated with vitamin E in the initial part of the study compared to control but there was no significant difference between the groups given two different doses of vitamin E, then. For both groups, the level of MDA decreased significantly at 20 weeks and increased again and similarly reaching its peak value at 40 weeks. However, the group treated with 60 mg kg⁻¹ diet vitamin E had a significantly lower (p<0.05) peak value compared to the group treated with 120 mg kg⁻¹ diet vitamin E. The group supplemented with the higher dose of vitamin E maintained a significantly higher MDA level (p<0.05) at the end of the study as compared to the lower dose as well the control.

There was a significant increase (p<0.05) in GPx activity in the control group at week 30 compared to baseline, but decreased at week 40 and later increase again until the end of the study which is week 70 (Fig. 2). It can be seen that the group supplemented with vitamin E at 120 mg kg⁻¹ diet showed a significantly higher activity (p<0.05) at week 50 as compared to the lower dose (60 mg kg⁻¹ diet) and control group. A significantly high (p<0.05) Cat activity in all the three groups can be seen at week 30 (Fig. 3) but significantly decreased (p<0.05) at
Fig. 4: Activity of SOD activity progressive with age. SOD activity increased significantly until week 40 in control group and decreased subsequently. In supplemented group, SOD activity initially reduced until week 40, but a sharp increase in week 50 and subsequently decreased with age. However, there is no significant difference between the 2 supplemented groups.

week 50 compared to peak values. Supplementation with vitamin E did not show much age-related changes in activity as compared to the control, however there was a significantly higher (p<0.05) activity at week 30 with the higher dose vitamin E. In control group, SOD activity increased significantly (p<0.05) at 13 months of age (30 weeks) compared to baseline (Fig. 4) and decreased significantly (p<0.05) as compared to its peak value at week 50. Meanwhile, supplementation with vitamin E increased its activity rapidly from week 40 reaching its peak value in week 50 which is significant (p<0.05) compared to control, but decrease again in subsequent weeks. However there were no significant changes noted in SOD activity between the groups supplemented with the two different doses of vitamin E.

DISCUSSION

The changes in aging results from an increase in oxidative damage to lipids, proteins, DNA or from the effect of the oxidative stress on the regulation of genes (Rikans and Hornbrook, 1997). Antioxidants may play an important role in preventing free radical damage associated with aging by interfering directly in the generation of radicals or by scavenging them (Poulin et al., 1996). The first line antioxidant defense system includes enzymes such as SOD, GPx and Cat and non-enzymatic antioxidant such as reduced Glutathione (GSH), protein-SH, vitamin E, C and β-carotene and uric acid (Cutler, 1991). Many studies have indicated that age-related changes in antioxidant defence are variable and inconsistent in finding and appear to depend on species, strain, sex and tissue studied (Rikans and Hornbrook, 1997).

In this study, the significant increase in MDA levels in control group at week 40 may be due to the higher rate of lipid peroxidation and the elevation of free radicals production as a result of the rats being very active at this age. It has been shown that the defense mechanisms and antioxidant status maybe affected with age (Cutler, 1991). MDA levels were decreased after week 40 until at the end of 70 week study. It could be due to lesser activity of the rats as they aged as well reduced dietary intake which has been shown to reduce lipid peroxidation (Sohal and Weindruch, 1996).

Gpx activity in the control group was found to be stable in rats aged up to week 20 which may be due to low free radical levels in the younger group. GPx activity reduced at week 40, but started to increase again with age. Cat activity increased significantly (p<0.05) at week 30 in the control group when there was higher oxidative stress as indicated by a high MDA level. It then decreased slightly with age. The lower levels of peroxyl radicals will stimulate the activity of GPx which has been reported to be the important antioxidant enzyme in protection against low levels of oxidative stress working synergistically with Cat where Cat deals with high oxidative stress level (Mates et al., 1999). SOD and Cat activities increased at week 30 and declined after 16 months of age which may due to the lower production of superoxide radicals in accordance with the decrease rate of metabolism in older rats.

Vitamin E is an ubiquitous component present among the lipid constituents of cell membranes and lipoproteins. Its major role is regarded to be as an antioxidant acting as scavenger of free radicals that can damage tissues and especially the unsaturated lipids. The second mode of action is to stabilize the structure of
membranes by forming complexes with destabilizing molecules (Wang and Quinlan, 1999). Administration of large amounts of vitamin E and nutrients has been shown to increase the average life expectancy in animals (Duthie et al., 1996). Vitamin E deficiency leads to lower erythrocyte life span, neurological dysfunction and certain forms of cancer (Ali and Ashok, 1999).

Our study showed that supplementation with vitamin E caused a reduction in the MDA level in the group supplemented with 60 mg kg\(^{-1}\) diet vitamin E but the opposite effect was obtained for the higher dose (120 mg kg\(^{-1}\) diet vitamin E). This result could be attributed by the prooxidative effects of vitamin E (Kamal and Applqvist, 1996) and prolonged high dose vitamin E supplementation may give rise to a prooxidative effect (Oski, 1980). Supplementation with higher dose of vitamin E caused a higher activity of GPx compared to the lower dose and control group. This increase in activity proved a synergistic effect between vitamin E and GPx activity in neutralizing free radical formed especially the peroxyl radicals (Poulin et al., 1996). The results pattern were found to be similar to those of SOD. McCall and Frei (1999) indicated that the doses of vitamin E and vitamin C used to reduce most indices of lipid peroxidation in smokers and non-smoker were 100 mg day\(^{-1}\) 1000mg day\(^{-1}\) respectively. Meanwhile in Cat. supplementation with vitamin E at two different doses did not show any significant changes in its activity as compared to the control.

CONCLUSION

In conclusion, the results suggested that vitamin E given as a life long supplementation in rats at 60 mg kg\(^{-1}\) diet has protective effect on the aging process by reducing lipid peroxidation, but a higher dose of 120 mg kg\(^{-1}\) diet appeared to be prooxidative. However there appeared to be a balance between both the antioxidant enzymes activities and vitamin E in coping with oxidative stress during the aging process.

REFERENCES


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