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Modulation of restraint stress induced oxidative changes in rats by antioxidant vitamins

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Abstract

In the present study we examined immobilization stress-induced antioxidant defense changes in rat plasma and also observed the antioxidant effects of pre and post vitamins A, E and C administration (15 mg/Kg of body weight) individually and in combination (vit E + C) on these alterations.

Following immobilization stress the circulating activities of superoxide dismutase, catalase and glutathione-S-transferase were decreased, while the level of thiobarbituric acid reactive substances (TBARS) was increased as compared to non-stressed control rats.

Post treatment with individual vitamins A, E and C (after exposure to stress) resulted in a less marked alteration of plasma TBARS levels and activities of SOD, GST and catalase as compared to pre vitamin stress or stress alone treatments. Both pre and post vitamin treatments were effective in preventing stress induced derangement of free radical metabolism with a relative dominance by latter. The combined treatment with vitamin E and C did not show any additive antioxidant effect on restraint stress induced altered free radical metabolism, rather a predominant effect similar to vitamin E alone was observed. The prevention of oxidative stress generated in response to restraint stress by the vitamins can be summarized as: vitamin (E + C) i.e. vit E > vit C > vit A, thus combined vitamin (E + C) treatment though showed maximum preventive effect, but was similar to vitamin E treatment alone, in terms of the circulating activities of SOD, GST, catalase and TBARS levels.

Keywords: Restraint stress; Vit A; Vit E; Vit C; Superoxide dismutase; GST; Catalase

1. Introduction

Immobilization/restraint stress is an easy and convenient method to induce both psychological (escape reaction) and physical stress (muscle work) resulting in restricted mobility and aggression [1,2]. Recently various stresses have been associated with enhanced free radical generation causing oxidative stress [3]. One of the most important consequences of the generation of free radical is the peroxidation of membrane lipids.Moreover, stress has been suggested to decrease the level of glutathione (GSH) and vitamin C which play an important role in protection of tissues from oxidative damage [5,6]. Next to the huge group of polyphenols,especially tocopherols, ascorbic acid and carotenoids have been associated with antioxidative properties [7].

In the present study the oxidative stress generated by the restraint stress was measured in terms of free radical scavenging enzyme activities like superoxide dismutase, catalase, glutathione-S-transferase and thiobarbituric acid reactive substances (TBARS).The antioxidative potential of vitamin A, E, C alone and in combination (vit E + C) was also studied on restraint stress induced oxidant/pro-oxidant status of rats.

The results of the study are likely to contribute to understanding the potential of antioxidant vitamins in preventing/alleviating stress induced diseases involving oxidative damage to cellular constituents.

2. Methods and materials

For the present study male Wistar rats weighing 180–200g were selected, the animals were housed in groups cages,purina diets and tap water were supplied to them ad libitum. Prior to the commencement and throughout the experiment the rats were housed at 24±3°C room temperature and 12 h light/dark cycles. All the reagents and chemicals were purchased from commercial sources.

Immobilization stress was accomplished by placing individual animals in wire mesh cages of their size attached to a wooden board. The rats were deprived of food and water during stress exposure [8]. The animals were subjected to

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Table 1
Effect of Vitamin A, E and C treatments individually and in combination (vitamin E + vitamin C) on the circulating levels of SOD, GST, catalase and lipid peroxidation in restraint stress treated rats (values are ± SEM)

		SOD unit/mg of protein	Catalase U/mg of protein	GST U/mg of protein	MDA nmoles/ litre
Control rats (10)		5.44 ± 0.351	0.60 ± 0.114	0.50 ± 0.170	0.64 ± 0.035
Stress treated rats alone (10)		3.22^{d} ± 0.291	0.40^{d} ± 0.02	0.23^{d} ± 0.017	0.97^{d} ± 0.043
Pre vitamin stress treated rats (40) 10 rats each	Vit A	3.45^{a} ± 0.21	0.47^{c} ± 0.022	0.30^{b} ± 0.034	0.76^{a} ± 0.024
	Vit E	3.8^{b} ± 0.070	0.52^{a} ± 0.016	0.42^{a} ± 0.037	0.77^{b} ± 0.030
	Vit C	3.55^{a} ± 0.153	0.44^{b} ± 0.020	0.29^{c} ± 0.024	0.72 ± 0.031
	Vit (E + C)	3.86^{b} ± 0.129	0.53^{a} ± 0.011	0.49^{a} ± 0.024	0.68^{a} ± 0.022
Post vitamin stress treated Rats (40) 10 rats each	Vit A	3.50^{c} ± 0.116	0.55^{d} ± 0.011	0.36^{c} ± 0.030	0.68 ± 0.035
	Vit E	4.2^{d} ± 0.24	0.53^{c} ± 0.013	0.43^{b} ± 0.036	0.65 ± 0.02
	Vit C	4.01^{c} ± 0.20	0.49^{c} ± 0.015	0.32^{d} ± 0.025	0.53^{d} ± 0.028
	Vit. (E + C)	4.42^{d} ± 0.125	0.53^{c} ± 0.016	0.40^{c} ± 0.033	0.63^{b} ± 0.022s

No. of experimental rats are indicated in the parenthesis. [a] a' $p < 0.05$, [b] b' $p < 0.02$, [c] c' $p < 0.01$, [d] d' $p < 0.001$ where a, b, c, d as compared to non stressed control rats where a', b', c', d' stressed treated rats.

6hr stress then sacrificed after 30 min by giving sodium pentobarbital (i.p., 50 mg/Kg of body weight). Controls were handled at the same time as the stressed animals and were placed in individual cages during the corresponding time.

For the stress and vitamins A, E, C and E + C treatment studies the animals were divided into two broad groups,one received vitamins (15mg/Kg of body weight) dissolved in olive oil 30 min prior to 6 hr stress session (pre vitamin stress treated), while the other groups received individual vitamins (A, E, C) and combined vitamins (E + C) 30 min after the stress session (post vitamin stress treated).

The rats were sacrificed 30 min after the termination of experiment by giving Sodium Pentobarbital (15mg/Kg of body weight, i.p) and immediately exsanguinated. The heparinized blood was centrifuged (5000 rpm, 15 min) and plasma was quick frozen and stored at −40°C until assay.

The plasma was subjected for the assay of Catalase [9], SOD [10], Glutathione-S-Transferase [11] and thiobarbituric acid reactive substances [12] by standerdised methods. The protein contents were estimated by the method of Lowry et al [13].

3. Statistical analysis

Rigorous statistical analysis were performed for the control/baseline levels of the enzymes under study with respect to the treatments given to the rats. It was performed using one way ANOVA test at $p = 0.05$ on the data obtained by the repeated investigations. Paired t tests were also performed at $p = 0.05$ to decide either the results are significantly different or not, followed by pair wise comparison (Tukey's honestly significant Post hoc analysis). Similar statistical treatments were also given to the data obtained for the enzyme activities from pre and post vitamin stress treatments with respect to the stress alone or non-stresed controls. The results obtained are summarized in Table 1.

4. Results

Six hours of immobilization stress resulted in a significantly decreased circulating activities of SOD ($F_{1\text{-}9} = 6.658$ $p = .018$), GST ($F_{1\text{-}9} = 148.04$ $p = .02$) and catalse ($F_{1\text{-}9} = 61.99$ $p = .001$) while the level of malondialdehyde were significantly enhanced ($F_{1\text{-}9} = 34.63$ $p = .0001$) in comparision to non-stressed control rats.The individual vitamin A,E, and C and combined (vit E + C) treatment both prior to (pre vitamin stress treated) or after stress (post vitamin stress treated) resulted in a less significant alteration of these parameters if compared with stressed or non-stressed controls, i.e a reversion towards control values was observed. The post vitamin treatments were found more effective than pre vitamin treatments in reverting the stress induced altered SOD, GST, catalase and TBARS levels towards their control values. However, the post stress oral administration of vitamin E (15mg/kg of body weight) was found more effective in restricting the stress induced decrease of SOD ($F_{1\text{-}9} = 8.57$ $p = .002$), GST ($F_{1\text{-}9} = 43.37$ $p = .03$), catalase ($F_{1\text{-}9} = 28.37$ $p = .005$) and increase of TBARS ($F_{1\text{-}9} = 41.14$ $p = .002$) as compared to stress alone or other vitamin treatments. While the combined vitamins (E + C) treatments did not show any additive effect rather an effect similar to vitamin E alone was observed.

5. Discussion

In the present study an attempt has been made to evaluate the effect of antioxidant vitamins A,E and C alone and in combination (vit E + C) on modulation of restraint stress induced oxidative changes in terms of the measurements of circulating activities of SOD, GST, catalase and TBARS levels in rats.

Immobilization/restraint stress is a well known method for the production of chronic stress [14]. This study was of interest as human beings are exposed to both emotional and

physical stress daily in their life [15]. The circulating activities of SOD, GST and catalase were decreased while the level of TBARS was increased in stress treated rats as compared to unstressed control group. Various antioxidants and free radical scavenging enzyme systems exist in the cell to protect it against the damaging effects of free radicals produced as a part of normal cell respiration and other cellular processes [16]. Free radicals and free radical reactions are involved in the etiology and development of a number of diseases, especially those that are life limiting [17]. Restraint stress has been shown to bring about antioxidant defense changes in the plasma of rats [18]. Superoxide dismutase, catalase and GSH play an important role in the detoxification of oxyradicals and their products [19]. In order to maintain the stability of a living organism it is necessary to reach a balance between the oxidative actions and the antioxidant defense i.e anti-FRS. Enhanced free radical production with lipid peroxidation has been observed during stress [20]. The decreased activities of SOD, GST and catalase as observed in the present study may be responsible for the elevated lipid peroxidation as represented by increased TBARS levels is stress [21]. Thus, restraint stress is found capable of generating severe oxidative stress in rats.

Both enzymatic in vivo SOD, GST, catalase [9,10,11] and non enzymatic (vitamin A, E and C) natural antioxidant defense mechanisms exist. However, the antioxidant mechanism fails either due to excessive production of free radicals or decreased activities of scavenging enzymes, or both causing lipid peroxidation. Since lipid peroxidation is a self propagating chain reaction the initial oxidation of only a few lipid molecules can result in significant tissue damage and diseases. A potential role for the antioxidant micronutrient (vitamin C, vitamin E and vitamin A) in modulating oxidative stress thus generated may determine their clinical usefulness. The treatment of rats both prior or after stress with these vitamins showed an increase in the activities of SOD, GST and catalase with a decrease in lipid peroxidation. Post vitamin treatment was found more effective than pre treatment in alteration of these parameters towards their control values. Vitamin A, E and C act as an effective antioxidant of major importance for protection against diseases and degenerative processes caused by oxidative stress [22]. In the present study these vitamins showed their antioxidant potential as radical scavengers by enhancing the activities of SOD, GST and catalase and thus decreased lipid peroxidation as reported by others too [23].

The antioxidant potential of vitamins (E + C) together though was found effective but not additive as expected, rather a predominant effect of vitamin E alone was observed.Vitamin E appears to be the first line of defense against peroxidation of polyunsaturated fatty acids present in cellular and subcellular membrane phospholipids. Vitamin C and E have long been associated with stress. A redistribution of vitamin C has been reported during stress with localized enhanced concentration near adrenal S. Increased knowledge in the use of micronutrients like Vitamin A, E and C as an antioxidant under normal and stressful conditions will have an impact on both clinical and dietetics practice and public health nutrition guidelines.

6. Conclusion

Restraint stress was found to induce oxidative stress through decrease of SOD, GST, catalase and increase of lipid peroxidation as shown by enhanced thiobarbituric reactive substances levels. The pre and post-stress oral administration of antioxidant vitamins A,E and C individually and in combination (vit E + C)were effective in protecting restraint stress induced oxidative changes. The combined vitamin treatment did not show any additive effect, but the post stress vitamin treatments were found more effective than pre vitamin treatments, as far as the alteration in the activities of free radical metabolising enzymes and lipid peroxidation was concerned. Vitamin E treatment was found most effective in preventing/restoring the stress induced decrease of SOD, GST, catalase activities and increase of MDA levels.

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