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Effects of short term administration of vitamin c on lung antioxidants in cigarette smoke exposed rats

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ABSTRACT

Male wistar rats were exposed to cigarette smoke along with supplementation of vitamin C at two doses (100mg and 200mg/kg body wt) for a period of one month. At the end of the experiment the rats were sacrificed and blood and lung tissues were collected for evaluation of antioxidant status. The depleted levels of antioxidant enzymes like SOD and Catalase were found to be elevated after treatment with vitamin C (200mg/kg body wt) ($p<0.005$). The smoke exposure was also found to increase the values of lipid peroxidation, hydroperoxides and conjugated dienes. Elevated values of all these were found to become normal with the treatment of vitamin C at a dose of 200mg/kg body wt, indicating the short term beneficial effect of vitamin C as a free radical scavenger against cigarette smoke.

INTRODUCTION

Smoking is a major health hazard that leads to several life threatening diseases. Cigarette smoke contains large amounts of free radicals and pro-oxidants, which in biological systems can undergo redox cycling to produce reactive oxygen species [1]. These Reactive oxygen species (ROS) are responsible for oxidative stress and membrane damage which lead to many of the diseases produced by smoking [2]. In addition, superoxide anion radical derived from cigarette smoke may react with endothelium- derived nitric oxide directly or via formation of peroxy nitrate, a highly reactive intermediate with cytotoxic activity [3]. Oxygen derived free radicals within the vasculature may also initiate the oxidation of lipoproteins, which in turn may interfere directly with vascular function, including vessel relaxation [4]. Further more, free radicals in cigarette smoke may increase the depletion of natural antioxidants, such as vitamin C, and there by increase their own pro-oxidant potential, indeed some of the studies have proved that plasma levels of ascorbate are lower in smokers compared with healthy control subjects [5].

Though the effect of vitamin C on endothelial dysfunction in relation to coronary artery diseases has already been studied, its role as an effective antioxidant in the respiratory system is still under experimental stage. The present study is aimed at the short term beneficial effect of vitamin C supplementation on lung antioxidants in rats exposed to cigarette smoke for a period of one month.

MATERIALS AND METHODS

Chemicals : Nitroblue tetrazolium (NBT), NADPH, Glutathione (GSH) and 5',5'-dithio-bis (2-nitro benzoic acid) (DTNB) were purchased from Sisco Research Laboratory, Mumbai. Thiobarbituric acid (TBA) was obtained from Hi Media, Mumbai. Vitamin C as Celine was purchased from Amala Hospital Pharmacy. All other chemicals and reagents used were of analytical reagent grade. Vitamin C was dissolved in distilled water for *in vivo* studies.

Experimental animals : Male wistar rats weighing 200 to 250gms were purchased from Veterinary college Mannuthy, Thrissur. They were housed in ventilated cages in air-controlled rooms and fed with normal rat diet (Sai Durga feeds, Bangalore, India) and water ad libitum. All the experiments were carried out after getting ethical clearance from Institutional animal Ethics Committee.

Experimental Design : Animals were divided into 4 groups of 6 rats each as follows.

GROUP I : normal controls

GROUP II : smoke exposed for 30 days

GROUP III : smoke exposed + 100 mg/kg body weight of vitamin C dissolved in water for 30 days

GROUP IV : smoke exposed + 200 mg/kg body weight of vitamin C dissolved in water for 30 days

The rats were exposed to smoke and vitamin C was given at different doses as above. The doses were decided after conducting a pilot study. The animals were exposed to cigarette smoke in a specially made thermocol box with suction pump attached. They were given cigarette smoke for at least 20minutes in each day for one month. After the exposure to smoke they were transferred into fresh cages. The cigarette used was of standard size. The rats were subjected to inhale the cigarette smoke in the closed chamber for 20minutes. All the animals in each group were exposed to smoke simultaneously.

Vitamin C was administered orally for a period of 30 days immediately after the smoke exposure as per the dose decided. At the end of the experiment, animals of all the groups were sacrificed and blood collection was done by heart puncture. Lung was excised and thoroughly washed in ice cold saline (4°C, 0.9%). Lung homogenate was prepared in ice cold Tris-HCl buffer (0.1M, pH-7.4) and cytosolic sample of lung was prepared by using, Remi Cooling Centrifuge (Rotor: C-24BL, 1,00,000g) for 30mins at 4°C. The blood, serum and lung homogenate were used for the biochemical analysis.

Biochemical estimations: Hemoglobin was estimated by cyanmethemoglobin method using Drabkin's solution [6] and by taking the absorbance at 540 nm. The readings were taken with respect to the standard hemoglobin solution, provided within the kit. The total WBC count was done by DC detection [7] method using a hemocytometer. Albumin was detected by measuring the blue green complex that is formed by the reaction of albumin with bromocresol green at slightly acidic pH, which is measured photometrically at 600nm [8]. The total protein in the serum was assayed by Biuret method [9].

Superoxide dismutase (SOD) (EC 1.15.1.1) of blood and lung tissue of animals exposed to cigarette smoke and treated with vitamin C for 30 days along with normal controls were done by Nitroblue tetrazolium reduction method of Mc Cord and Fridovich [10]. The values were expressed in Units/gm Hb in the case of blood and Units/mg protein in the case of lung. Catalase (CAT) (EC 1.11.1.6) activity in blood and tissue homogenate were done by the method of Hugo Aebi [11] by measuring the rate of decomposition of hydrogen peroxide at 240nm. A decrease in absorbance was observed after addition of hydrogen peroxide to the reaction mixture containing either the tissue homogenate or the erythrocyte sediment used as the source of catalase. Units of activity were determined from the E_{max} (240nm) of hydrogen peroxide. Reduced Glutathione (GSH) activity in lung tissue homogenate was measured by the method of Moron et al [12], based on the reaction with 5-5' dithiobis (2-nitrobenzoic acid). Values were calculated from a standard graph of GSH treated with same reagent.

Lipid peroxidation (LPO) in lung tissue was estimated using TBA method of Ohakawa [13] by using 1,1,3,3-tetra methoxy propane as standard against absorbance. LPO in serum was done by TBA method as modified by Yoshioka et al [14] using TCA and TBA.

Hydroperoxides and conjugated dienes in lung tissues were done by the method of John and Steven [15]. In both the tests the samples were first extracted in chloroform and methanol and lower layer is taken to dryness. The remaining lipid residue was dissolved in 1.5 ml of cyclohexane, and absorbance was taken at 233nm. For the estimation of hydroperoxides the lipid residues were treated with hydrogen iodide and cadmium acetate. The absorbance was measured at 353 nm and concentration was determined from Emax. The protein in the tissue homogenate was estimated by Lowry's method [16].

STATISTICAL ANALYSIS

Statistical analysis of the values obtained was performed using the student t-test. The values were expressed as mean $\pm$ SD. The p values less than 0.005 was considered as statistically significant.

RESULTS AND DISCUSSION

Table I represents the values of Hb, Total WBC count, albumin, total protein, SOD, catalase and lipid peroxidation in the blood and serum of control as well as in the treated groups. The value of Hb was found to be elevated in the smoke exposed group when compared to that of the control ($p<0.005$). After treatment with vitamin C the value of Hb was found to come back to the normal level when compared to smoke exposed group. Out of the two doses of vitamin C given, 200mg/kg body wt was found to bring the Hb level to normal controls and was found to be statistically significant when compared to smoke exposed groups ($p<0.005$). The total WBC count was found to be decreased in smoke exposed group when compared to that of the normal control and was not found to be statistically significant. The treatment with vitamin C did not enhance the WBC count in treatment groups. The value of albumin, a natural antioxidant present in the body was not altered in smoke exposed as well as in the drug treated groups. The value of total protein was also remained the same in all the 4 groups.

The value of SOD was found to be significantly decreased in the smoke exposed groups when compared to normal controls ($p<0.005$) and the value was significantly elevated after treatment with vitamin C at a dose of 200mg/kg body wt ($p<0.005$). However the values of CAT were remained almost the same in all the four groups. The lipid proxidation was found to be slightly elevated in the serum of smoke exposed rats when compared to normal controls and is not statistically significant. The oral dose of vitamin C at a concentration of 200mg/kg body wt was found to decrease the LPO levels in the serum of rats when compared to smoke exposed group.

Table II represents the values of SOD, CAT, GSH, LPO, conjugated dienes and hydroperoxides in lung tissues of normal, smoke exposed and vitamin C treated animals for a period of one month. The values of SOD and Catalase were found to be decreased in smoke exposed group when compared to control and is found to be significantly ($p<0.005$) increased after vitamin C treatment (200mg/kg body wt) when compared to smoke exposed groups. However the tissue GSH did not show much change in smoke exposed as well as in vitamin C treated groups when compared to controls. The values of LPO, conjugated dienes and hydroperoxides were found to be increased in smoke exposed groups and this came back to near normal values after vitamin C treatment at a dose of 200mg/kg body wt.

The necessity to improve the health in smokers is very essential as many are dying at an early age. Further, it is found that the rate of smoking cessation remaining very low in all population and this contributes to early mortality [17]. In this study we could able to find out that a single dose of vitamin C supplementation

Table 1: The values of Hb, WBC, Albumin, Total protein, SOD, Catalase and Lipid peroxidation in blood and serum of normal, smoke exposed as well as in Vitamin C treated rats.

Parameters	Normal (Group I)	Smoke Exposed (Group II)	Vitamin C 100mg/ Kg body wt (Group III)	Vitamin C 200mg/ Kg body wt (Group IV)
Hb (gm%)	9.56 + 0.76	13.19 + 1.70*	11.44 + 2.27	8.48 + 1.23*
WBC (count/cmm)	6180 ± 1080	5280 ± 1209	4810 ± 818.9	4670 ± 1627
Albumin(mg/ml)	5.23 + 0.39	5.50 + 0.33	5.47 + 0.31	5.35 + 0.27
Total protein (mg/ml)	6.53 ± 0.31	6.41 ± 0.54	5.96 ± 0.52	5.75 ± 0.49
SOD (U/gm Hb)	747.8 + 129.30	495.64 + 169.40*	596.38 + 237.16	1183.18 + 188.28*
Catalase (K/gm/Hb)	40.94 ± 7.20	33.15 ± 1.30	35.38 ± 11.90	38.92 ± 12.10
LPO (U/ml)	8.78 + 0.52	9.14 + 0.26	7.54 + 1.90	6.33 + 2.71

Values are expressed as mean + SD of 6 animals in each group
*P<0.005 Group II is compared with Group I Group IV is compared with Group II

Table 2: Values of SOD, CAT, GSH, LPO, Conjugated dienes and hydroperoxides in lung tissue of normal, smoke exposed and vitamin C treated rats.

Parameters	Normal (Group I)	Smoke Exposed (Group II)	Vitamin C 100mg/ Kg body wt (Group III)	Vitamin C 200mg/ Kg body wt (Group IV)
SOD (U/gm protein)	100.5 + 38.40	88.12 + 7.60	108.16 +39.00	189.00 + 35.70*
Catalase (K/gm/Hb)	22.01 + 2.81	16.02 + 3.30	22.10 + 2.42	29.13 + 5.50*
GSH (nmol/ml)	30.80 + 1.30	30.00 + 1.22	32.20 + 3.83	35.40 + 2.61
LPO (U/ml)	34.15 + 9.69	38.94 + 8.78	32.47 + 5.48	31.22 + 5.64
Conjugated Dienes (nmol/ml)	15.12 + 1.30	18.20 + 4.24	14.85 + 1.06	13.72 + 1.37
Hydroperoxides (nmol/ml)	61.38 + 19.80	96.78 + 24.86	85.16 + 12.05	65.50 + 17.53

Values are expressed as mean + SD of 6 animals in each group
**p<0.005 Group II is compared with Group I Group IV is compared with Group II

for a period of one month improves the lung antioxidant status very effectively though the study was conducted only for one month. Previous studies using high dose of parenteral administration of vitamin C have shown that its infusion acutely improves endothelial function in smokers [18] and in this particular study we could able to prove its beneficial effect on the lung antioxidant system. When the smoke was given for a period of one month to the rats, Hb was found to be slightly elevated, as a mechanism for increased transport of oxygen to counteract hypoxia, and supplementation with vitamin C could able to bring it back to normal level.

The body's defense mechanism against the free radicals is mainly reflected via the antioxidant enzymes, which increases the body's antioxidant status and prevent the diseases up to some extent [19]. However the smoke exposure leads to acute lung inflammation through its influence over oxidants/antioxidants imbalance [20]. This is well reflected in the experiment as the smoke exposure significantly decreased the activity of SOD in the blood and lung tissues. The Catalase activity was also found to be decreased in the lung tissues of the rats exposed to smoke when compared to normal controls. The vitamin C supplementation at a dose of 200mg/ kg body wt for a period of one month could able to enhance these antioxidant enzymes in the lung tissues as well as in the blood. The elevated values of lipid peroxidation as well as the hydroperixides, the indicators of cell injury by free radicals from smoke, were found to be decreased with vitamin C treatment at a dose of 200mg/kg body wt indicating its role in tissue repair and restoration via the collagen formation.

CONCLUSION

On the basis of the results, it can be concluded that the short term administration of vitamin C as a single dose could able to enhance the antioxidant system present in the lung tissue and it could able to prevent the free radical attack by cigarette smoke in lung tissues very effectively and can be given as an oral supplement to improve the antioxidant status

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