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Effect In Vivo of Cigarette Smoke on Lipid Peroxidation and Antioxidant status in Male *albino* Mice

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Abstract:

Cigarette smoke (CS) contains free radicals that are considered to be a major group of carcinogens. Wistar mice were exposed to cigarette smoke for 16 weeks, 2cigarettes for 15 min/ twice daily, 6 days/wk. The control animals (C) inhaled clean room air. Biochemical markers in blood and lung tissues were analyzed. There was gradual increase in the lipid peroxidation indicated by augmented levels of thiobarbituric acid reacting substances (TBARS) in the lung tissue of cigarette smoke exposed animals when compared with that of control group. The enzymatic antioxidant levels such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were decreased significantly in CS exposed animals. The activity level of glutathione-S-transferase (GST) was increased in CS group when compared with that of control. The Non- enzymatic antioxidants such as vitamin-C and vitamin-E were decreased significantly in the serum of CS exposed mice. These results revealed that chronic exposure to cigarette smoke enhances oxidative stress in mice.

Keywords: *Oxidative stress, SOD, Cigarette smoke, TBARS, COPD.*

Introduction:

Cigarette smoke is a complex mixture of over 4700 chemical compounds including high concentrations of oxidant and free radicals present in gas phase and the tar phase of smoke. Those in the gas phase are both organic and inorganic including reactive oxygen species (ROS) and free radicals, aldehydes, peroxides and oxides nitrogen [1]. Cigarette smoke also generates ROS indirectly from activated polymorph nuclear leucocytes or pulmonary alveolar macrophages [2]. The increased production of ROS by smoke can produce a condition of oxidative stress that can result in the oxidation of lipids, induction of DNA single-strand breakage, inactivation of certain proteins, and the disruption of biological membranes [3, 4]. Cigarette smoking is known to be associated with various chronic pulmonary and cardiovascular diseases ranging from inflammation to cancer [5, 6, 7]. In the present investigation the CS generated oxidative stress was assessed in the albino mice.

Materials and methods:

Animals

Adult male albino mice of Wistar strain weighing around 25-30g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25 ± 2^0 C and 55-65% relative humidity. A 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India) and had free access to water. The experiments were carried out in accordance with the guidelines provided by the Institutional Animal Ethical Committee.

Cigarette smoke exposure

Mice were divided into two groups of eight animals each. Group I animals (control) were air exposed. Group II animals (experiment) CS exposed. The animals were exposed to side-stream CS of two cigarette for 15 minutes, twice daily six days a week for 16 weeks as described earlier [8,9]. The polypropylene cage was covered by a rectangular acrylic chamber (size 45 cm^3) with two holes about 2 cm diameter each. A burning cigarette was introduced through one hole and fixed to a holder and air with a pressure of 0.4 kg/cm^2 was passed through the other. The 15 minutes CS exposure time was chosen because it is the time enough to burn the cigarettes completely. At the end of experiment period

Table 1: Levels of (TBARS), (SOD), (CAT), (GPX), and (GST) in the Lung tissue.

Parameters	Control	Cigarette smoke group	% of changes (Control Vs CS Exposed)
TBARS(nm/100g tissue)	21.12 ± 0.61	55.77± 3.40	+164.06*
SOD(Unit[1]/mg protein)	2.03 ± 0.68	0.39 ± 0.12	-80.78*
CAT(Unit[2]/min/mg protein)	10.33 ± 1.32	5.70 ±0.88	-44.82*
GPX(Unit[3]/mg protein)	12.79 ± 1.66	8.26 ± 1.73	-35.41*
GST(Unit[4]/min/mg protein)	3.97 ± 0.63	6.40 ± 1.64	+61.20*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001.SOD – U_1- One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute.CAT – U_2- µmoles of hydrogen peroxide consumed per minute.GPX – U_3- µg of glutathione consumed per minute. GST – U_4- µmoles of CDNB – GSH conjugate formed per minute

Table 2: Plasma levels of vitamin-C and vitamin-E.

Parameters	Control	Cigarette smoke group	% of changes (Control Vs CS Exposed)
Vitamin-C(mg/dl)	0.70± 0.39	0.20 ± 0.01	-71.42*
Vitamin-E(mg/dl)	11.05 ± 0.63	3.46 ± 0.48	+68.68*

Data are expressed as Mean ±SD of 6 individual observations. Statistical significance * P<0.001

mice were sacrificed by cervical dislocation under anesthesia. Blood samples and lung tissue were collected.

Estimation of lipid peroxidation

The lipid peroxidation was assessed in the lung tissue measuring the levels of thiobarbituric acid reacting substances (TBARS) by the method of Nichans and Samuelson [10], tissue levels of enzymatic antioxidants *viz.* SOD, CAT, GPX, GST by the methods of Kakkar *et al.* [11] Sinha [12], Rotruck *et al*. [13] and Habig et al. [14] respectively. Levels of non-enzymatic antioxidants such as vitamin-C and vitamin-E by the methods of Roe and Kuether [15] and Baker *et al.* [16].

Statistical analysis

The results were expressed in mean ± standard deviation. Statistical analysis was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS) version 9.5.

Results:

The levels of thiobarbituric acid reacting substances and activity levels of superoxide dismutase, catalase, and glutathione peroxidase in lungs of control and experimental animals have been summarized in Table 1. The levels of TBARS in lung homogenates were found to be significantly higher when compared to that of control, the SOD, CAT and GPX activities in the lung tissues were decreased significantly in mice exposed to in vivo cigarette smoke. Cigarette smoke exposure significantly increased the GST enzyme activity in lungs of mice. The levels of plasma non-enzymatic antioxidants *viz*. vitamin-C, and vitamin-E were significantly decreased in cigarette smoke exposed animals when compared to that of control Table 2.

Discussion:

Oxidative stress is thought to play an important role in the pathogenesis of COPD, not only through direct injurious effects, but also by involvement in the cellular and molecular mechanisms that control lung inflammation [17]. Oxidative stress results from an oxidant–antioxidant imbalance: an excess of oxidants and/or a depletion of antioxidants. In the present study the TBARS levels, a measure of lipid peroxidation were determined in the lung tissue. The increased levels of TBARS in CS exposed animals envisaged the increased levels of lipid per oxidation. The tissue antioxidant SOD which is primary enzymatic defense in the lungs against the damaging effects of $O_2^{\bullet -}$, by converting $O_2^{\bullet -}$ into H_2O_2, which is a substrate for CAT and GPx. If SOD activity is inadequate, $O_2^{\bullet -}$ can interact with NO to form peroxynitrite ($ONOO^{\bullet -}$) which can react to form the potent $^{\bullet}OH$ and nitrogen dioxide ($NO_2^{\bullet}$) radicals, which are highly damaging to cell proteins, lipids, and DNA [18]. The activity levels of SOD, CAT and GPX in the tissue of CS exposed animals significantly decreased indicating the deranged antioxidant defense system. The tissue enzyme Glutathione -S- transferase (GST) principally involved in the detoxification processes by catalyzing the reactions in which Glutathione (GSH) is conjugated with electrophiles to form thioether [19]. Oxidative stress induced toxicity initially increase GST activity. The increased activity levels of GST in the present study might be due to the increased oxidative stress due to CS exposure. The non-enzymatic antioxidants, vitamin-C and vitamin-E play vital role in preventing oxidative stress. Ascorbic acid (vitamin C) is the major essential antioxidant [20, 21]. It is present extracellularly in high concentration in the blood plasma. The depleted levels of vitamin-C in the CS exposed animals might be either increased turn over of the vitamin due to increased oxidative stress or increased consumption during recycling of vitamin E or ß carotene that are directly oxidized in the course of scavenging the free radicals and reactive oxidant species (ROS) that are generated due to CS. The tocopherol (vitamin-E) has been known as dietary antioxidant for a long time and can eliminate free radical damage. The depleted plasma vitamin E levels in CS exposed group indicative of increased oxidative stress.

Conclusion:

In conclusion the present study revealed that the CS exposure induced deranged antioxidant defense mechanism leading to increased oxidative stress in albino mice.

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