FREE RADICAL GENERATION AND LIPID PEROXIDATION AMONG THE DRY CELL INDUSTRY WORKERS EXPOSED TO CARBON

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ABSTRACT

A total of 75 healthy male dry cell industry workers exposed to carbon for 5, 8 and 10 years were compared with 48 controls matched for age and economic status with respect to free radical generation, lipid peroxidation and antioxidant levels. Lymphocytes were separated from the whole blood and used for estimation of free radicals and antioxidants. Plasma lipid peroxidation products were estimated in the form of thiobarbituric acid reactive substances. Significant increase in free radical generation and lipid peroxidation products were observed in carbon exposed population than controls, and the increase was found to be significant with increase in the period of exposure. The levels of antioxidants i.e., superoxide dismutase (SOD) and catalase were found to decrease with the increase in exposure to carbon in industrial workers. These results suggest that exposure to carbon augments free radical generation, lipid peroxidation and promotes decline in antioxidant enzymes.

KEY WORDS : Free radicals, Lipid peroxidation, Superoxide dismutase, Catalase, Carbon exposure.

INTRODUCTION

Reactive oxygen species play an important role in various kinds of cell toxicity, including cytotoxicity, chromosomal aberrations, sister-chromatid exchanges, lipid peroxidation as well as carcinogenesis (1). Majority of carcinogens are found either to be free radicals involved in free radical reaction or ones that generate free radical intermediates (2). Free radicals induced by radiation, damage the sugar phosphate chains of DNA and cause strand breaks (3). The free radicals generated by metal ions also promote lipid peroxidation and DNA damage (4).

Exposure of cells to hexavalent chromium or divalent nickel significantly increases the frequency of DNA strand breakage and protein cross linking (5). *In vitro*, welding fumes produced during manual metal-arc and metal-active gas welding, increase the frequency of sister-chromatid exchange and chromosomal aberration (6). It has been postulated that antioxidants like vitamin E, superoxide dismutase (SOD), catalase (CAT) and turmeric components significantly protect the DNA damage caused by reactive oxygen species induced by smoke condensate (7). Studies from our laboratory show that exposure to various environmental toxicants and industrial wastages elevated the levels of lipid peroxidation products, lipids and lipoproteins, with a decline in antioxidant status (8,9).

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During the past two decades there have been an increasing number of reports on genotoxicity and its role in disease (10). Because of the extensive occupational exposure to humans, much attention has been given to this end. The objective of this study is to evaluate the effect of carbon exposure on free radical generation, lipid peroxidation, and antioxidant defense systems in dry cell industry workers.

MATERIALS AND METHODS

Subjects

Blood samples were taken from 75 men who had been employed full time (8hrs per day) as fitters exposed to carbon in the dry cell industries of Tirupati and Nellore towns of Andhra Pradesh. All were involved in the manufacturing of carbon rods. The subjects were divided into three groups, depending upon their period of exposure to carbon i.e. 5 years, 8 years and 10 years. A control group of 48 non-exposed men was included whose age distribution was standardised to those of the exposed group. The objectives of the study were explained to the subjects before obtaining their consent. Socio-economic status and manual labor was matched in both test and control groups. Body mass indices and haemoglobin levels in these two groups revealed insignificant variations (Table 1). Venous blood (10ml) was collected in the morning

from all the workers and controls into disposable vials containing EDTA. Plasma was separated from whole blood on centrifugation at 5000 rpm. Lymphocytes were separated by dextran sedimentation technique(11).

Techniques

Free radicals

Superoxide anion: Superoxide anion ($O_2^{\cdot-}$) can reduce nitroblue tetrazolium (NBT) to the insoluble formazan(12). The ability to reduce NBT was assayed by incubating lymphocytes with 0.1% NBT dissolved in PBS (phosphate buffered saline) for 20 min at 37°C. The assay was terminated by adding 0.6 ml of glacial acetic acid and the extracted NBT dye was read at 560 nm.

Hydrogen peroxide: Hydrogen peroxide (H_2O_2) released by lymphocytes was estimated by the horse-radish peroxidase method(13). To the lymphocytes 0.5 ml of phenol red solution (1%) containing peroxidase enzyme (3.75 units/assay) was added and incubated at 37°C for 20 min. The reaction was terminated by adding catalase (150 units/assay) and the optical density readings were taken at 610 nm. 1×10^6 lymphocyte cells/assay were taken for both superoxide anion and H_2O_2 assay.

Table 1. Detailed history of carbon exposed and non- exposed populations.

S. No.	Population	Sample size	Mean age (yrs)	Duration of exposure	Hb values g/dl	BMI W/H ²
1.	Control	48	34.35 ±6.03	—	12.91 ±1.41	19.81 ±2.95
2.	Carbon exposed	25	36.92 ±4.12	5years	14.03 ±2.12	20.55 ±3.14
3	"	25	35.14 ±6.72	8 years	13.25 ±1.79	19.69 ±2.17
4	"	25	35.60 ±4.91	10 years	13.55 ±2.01	21.01 ±3.04

Data as Mean ± S.D.

Antioxidants

Superoxide dismutase : Superoxide dismutase activity was assayed according to the method of Misra & Fridovich (14). The assay medium contained 50 mM sodium carbonate, bicarbonate buffer (pH, 9.8), 0.1 mM EDTA, 0.6 mM adrenaline in a total volume of 3 ml. Adrenaline was the last component to be added and the adrenochrome formed in the four minutes was recorded at 470 nm. One unit of SOD activity was defined as the amount of enzyme needed to cause 50% inhibition of adrenaline autoxidation at pH 9.8.

Catalase : Catalase assay was carried out by the method of Beer and Sizer(15). The decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240 nm.

Lipid peroxidation products : Thiobarbituric acid was added to plasma sample under acidic condition, and the absorption of color that developed after heating was estimated spectrophotometrically at 535 nm(16). 1, 1, 3, 3-tetraethoxypropane was used as internal standard and the concentration was expressed in nmol of malondialdehyde per ml of plasma.

RESULTS AND DISCUSSION

Superoxide, hydrogen peroxide, lipid peroxidation products and antioxidant enzymes by blood mononuclear cells obtained from carbon exposed and non-exposed population were shown in Table 2. Superoxide anion and H_2O_2 generation were found to be significantly elevated in carbon exposed than the non exposed population, whereas the increase in antioxidant enzymes such as SOD and CAT was insignificant. There is an increase in the formation of lipid peroxidation products following exposure to carbon.

Mean production of free radicals, TBARS and antioxidants were calculated for each of the three groups of workers exposed to carbon for 5, 8 and 10 years and shown in Figs. 1 and 2. It is evident from these results that there is an increase in the generation of superoxide anion and H_2O_2 , with increase in the period of exposure to carbon. The same trend was also observed with regard to the formation of lipid peroxidation products. But, in the case of antioxidant levels the picture is found to be reverse.

The results of the present study clearly show that there is an increase *in vitro* free radical

Table 2. Superoxide anion, hydrogen peroxide, lipid peroxidation and antioxidant enzymes in carbon exposed and non-exposed populations.

S. No.	Parameter	Carbon exposed population n = 75	Non exposed population n = 48
1.	Superoxide anion μ moles/ 1×10^6 cells	4.25 ± 2.12	2.80* ± 1.75
2.	Hydrogen peroxide μ moles/ 1×10^6 cells	3.71 ± 2.20	1.56* ± 1.19
3.	Lipid peroxidation products nmol/ml plasma	4.09 ± 1.12	3.03* ± 1.45
4.	Superoxide dismutase U/mg protein	60.71 ± 27.04	57.83 ± 21.83
5.	Catalase U/mg protein	41.59 ± 23.50	40.73 ± 23.50

n = sample size * p < 0.05.

Fig. 1: Free radical generation by blood lymphocytes and levels of lipid peroxidation products in plasma of carbon exposed population. Differences between the exposure groups were significant at $P < 0.05$.

Fig. 2: Lymphocyte antioxidant levels of carbon exposed population. Differences between the exposure groups were significant at $P < 0.05$.

generation of lymphoid cells in carbon exposed population. This is supported by the observation that there is an increase in the levels of TBARS with exposure to carbon. Other studies show an increased generation of superoxide anion and H_2O_2 , with elevated oxidative damage products such as protein carbonyls, lipofuscin and n-pentane(17). Population exposed to various industrial toxicants also exhibit higher hydroxyl radicals, DNA damage and sister chromatid exchange(18). These and our results suggest that exposure to carbon, may also increase the risk for carcinogenesis and other degenerative diseases(19). The depletion of lymphocyte antioxidant enzymes i.e., superoxide dismutase and catalase in the carbon exposed population, is probably secondary to the oxidative damage. Since exposure to toxicants is associated with free

radical mediated tissue damage, the antioxidants should be in greater demand during exposure(20). Similar results were obtained by Reddy et al(21) on Finnish iron foundry workers exposed to polycyclic aromatic hydrocarbons. In conclusion, results of this study lead to the hypothesis that exposure to carbon augment in a dose dependent fashion leads to free radical generation and lipid peroxidation.

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