Effect of cigarette smoke on lipid peroxidation and antioxidant enzymes in albino rat

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Received: September 1998; revised 3 June 1999

Effect of cigarette smoke on lipid peroxidation (LPX) and antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione-S-transferase (GST) in various organs like brain, heart, lung, liver and kidney of the albino rats exposed to cigarette smoke for 30 min/day for a period of 30 days was assayed. It was observed that the lipid peroxide levels in liver, lung and kidney were enhanced in case of animals exposed to cigarette smoke, whereas brain and heart did not show any change as compared to control animals. The activity of the antioxidant enzymes was also elevated in liver, lung and kidney of the test animals whereas, brain and heart did not show any change in the activity of all of these antioxidant enzymes except glutathione-S-transferase which was increased in brain also. The level of reduced glutathione (GSH) was lowered in liver, lung and kidney of the tested animals when compared with the control animals but there was no significant change in brain and heart. The results of our study suggest that cigarette smoke induces lipid peroxidation in liver, lung and kidney, and the antioxidant enzymes levels were enhanced in order to protect these tissues against the deleterious effect of the oxygen derived free radicals. The depletion of reduced glutathione in these organs could be due to its utilization by the tissues to mop off the free radicals.

Lipid peroxidation (LPX) has gained more importance nowadays because of its involvement in pathogenesis of many diseases like atherosclerosis 1, cancer 2, diabetes mellitus 3 and myocardial infarction 4 and also in ageing. 5 . Free radicals or reactive oxygen species (ROS) are produced in vivo from various biochemical reactions and also from the respiratory chain as a result of occasional leakage. 6 . These free radicals are the main culprits in lipid peroxidation. A free radical may be an atom or a molecule with one or more unpaired electrons like superoxide anion radical, hydroxyl radical, peroxyl radical and alkoxyl radical. The free radicals are capable of independent existence and can cause oxidative tissue damage. 7 . The non-radical oxidants like hydrogen peroxide and hypochlorous acid which do not possess unpaired electrons are also capable of initiating oxidative tissue damage. 8 . Oxidative stress can increase the rate of production of free radicals and hence induces lipid peroxidation. Antioxidants are natural defense mechanisms existing in our system and these are capable of scavenging the deleterious free radicals. These antioxidants are of three types: (a) antioxidant enzymes like superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), glutathione peroxidase (EC 1.11.1.9), glutathione-S-transferase (EC 2.5.1.18) etc., (b) Antioxidant vitamins like beta carotene (precursor of vitamin A), alpha tocopherol (vitamin E) and ascorbic acid (vitamin C) and (c) Non protein thiol/ reduced glutathione. The deleterious effect of the free radicals are kept under check by a delicate balance between the rate of their production and the rate of their elimination by the different defense mechanisms, and any shift in this delicate balance will lead to cellular damage. 9 . Cigarette smoke is produced by incomplete combustion of tobacco. It is a heterogeneous aerosol containing more than 4000 substances. 10 . Cigarette smoke contains various cytotoxic, mutagenic and carcinoogenic agents like biphenyl and polycyclic aromatic hydrocarbons. Besides a large variety of toxic compounds, it also contains many oxidants like oxygen, nitrous oxide etc and free radicals. Hence, it is capable of initiating or promoting oxidative damage. 11 . Though there are lot of reports regarding effect of cigarette smoke on lipid peroxidation and antioxidant enzymes, the effect of cigarette smoke on lipid peroxidation and antioxidant enzymes in various organs are lacking. Hence, we have undertaken this study to investigate

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various organs under oxidative stress due to cigarette smoke.

Materials and Methods

Bovine serum albumin, 5, 5-dithio-bis-(2-nitrobenzoic acid), glutathione-reduced form, glutathione reductase, NADH-reduced form (disodium salt), NADPH-reduced form (tetrasodium salt) and 1, 1, 3, 3-tetramethoxy propane were purchased from Sigma Chemical, St. Louis, USA. Hydrogen peroxide solution (30%) was obtained from Qualigen Fine Chemicals, Bombay. 1-Chloro-2, 4-dinitrobenzene (CDNB), EDTA, Folin & Crocetean's phenol reagent, thiobarbituric acid and trichloroacetic acid were purchased from Loba-Chemie, Bombay. All other chemicals were of analytical grade and purchased locally.

Inbred adult male albino rats weighing from 200-250 g from our animal house were used for the experiment. Animals (5 rats/cage) were kept in polypropylene cages of size 43.5x29.0x16.0 cm and fed with standard pellet diet supplied by National Institute of Nutrition, Hyderabad and water ad libitum. Animals were divided in to two groups each group comprised of 10 animals. Group I served as control and group II served as test animals. The animals were exposed to cigarette smoke by keeping a bottom less rectangular metal container on top of the polypropylene cage containing rats. This metallic container contains two holes of about 3 cm diameter, one in the front and the other at the back of the container. A burning cigarette was introduced through one hole and air through another hole. The animals were exposed to the smoke of one burning cigarette for 15 min, twice daily, at the same time every day, morning and evening (10.00 A.M. and 4.00 P.M.) for 30 days. The same brand of a locally available cigarette was used throughout this experiment. Animals were sacrificed at the end of the experimental period by cervical dislocation, the organs like brain, heart, liver, lung, and kidney were dissected out, washed in ice cold PBS and frozen immediately in deep freezer until further use. The organs were minced and 10% homogenate (1g, w/v) in 50 mM, phosphate buffer, pH 7.4, in cold was prepared using teflon-glass homogeniser. The homogenate was centrifuged at 1000 g for 15 min, the supernatant was stored at 4°C and used for various biochemical assays.

The lipid peroxide level in various tissue homogenates was assayed in terms of thiobarbituric acid reactive substances (TBARS)/malondialdehyde (MDA) using 1, 1, 3, 3-tetramethoxy propane as standard. The lipid peroxidation was expressed as μmole of MDA formed per mg protein. Antioxidant enzymes such as catalase, glutathione peroxidase, glutathione-S-transferase and superoxide dismutase were assayed in various tissue homogenates. Total protein in various tissue homogenates was assayed by the Lowry’s method. Mean and standard deviation values were calculated and statistical significance of difference between the control and test groups was calculated using Student’s t test.

Results and Discussion

Table 1 shows the effect of cigarette smoke on lipid peroxide level in liver, lung, kidney, heart and brain of rat. The lipid peroxide level was increased in liver, lung and kidney by 109, 67 and 51% respectively over the control values. Whereas, brain and heart did not show any significant change. Elevation in catalase activity in liver, lung and kidney of the test animals over the control animals by 61, 128 and 39% respectively was observed as shown in Table 2 and this table also shows increase in glutathione peroxidase activity in liver, lung and kidney of test animals by 157, 38 and 25% respectively over the control animals. Increase in glutathione-S-transferase activity in liver, lung, kidney and brain of test animals by 160, 18, 35 and 44% respectively over the control animals was noticed as shown in table 2. Superoxide dismutase activity was also increased in liver, lung and kidney of test animals by 21, 42 and 55% respectively over the control values as depicted in Table 2.

The results show that the activity of the antioxidant enzymes were increased in all tissues studied except heart and brain with the exception that the glutathione-S-transferase was also increased (44%) in brain as shown in Table 2. The levels of glutathione was decreased in liver, lung and kidney by 41, 23 and 28% respectively in test animals when compared with the control animals as shown in Table 1.

The results of our study clearly show that the cigarette smoke induces lipid peroxidation in liver, lung and kidney. However, there was no alteration in lipid peroxide levels in brain and heart. There was a tremendous increase (109%) in lipid peroxidation in
It has been suggested that the metabolism of toxic compounds including free radicals mainly, though not exclusively, occurs in liver. The metabolites from liver may diffuse into various extrahepatic tissues, causing lipid peroxidation and cellular injury. In lung tissue also there was a marked increase (67%) in lipid peroxidation level. Though the lung is the first target organ for cigarette smoke and its massive surface area also makes it a major target organ for the oxidative stress due to cigarette

### Table 1—Effect of cigarette smoke on lipid peroxidation and level of GSH in various organs of rat

<table>
<thead>
<tr>
<th>Organ</th>
<th>Lipid peroxidation</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>Liver</td>
<td>2.618±0.582</td>
<td>5.471±0.782* (109%↑)</td>
</tr>
<tr>
<td>Lung</td>
<td>2.084±0.437</td>
<td>3.476±0.759* (67%↑)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.326±0.445</td>
<td>3.507±0.445** (51%↑)</td>
</tr>
<tr>
<td>Heart</td>
<td>2.167±0.301</td>
<td>2.449±0.533 NS</td>
</tr>
<tr>
<td>Brain</td>
<td>2.975±0.368</td>
<td>2.794±0.495 NS</td>
</tr>
</tbody>
</table>

- *Expressed as nmole of MDA formed/mg protein.
- **Expressed in μg/mg protein.
- Figures in parentheses show percent increase (↑) or decrease (↓) as compared to control.
- P-value: *<0.0001; **<0.0001; NS Not significant.

### Table 2—Effect of cigarette smoke on the activities of the antioxidant enzymes in the various organs of rat

<table>
<thead>
<tr>
<th>Organ</th>
<th>Catalase</th>
<th>Glutathione peroxidase</th>
<th>Glutathione-s-transferase</th>
<th>Superoxide dismutase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>Liver</td>
<td>33.519±</td>
<td>53.861±</td>
<td>0.0413±</td>
<td>0.00347±</td>
</tr>
<tr>
<td></td>
<td>2.344</td>
<td>3.896*</td>
<td>0.05029</td>
<td>0.0192</td>
</tr>
<tr>
<td></td>
<td>(61%↑)</td>
<td>(157%↑)</td>
<td>(160%↑)</td>
<td>(21%↑)</td>
</tr>
<tr>
<td>Lung</td>
<td>11.723±</td>
<td>25.442±</td>
<td>0.0466±</td>
<td>0.0192</td>
</tr>
<tr>
<td></td>
<td>1.109</td>
<td>2.329*</td>
<td>0.00509</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td>(128%↑)</td>
<td>(38%↑)</td>
<td>(18%↑)</td>
<td>(16%↑)</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.863±</td>
<td>12.326±</td>
<td>0.0289±</td>
<td>0.0107±</td>
</tr>
<tr>
<td></td>
<td>0.924</td>
<td>0.896*</td>
<td>0.00243</td>
<td>0.0010±</td>
</tr>
<tr>
<td></td>
<td>(39%↑)</td>
<td>(35%↑)</td>
<td>(35%↑)</td>
<td>(35%↑)</td>
</tr>
<tr>
<td>Heart</td>
<td>7.788±</td>
<td>7.335±</td>
<td>0.0212±</td>
<td>0.0247±</td>
</tr>
<tr>
<td></td>
<td>0.862</td>
<td>0.635 NS</td>
<td>0.00151</td>
<td>0.00197</td>
</tr>
<tr>
<td></td>
<td>(44%↑)</td>
<td>(44%↑)</td>
<td>(44%↑)</td>
<td>(44%↑)</td>
</tr>
<tr>
<td>Brain</td>
<td>10.947±</td>
<td>10.393±</td>
<td>0.0292±</td>
<td>0.0294±</td>
</tr>
<tr>
<td></td>
<td>0.785</td>
<td>1.101 NS</td>
<td>0.00227</td>
<td>0.00226</td>
</tr>
<tr>
<td></td>
<td>(44%↑)</td>
<td>(44%↑)</td>
<td>(44%↑)</td>
<td>(44%↑)</td>
</tr>
</tbody>
</table>

- One unit of enzyme activity is the amount of the enzyme required to disproportionate hydrogen peroxide at the rate of 10⁻³ absorption/sec.
- One unit of enzyme activity is defined as the amount of the enzyme required to oxidize 1 μ mole of NADPH/min.
- One unit of enzyme activity is the amount of the enzyme required to conjugate 1 μ mole of CDNB with GSH/min.
- One unit of enzyme activity is defined as the amount of the enzyme required to inhibit the optical density at 560 nm of chromogen production by 50%/min.

Figures in parentheses show the percent increase (↑) as compared to control.

P-value: *<0.00001; **<0.0001; †<0.001; ‡<0.01
smoke, the increase in lipid peroxidation is less than liver tissue. It shows that the effect of cigarette smoke on lung is less than liver. The mucin in the fluids lining the respiratory tract presumably provide the initial as well as additional defense mechanism against the oxidative stress so, it could minimise the impact of oxidative stress due to cigarette smoke on the lung that is why the lipid peroxidation level in lung is less than liver. Besides, mucin can reinforce the antioxidants present in the fluids lining the respiratory tract. Gupta et al. already reported that cigarette smoke increased lipid peroxidation in the rat lung but there was no change in the activity of the various antioxidant enzymes. They also reported that the activity of only superoxide dismutase was increased significantly by the cigarette smoke in the rat liver. Kidney, one of the important excretory organs, also showed an increase (51%) in lipid peroxidation next to liver and lung. The study showed that cigarette smoke did not induce lipid peroxidation in heart and brain, it suggests the existence of a more potent antagonistic mechanism in these vital organs anyhow, further study is required to substantiate this view. It has also been reported that the myocardium utilizes glucose in preference to fatty acids during stress for its energy requirement and this decreased utilization of fatty acids by the myocardial tissue may be one of the reasons for no change in lipid peroxide level in the heart.

The present study revealed that the activity of the antioxidant enzymes were increased in liver, lung and kidney of the rats exposed to cigarette smoke. The increase in activity of the glutathione peroxidase and related enzyme systems in the lung tissue of the rat exposed to cigarette smoke was also reported by York et al. The antioxidant enzymes can detoxify the deleterious reactive oxygen species so, increase in the level of these enzymes could be a defense mechanism against the free radicals. The enzyme levels are elevated in the various tissues in order to cope with the tremendous increase in the production of the reactive oxygen metabolites under the oxidative stress. There was no increase in lipid peroxidation in brain tissue but only glutathione-transferase activity was elevated. It appears that the lipid peroxidation induced by cigarette smoke in the brain tissue was so minimal so that it did not warrant an array of antioxidant enzymes to cope with. So, increase in activity of only one antioxidant enzyme (GST) was adequate to keep the lipid peroxidation under control in the brain tissue. Reduced glutathione plays very important role in various biochemical process including lipid peroxidation and it provides a defense mechanism for tissues against the reactive oxygen species. GSH also forms one of the substrates for the two antioxidant enzymes, glutathione peroxidase and glutathione-s-transferase. The level of glutathione was found to be reduced in liver (41%), lung (23%) and kidney (28%) of the test animals whereas, there was no significant change in brain and heart. The decrease in the level of glutathione in kidney of the rats exposed to cigarette smoke was also reported by Anand et al. The depletion of glutathione was associated with increase in lipid peroxidation in all these organs. The decrease in glutathione level in these tissues may be a consequence of enhanced utilization of this compound by the antioxidant enzymes, glutathione peroxidase and glutathione-s-transferase. The maximum depletion (41%) of glutathione was observed in liver and it could be attributed to the efflux of glutathione from liver to various extra hepatic tissues for their protection against the reactive oxygen metabolites besides it’s consumption by the antioxidant enzymes as stated already. Thus, we have noticed a direct correlation between increase in lipid peroxidation and decrease in glutathione level. Similar observation has been made already by various workers under different stress conditions.

To conclude, our findings clearly show that though there was an increase in the activity of antioxidant enzymes in liver, lung and kidney to protect them against the lipid peroxidation induced by the cigarette smoke, it was not adequate to cope with the copious production of free radicals, that is the reason why the lipid peroxidation could not be kept under control in these tissues. The increase in activity of these antioxidant enzymes also increased the utilisation of glutathione hence, there was a concomitant decrease in the level of glutathione in these tissues.

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