Effects of Benzo(a)pyrene and Ethanol on Oxidative Stress of Brain, Lung Tissues and Lung Morphology in Rats

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Abstract

Ethanol and benzo(a)pyrene cause lipid peroxidation either by producing the reactive oxygen species or decreasing the activities of antioxidant enzymes that lead to cellular damage and cellular dysfunction. In this study, we investigated both physiological and histological changes in lung and physiological changes in brain after the administration of benzo(a)pyrene and ethanol both separately and together. Male Sprague Dawley rats were divided into four groups, each containing seven rats as follows: Group I (control), group II (benzo(a)pyrene, [B(a)P]), group III ([B(a)P] + ethanol (EtOH)) and group IV (EtOH). Superoxide dismutase (SOD) activity, levels of glutathione (GSH), malondialdehyde (MDA) as well as histological examinations were evaluated to demonstrate the damages in lung and brain tissues following the administration of [B(a)P] and EtOH. SOD activities of lung and brain tissues in group II and group III decreased significantly, compared to that in group I and group IV, respectively. GSH levels of both the lung and brain tissues in the experimental groups were lower when compared to the control group. MDA levels of lung tissues in group II and III were significantly higher than that in the control group. Moreover, MDA levels in the brain tissues of all the experimental groups were higher than that in the control group, but these values were only significantly higher in group II and IV. In the second study group, [B(a)P] administration resulted in lung damage. On the other hand, lung tissue of the third experimental group showed moderate damage, and lung tissues of the fourth group was less severely damaged. [B(a)P] and EtOH administration alone or together caused changes in the GSH, MDA levels and SOD enzyme activity in the lung and brain tissues. We also noted that [B(a)P] and EtOH caused different degrees of histological changes.

Key Words: brain, lung, ethanol, benzo(a)pyrene, SOD, MDA

Introduction

A human being can be simultaneously exposed to many hazardous chemical substances in his or her natural as well as occupational living environments and by consuming canned food (7, 9, 16). Many pollutants are known to induce the formation of reactive oxygen species in an organism during biotransformation via the redox-cycle (7, 27, 28). Evidence showed that free radicals and reactive oxygen species can contribute to various diseases including cancer, neurodegenerative disorders, cataract and AIDS etc. (4, 21). On the other hand, a living organism has antioxidant enzymes which are essential in preventing the cellular damage caused by the free radical and free radical mediated lipid peroxidation, but many chemical substances can disrupt
this balance in favor of oxidant substances (23, 28).

Lung cancer is the most common type of cancer in both men and women, accounting for 29% of all cancer related deaths (20). Previous studies have reported that occupational exposure to polycyclic aromatic hydrocarbons is one of the main suspected factors of excessive mortality by lung cancer (5, 7). [B(a)P] is a polycyclic aromatic hydrocarbon compound which is present in the natural environment. It is a common product resulting from incomplete combustion of organic matters such as cigarettes, traffic clustering and industrial production (25). [B(a)P] is an indirect toxicant substance, but it has to be metabolized for soluble metabolites for toxic effect. It affects cells in many ways, including covalent modifications of DNA, participation in redox cycling, alteration of cellular signaling pathways and growth factor signaling pathways. During metabolism of polycyclic aromatic hydrocarbon, highly reactive oxygen species such as superoxide anion (O2⁻) could be generated (6, 7, 25). EtOH permeate all tissues of the body and affects most vital functions of all organs. Acute or chronic alcohol administration to experimental animals could alter the balance between oxidant substances and antioxidant enzymes in favor of oxidant substances, by inducing pro-oxidants or by decreasing the antioxidant enzyme levels or by a combination of both. Therefore, many toxic effects of EtOH are linked to its metabolism. In other words, ethanol causes production of free radicals or reactive oxygen species depending on its metabolism (17, 23, 26). Like [B(a)P], EtOH can contribute to a variety of diseases including cancer, neurodegenerative disease, cataract, etc. (1, 18). Previous reports suggest that there is a positive relation between breast cancer and alcohol ingestion but its precise mechanism is still unclear (2, 22).

In the present study, firstly we aimed to examine the oxidative effect of [B(a)P] or EtOH administration on rat’s lung and brain tissues. Secondly, we performed a detailed histological examination of the lung tissues.

Materials and Methods

Animals and Treatment

Male Sprague Dawley rats (155-220 g) were used in this study. The rats were bred in Animal Laboratory Unit at Inonu University and kept for two days before the experiments for acclimatization to the experimental conditions with free access to food (standard diet) and water, but food was withdrawn 24 h before the experiment. The experimental protocol was conducted after obtaining permission from local ethical committee (04.26.2004/2).

The animals were divided into four groups of seven and maintained as follows:

In the control group 1 (C), the rats received intraperitoneal (i.p.) corn oil; in group 2 the rats received i.p 200 mg/kg [B(a)P] with corn oil; in group 3 EtOH were administered to the rats at a dosage of 3 g/kg body weight (bw) i.p. and 200 mg/kg B[a]P with corn oil; in group 4 the rats were given EtOH at a dosage of 3 g/kg (bw) i.p.

EtOH was administered 2 h before the injection of [B(a)P] in [B(a)P]+EtOH group. Twenty-four h after the injection of [B(a)P], the rats were sacrificed. Lung and brain of each rat were promptly removed and a part was used to assess the activity of SOD, and the concentrations of GSH and MDA in the tissues.

Assessment of Malondialdehyde (MDA)

The MDA concentrations of the lung and brain homogenates were assessed spectrophotometrically by measuring the presence of thiobarbituric acid reactive substances. Three ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid solution were added to 0.5 ml of plasma pipetted into a tube. The mixture was heated in boiling water for 45 min. After cooling, the color was extracted into 4 ml of n-butanol. The absorbance was measured in spectrophotometer (shimadzu UV-1601, Kyoto, Japan) at 532 nm. The amount of lipid peroxides was calculated as thiobarbituric acid reactive substances of lipid peroxidation. The results were expressed as nanomole per g wet tissue (nmol/g wet tissue) from a standard calibration curve prepared with a standard solution of 1, 1, 3, 3-tetramethoxypropane (13).

Assessment of Superoxide Dismutase Activity (SOD)

Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was assessed using the method of Sun, et al. (24). The method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as units per milligram protein (U/mg protein).

Assessment of Glutathione (GSH)

Concentration of Glutathione was assessed by a spectrophotometric method. The principle of the method is based on reaction taking place among 5,5'-dithiobis-(2-nitrobenzoic acid, DTNB) and thiols. Results are expressed as nmol/mg tissue (3).

Assessment of Protein Content

Protein were measured at all stages according to the Lowry’s method (12).
Histological Examination

For histological evaluation, the peripheral lung tissue sections were fixed in phosphate buffered (10% formalin), dehydrated and embedded in paraffin. Tissues were sectioned at 5 \( \mu \)m, stained with hematoxylin and eosin (H&E).

Statistical Analysis

The data obtained were analyzed by one-way analysis of variance (ANOVA) and Tukey-Kramer posthoc tests for the significant interrelation between the various groups using Instat computer software. \( P < 0.05 \) was considered to be significant from the control.

Results

SOD enzyme activities, GSH and MDA concentrations in lung and brain tissues of the rats of control and experimental groups are shown in Table 1.

There was a significant increase in concentration of MDA in the tissues of rats which received B(a)P and EtOH either separately or together when compared to the control rats. However, MDA concentration of lung tissue of group IV did not differ from that of control group.

Table 1. Effect of ethanol and benzo(a)pyrene on lipid peroxidation enzymatic and non-enzymatic antioxidant in lung and brain of the rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parameters</th>
<th>Control</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung MDA (nmol/g tissue)</td>
<td>93.9 ± 5.5</td>
<td>124.4 ± 4.9*</td>
<td>134.4 ± 3.4NS</td>
<td>91.5 ± 4.4NS,b+</td>
<td></td>
</tr>
<tr>
<td>SOD (U/mg prot)</td>
<td>15.53 ± 0.85</td>
<td>10.75 ± 0.37*</td>
<td>13.14 ± 0.77bNS</td>
<td>15.22 ± 0.66aNS,b#</td>
<td></td>
</tr>
<tr>
<td>GSH (nmol/g tissue)</td>
<td>0.90 ± 0.04</td>
<td>0.65 ± 0.02a</td>
<td>0.79 ± 0.04b#</td>
<td>0.79 ± 0.02aNS,b#</td>
<td></td>
</tr>
<tr>
<td>Brain MDA (nmol/g tissue)</td>
<td>388.8 ± 9.10</td>
<td>425.0 ± 6.7#</td>
<td>391.9 ± 7.5bNS</td>
<td>444.3 ± 10.2bNS</td>
<td></td>
</tr>
<tr>
<td>SOD (U/mg prot)</td>
<td>22.42 ± 0.95</td>
<td>15.77 ± 0.58a*</td>
<td>13.45 ± 0.78bNS</td>
<td>14.86 ± 0.50a,bNS</td>
<td></td>
</tr>
<tr>
<td>GSH (nmol/g tissue)</td>
<td>0.90 ± 0.03</td>
<td>0.58 ± 0.04a*</td>
<td>0.57 ± 0.03bNS</td>
<td>0.61 ± 0.02a,bNS</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Normal lung morphology of the control group. (H&E), \( \times 66 \)

Histological Examination

For histological evaluation, the peripheral lung tissue sections were fixed in phosphate buffered (10% formalin), dehydrated and embedded in paraffin. Tissues were sectioned at 5 \( \mu \)m, stained with hematoxylin and eosin (H&E).

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Results

SOD enzyme activities, GSH and MDA concentrations in lung and brain tissues of the rats of control and experimental groups are shown in Table 1.

There was a significant increase in concentration of MDA in the tissues of rats which received B(a)P and EtOH either separately or together when compared to the control rats. However, MDA concentration of lung tissue of group IV did not differ from that of control group. Table 1 shows the activities of enzymatic antioxidant SOD in lung and brain tissues of experimental animals. Only EtOH administration did not cause any significant decrease in the lung tissue, whereas either treatment of [B(a)P] or [B(a)P] + EtOH significantly decreased the SOD activities when compared with the control group (\( P < 0.001 \)). In the brain tissues, SOD activities of experimental groups were lower than that of the control group.

Levels of GSH in lung and brain tissues of experimental groups are presented in Table 1. When compared with control group, there was a significant decrease in GSH concentrations of both tissues of rats to which either [B(a)P] or [B(a)P] plus EtOH or only EtOH had been administered.

Light Microscopic Examination Results

Morphological structure was normal in the control group (Fig. 1), but there were mild changes in the EtOH group (Fig. 2), moderate changes in the EtOH+ [B(a)P] group (Fig. 3, a and b) and severe changes in the [B(a)P] group (Fig. 4, a, b and c). Morphological changes including alveolar septal thickening, inflammatory cell infiltration, metaplasia and epithelial cell detachment of bronchi and bronchioles were clearly observed in the lung of the EtOH+ [B(a)P] (Fig. 3, a and b) and [B(a)P] (Fig. 4, a and b) groups. In addition to these findings in [B(a)P] group, some areas of the lungs showed dilation of many alveolar spaces and destruction of the septal walls (Fig. 4c). The affected alveoli were irregular and lost the normal alveolar structure. In the EtOH group morphological features were almost similar to the control group except the fact that mild interalveolar septal thickening and peribronchiolar inflammation were present (Fig. 2).
Fig. 2. Peribronchioler inflammation and slight interalveolar septal thickening are visible in the EtOH group. Notice that the epithelium of bronchioles are intact (H&E), × 66.

Fig. 3. (a) The alveolar septa were thickened significantly with infiltration of inflammatory cells. In addition desquamated epithelial cells are visible in the lumen of the bronchioles (arrows) in the EtOH+B(a)P group (H&E), × 66; (b) The bronchial epithelium of the EtOH+B(a)P group. The epithelium of the left side show metaplastic changes while the epithelium of the right side appear almost normal. (H&E), × 132.

Fig. 4. (a) Marked thickness of the alveolar septae, inflammatory cell infiltration and cellular debris in the lumen of the bronchioles are observed in the B(a)P group. (H&E), × 66; (b) Notice the pseudostratified epithelium (thick arrows) lining the bronchi transformed into stratified epithelium (thin arrows) in the B(a)P group. (H&E), × 132; (c) Alveolar space enlargement, thinning of the alveolar wall and destruction of the septal wall are apparent in the B(a)P group (H&E), × 66.
Discussion

The present study was conducted to assess the oxidative status of lung and brain of rats after the administration of either B(a)P or B(a)P plus EtOH or only EtOH. By examining the activities of SOD as well as GSH and MDA concentrations in both organs and histological structure of the lung tissues from all groups, not only did we aim to explain the probable mechanism of simultaneous action of B(a)P and EtOH, but also to evaluate whether the risk of cellular damage may be increased due to the influence of B(a)P and EtOH.

The results regarding the activities of SOD and GSH and concentration of MDA in the lung and brain tissues indicate that the administration of B(a)P or B(a)P plus EtOH or EtOH only might induce different levels of the oxidative stress.

SOD is essentially a protective enzyme which scavenges the superoxide ions produced as cellular byproducts during oxidative stress (17). Its decreased activity can lead to adverse effects because superoxide anions are extremely toxic and may accumulate in the cells. We assessed various decreases in SOD activity levels of lung and brain in the experimental groups (Table 1). These findings are parallel to literature (17, 20). Varying levels of SOD activities assessed in the tissues might be related to the repair rate of DNA damage since Kim and Lee (10) reported that SOD activities in organs of rats were inversely associated with oxidative damages to DNA and protein. Decreased activities of SOD in brains of rats of experimental groups might have been caused by the accumulation of superoxide radical and H$_2$O$_2$, thereby consuming the SOD activity. However, EtOH administration did not show the same effect on lung SOD activity. It is possible that DNA repair rate in lung tissue may not be as efficient as in brain tissue; therefore the type of the tissue might be an important factor (10). However, more details are needed to clarify the issue.

MDA is considered as an index of lipid peroxidation and produced during oxidative stress, increased concentration of which in intra- and extracellular membranes results in damage to the cells, tissues and organs. MDA concentration is an indicator which reflects level of lipid peroxidation in any organ. B(a)P or EtOH administration induced escalation of lipid peroxidation in the brain, but we did not assess the same status in the lung tissues of EtOH treated group. Increased lipid peroxidation of brain tissues from the experimental groups might be a consequence of increased formation of free radicals as well as inhibition of SOD and GSH activities by EtOH and [B(a)P] (22). Alternatively, antioxidant defence system in brain may be insufficient to provide complete protection and therefore enhances the processes of lipid peroxidation brought about by [B(a)P] or EtOH administration. That is, SOD enzyme and GSH are much lower in the central nervous system when compared to erythrocytes and peripheral tissue (8). The discrepancy between the tissues can be explained either by variations in lipid contents of the tissues or inefficiencies of their antioxidant defense systems (10, 17, 22).

MDA concentration in the lung tissues did not show significant difference between control group (I) and EtOH treated group (see Figs. 1 and 4). On the other hand, MDA concentration in lung tissues of group IV differed significantly from that of group II. It is clear that MDA concentrations are parallel to the histological changes (see Figs. 2, a, b, and c, 3, a and b). This means that high MDA concentrations indicate high lipid peroxidation concentration and as a result, oxidative damage will be greater.

GSH is the most abundant non-protein thiol source in the cells and serves many vital physiological functions including protection of cells from reactive oxygen species, detoxification of exogenous compounds, and amino acid transport (11, 14). Much of the pathology is associated with the decrease in intracellular GSH concentration (19). Therefore, GSH concentration is important for survival of the cells. It is also a substrate for glutathione peroxidase. In this study, we assessed significant changes in GSH concentrations of the lung and brain tissues of both control and experimental groups. A close relationship between decreased GSH level and increased MDA concentration has been found. These findings are partly consistent with the literature (17, 19). Many researchers have reported that administration of [B(a)P] or EtOH separately or together induced free radical formation which in turn altered antioxidant enzyme activities (1, 6, 7, 18, 25). Additionally, reactive oxygen species are removed by antioxidant enzymes. So, this status consumes and consequently changes antioxidant enzyme levels. But, the degree to which GSH concentration decreased differed between lung and brain tissue. This result might be related to activities of γ-Glutamylcysteine synthetase, and glutathione synthetase, both of which serve to synthesize GSH (14). Activities of these enzymes depend on the type of tissue in which they are present (15).

In conclusion, [B(a)P] and EtOH induced oxidative stress but the degree oxidative stress depends on the type of tissue and may be affected by the experimental duration, administration route, amount of agent administered, and animal species used. Therefore, further investigations are needed to explain the mechanisms responsible for the effect of [B(a)P] and EtOH on oxidative stress in different tissues.
References


