Pulmonary and genotoxicity of Bisphenol-A in Wistar albino rats

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ABSTRACT

Bisphenol A (BPA) is one of the common environmental endocrine disruptors with estrogenic properties and is the building block of carbonate plastic and a component of resin coatings. Wide spread use of BPA in consumer products has led to a great public concern since adverse effects of BPA on human and animal reproduction are suspected due to its estrogenic activity. The present experiment was designed to make a systematic study of experimentally induced BPA toxicity in both male and female Wistar albino rats at 500 and 250 mg /kg b.wt. to groups II & V and III & VI, respectively by mixing in sunflower oil for 12 weeks, with the objective of finding out the effect of BPA toxicity on DNA and resultant histopathological changes in lungs and trachea. DNA damage was evident as significant increase in micronuclei in poly chromatophilic erythrocytes of BPA treated groups when compared to control. Microscopically, lungs revealed congestion, fatty infiltration, peribronchial lymphoid aggregates and infiltration of eosinophils, vacuolated alveolar epithelial cells, infiltration of alveolar macrophages, hyperplasia of bronchiolar epithelium and focal alveolar epithelial cell proliferation with papillary projections and desquamated bronchial epithelial cells were found as constant lesion by the end of 12th week in a dose dependent manner.

KEY WORDS: Bisphenol A, DNA damage, lungs, micronuclei, rats

INTRODUCTION

Since last two decades, there has been increasing scientific concern and public debate regarding the adverse effect of chemical pollutants present in the environment that can interfere with the normal functioning of the endocrine system in animals and humans. Endocrine disrupting chemicals (EDCs) exposure mimics or interferes with hormone function, particularly estrogen function (Yeshvandra Verma and Suresh, 2009). The problem of man made chemicals released into the environment has also been of a great concern in recent years. A large numbers of environmental chemicals including polychlorinated biphenyls (PCBs), bisphenol A (BPA) and brominated flame retardants (BFRs) have been shown to disrupt the normal action of endogenous hormones in wild life and humans, leading to changes in hormone mediated actions (Keiko et al., 2009).

Bisphenol-A (BPA) is one of the common environmental endocrine disruptors with estrogenic properties and is the
building block of carbonate plastic and a component of resin coatings. It is being used in a wide variety of consumer products, including food and beverage packaging, compact disks, eye glass lenses, dental sealants, artificial teeth, cans, drums, reinforced pipes, adhesives, nail polish and carbonless papers used in receipts making BPA a ubiquitous part of our daily life (Richard et al., 1987 and Vandenberg et al. 2007). Wide spread use of BPA in consumer products has led to great public concern since adverse effects of BPA on human and animal reproduction are suspected due to its estrogenic activity. BPA has high affinity to estrogen related receptor (ERR-γ) which may be related to its ability to function as endocrine disruptor (WHO, 2009). The present study has been aimed at investigating the effects of BPA on DNA damage and lungs in rats.

**MATERIALS AND METHODS**

The present research work was carried out in Department of Veterinary Pathology, College of Veterinary Science, Tirupati during the year 2010 – 2011. Wistar albino rats with body weight around 150g were used for the present experiment.

Rats were acclimatized to the experimental conditions for one week, after acclimatization the animals were grouped and housed in standard poly propylene rat cages (three rats 1 cage) during the experiment. They were maintained at 25°C ±1°C and a 12:12 hour interval light / dark cycle throughout the experimental period for 12 weeks by taking necessary precautions and providing standard laboratory hygienic conditions and laboratory animal feed and water ad libitum. The approval of the institutional animal ethical committee was obtained prior to commencement of the experiment.

The Bisphenol A (4, 4’ – dihydroxy – 2, 2 – diphenyl propane) with a laboratory reagent grade was procured from the Sd Fine chemicals, Bombay with 98.7% purity. In the present experiment, Bisphenol A was fed to Wistar albino rats at 500mg / kg b.wt. and 250 mg/kg b.wt. to male (Groups II &III) and female rats (Groups V & VI) respectively by mixing in refined sunflower oil for 12 weeks. To the Groups I (male) and IV (female) rats, sunflower oil was given and were kept as controls. Six rats from each group were randomly sacrificed at every fortnight intervals after starting the experiment i.e. 2nd, 4th, 6th, 8th, 10th and 12th weeks.

**Genotoxicity studies:** DNA damage was assessed by micronuclear assay.

**Micronucleus assay**

At each sacrifice, lower abdomen and limbs were incised and the femur were cleaned and separated from the hip joint. The ends of the femur were trimmed and a blunt needle was pushed to pierce the marrow cavity. Bone marrow was flushed out into 0.9% saline. The suspension was made up to 5 ml in a centrifuge tube and centrifuged at 1000 rpm for 10 minutes. The clear supernatant was discarded, 2-3 drops of fetal calf serum were added and the pellet was mixed thoroughly. Smears were drawn on to precleaned slides using a drop of the suspension. The slides were air dried and fixed in absolute methanol. The slides were then stained with 0.125% acridine orange in Sorensen’s buffer (pH 6.8) and observed under fluorescent microscope by using green filter at 40x objective piece and counted 2000 B.M cells /smear and the no. of micro nucleated cells out of 2000 PCE (Polychromatophilic Erythrocytes) and NEC
(Normochromatophilic Erythrocytes) were counted per animal (Manjula et al., 2006).

A detailed postmortem examination was conducted on all the sacrificed rats in all the experimental groups and representative tissue pieces from thyroid gland were collected and preserved in 10% neutral buffered formalin for histopathological studies. Fixed tissues were processed by routine paraffin embedding technique. Sections of 5-6 microns thickness were cut and were stained with routine Haematoxylin and Eosin method (H&E). Special stains were employed where ever it was necessary. The results were analysed statistically by performing oneway ANOVA using data analysis tool pack.

RESULTS AND DISCUSSION

Genotoxicity: Micronuclear assay

The mean values of micronuclear assay in different treated groups (I to VI) were 2.25, 12.33, 9.99 2.11, 14.04 and 9.83 (No. of micronucleated cells/ 2000 PCE), respectively and are shown in Table.1. Statistically, striking increase was recorded in micronuclear assay values in BPA fed male and female groups when compared to corresponding control (Groups I & IV). No significant difference was noticed in values of micronuclear assay among male and female treated animals. Similar findings were recorded by Tiwari et al. (2007). Increased micronuclei formation may be due to DNA adduct formation (Tsutsui et al., 1998) and may be due to induction of point mutations, which lead to breaks in double standard DNA (Ruth et al., 2007).

Gross pathology

Lungs of the rats from the BPA treated groups revealed moderate to severe congestion, edema and emphysema (Fig.1) throughout the experimental period and emphysema was more conspicuous by the end of 12th week.

Table 1: Mean values of micronuclei assay (No. of micronucleated cells/2000 PCE) in rats of different experimental groups

<table>
<thead>
<tr>
<th>Experiment period (weeks)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
<th>Group VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.80</td>
<td>6.00</td>
<td>3.30</td>
<td>1.67</td>
<td>5.25</td>
<td>2.67</td>
</tr>
<tr>
<td>4</td>
<td>2.50</td>
<td>7.30</td>
<td>4.25</td>
<td>2.30</td>
<td>9.67</td>
<td>6.00</td>
</tr>
<tr>
<td>6</td>
<td>1.90</td>
<td>10.00</td>
<td>9.00</td>
<td>2.71</td>
<td>13.33</td>
<td>7.67</td>
</tr>
<tr>
<td>8</td>
<td>1.80</td>
<td>13.00</td>
<td>12.12</td>
<td>2.50</td>
<td>16.00</td>
<td>11.67</td>
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<tr>
<td>10</td>
<td>1.70</td>
<td>15.67</td>
<td>14.13</td>
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<tr>
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<td>22.00</td>
<td>17.12</td>
<td>1.67</td>
<td>21.00</td>
<td>17.30</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>2.25 ± 0.21a</td>
<td>12.33 ± 2.42de</td>
<td>9.99 ± 2.24cde</td>
<td>2.11 ± 0.18a</td>
<td>14.04 ± 2.41e</td>
<td>9.83 ± 2.19bcde</td>
</tr>
</tbody>
</table>
Histopathology

Group II

Microscopically, lungs of Group II animals revealed congestion, focal perivascular and bronchiolar lymphoid aggregates and mono nuclear cell (MNC) infiltration in between alveoli in majority of animals. Alveolar congestion, mild to moderate emphysema and thickened blood vessels were noticed by the end of two weeks. In addition peribronchial fatty infiltration (Fig. 2), hyperplasia of bronchial epithelium with desquamated cells in lumen and mucosal droplets, peribronchial and perivascular eosinophil infiltration (Fig. 3), widening of interstitial spaces with MNC and RBC, bronchiectasis and increased size of peribronchial lymphoid aggregate were evident by the end of 4th week. From 6th week onwards, bronchiectasis, moderate infiltration of eosinophils around bronchi and in between alveoli, peribronchial and perivascular connective tissue proliferation, vacuolated alveolar epithelial cells (Fig. 4), mild infiltration of alveolar macrophages and plasma cells were evident constantly in all animals.

During 10th week few animals from group II revealed desquamated bronchiolar epithelial cells into lumen, bronchitis characterized by MNC infiltration, increased mucin droplets in epithelial cells (Fig. 5), more hyperplasia of bronchiolar epithelium, stenosed blood vessels and emphysema with giant alveoli formation. In focal areas hyperplasia of alveolar epithelial cells giving papillary projections with few giant cells was also evident. Dysplasia of bronchial epithelium with bronchiolar hemorrhages, capillary proliferation, infiltration of eosinophils and lymphoid aggregation and prominent papillary projections in alveoli were noticed by the end of 12th week.

Group III

Histopathologically, congestion, thickened alveolar walls with mild MNC infiltration, mild perivascular and peribronchiolar eosinophil infiltration and peribronchial fatty infiltration were noticed by the end of 2nd and 4th weeks. During 6th and 8th weeks, bronchiectasis, alveolar hemorrhages and moderate peribronchial and perivascular mononuclear cell and eosinophils infiltration, hyperplasia of bronchiolar epithelium, infiltration of alveolar macrophages and vacuolated alveolar epithelium were noticed. Mild sub epithelial infiltration of eosinophils and MNCs and stenosed blood vessels were observed by the end of 10th and 12th weeks in addition to the above changes.

Group V

The lesions were similar as described in Group II were observed but proliferation of alveolar epithelium was more prominent (Fig. 6) in female rats than in male rats.

Group VI

The similar changes as described in Group III were noticed.

Trachea

In Group II, severe mononuclear cell infiltration and eosinophils in mucosa and sub mucosa (Fig. 7), disruption of mucosa and cystic dilatation of glands were the constant features in majority of animals throughout the experimental period. By the end of 12th week increased ossification of tracheal cartilage and sub epithelial capillary proliferation (Fig. 8)
were noticed. Whereas in Group III, mild MNC infiltration in mucosa and sub mucosa was noticed. In Groups V and VI the changes were similar to those observed in Groups II & III. NTP (1982) observed congestion, pneumonia, inflammation and chronic diffuse histiocytosis in rats treated with BPA. Nitschke et al. (1988) and European Union (2003) observed hyperplasia in lining epithelium, inflammation of submucosal tissues in the anterior nasal cavities and slight to moderate hyperplasia of goblet cells in lateral nasal wall. BPA exposure will enhance the allergic sensitization, eosinophilic airway inflammation and bronchial hyperactivity and promoted the development of experimental asthma in mouse pups (Terumi et al., 2010).

CONCLUSION

DNA damage was evident as significant (P<0.05) increase in micronuclei in PCE cells of BPA treated groups as compared to control. Microscopically, lungs revealed congestion, fatty infiltration, peribronchial lymphoid aggregates and infiltration of eosinophils, vacuolated alveolar epithelial cells, infiltration of alveolar macrophages, hyperplasia of bronchiolar epithelium and focal alveolar epithelial cell proliferation with papillary projections and desquamated bronchial epithelial cells were found as constant lesion by the end of 12th week in a dose dependent manner. Increased micronucleated polychromatophilic erythrocytes indicated the genotoxic action of BPA. Fatty infiltration in lungs indicates its effect on lipid metabolism. Infiltration of eosinophils in different organs is due to hypersensitivity reaction caused by BPA. It was concluded that BPA at 500mg/Kg b.wt was highly toxic to rats.

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![Fig. 1: Group II & III: Lungs showing congestion and edema](image1)

![Fig. 2: Group II: Section showing peribronchial fatty infiltration. Oil red ‘O’ stain x 70.](image2)
Fig. 3: Group II: Peribronchial and perivascular infiltration of eosinophils (Lungs)

Fig. 4: Group II: Vacuolated alveolar epithelial cells (Lungs)

Fig. 5: Group II: Section showing increased mucin droplets in epithelial cell (Lungs)
H & E: x 280

Fig. 6: Group V: proliferation of alveolar epithelial cell (Lungs)
H & E: x 280
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