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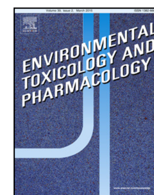

Depletion of mitochondrial enzyme system in liver, lung, brain, stomach and kidney induced by benzo(a)pyrene

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ABSTRACT

Mitochondrial dysfunction has recently received considerable attention as it plays an important role in adult human pathology caused by various drugs, endogenous agents and environmental agents. Benzo(a)pyrene (BaP), is a ubiquitous environmental contaminant mainly derived from anthropogenic activity during incomplete combustion of organic materials from various sources. The present study aimed to evaluate the effects of benzo(a)pyrene (BaP) on mitochondrial enzymes in the multiple organs including liver, lung, brain, stomach and kidney. ICR mice were exposed to different doses of BaP (2.5, 5 and 10 mg/kg body weight) through oral gavage and intraperitoneal injection treatment for 13 weeks consecutively. The induced mitochondrial damage in the examined organs was assayed in terms of significant increase in lipid peroxidation (LPO) and prominent decrease in antioxidant enzymes. Non enzymatic antioxidants and Krebs cycle's enzymes were also significantly decreased in mitochondria. Additionally, BaP induced the body growth retardation and decrease in relative liver weight, increase in relative lung, stomach, kidney and brain weights, and this was further certified through histopathological lesions. Liver and lungs were more prominently damaged by BaP. The mitochondrial depletion increased in BaP dose-dependent manner.

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1. Introduction

Mitochondria play an important role in converting organic materials into cellular energy in the adenosine triphosphate (ATP) form via oxidative phosphorylation and supply the cell with ATP to meet the bulk of needs. Studies have indicated that mitochondria are essential for cell life and decision-makers regulating cell death. Simultaneously, mitochondria are an important cellular source of oxygen radicals and are more easily affected by free radical attack to cause mitochondrial dysfunction (Kamaraj et al., 2011; Priya et al., 2011; Yang et al., 2011). Mitochondrial dysfunction has recently received considerable attention as it plays an important role in adult human pathology caused by various drugs, other endogenous agents and environmental agents (Priya et al., 2011).

Benzo(a) pyrene (BaP), a kind of polycyclic aromatic hydrocarbons (PAHs), is a ubiquitous environmental contaminant mainly derived from anthropogenic activity during incomplete combustion of organic materials from various sources (Hattemer-Frey and Travis, 1991). It is metabolically activated into phenols, epoxides, dihydrodiols, dihydrodiol epoxides, quinones, and the ultimate carcinogenic metabolite anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-BaP (BPDE) by both phase I and phase II biotransformation enzymes. Simultaneously, BaP is metabolized by cytochrome P-450 (CYP), especially CYP1A1, 1B1 epoxide hydrolase

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to *trans*-BaP-7,8 diol, then catalyzed by aldo-keto reductases (AKRs) to catechol. Following catalysis by NAD(P)H:quinone oxidoreductase l (NQO1) to δ-semiquinone anion radical and BaP-7,8-dione, excessive reactive oxygen species (ROS) are produced, including superoxide anion ($O^{2\bullet-}$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) (Palackal et al., 2001; Park et al., 2008).

ROS and other oxidants are controlled by various cellular defense mechanisms, such as enzymatic and non enzymatic antioxidants (Sivaranjani et al., 2013). Antioxidant systems of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are to eliminate the excessive ROS (Pan et al., 2006). Some of BaP metabolites are further transformed by conjugation with glutathione (GSH) that is a reaction catalyzed by glutathione S-transferases (GST). GST conjugates with glutathione to detoxify BaP within organism (Vieira et al., 2008). GST and GPx are GSH-dependent enzymes which generate glutathione disulfide (GSSG) in the process of radical detoxification. Glutathione reductase (GRD) is an enzyme which plays a crucial role for regeneration of GSH and ensures its availability to GST and GPx enzymes for their proper function (Gutierrez-Correa and Stoppani, 1997). Also, GST plays an important role in the prevention of lipid peroxidation (LPO). Recent reports have indicated that BaP could induce oxidative stress and cause LPO (Vieira et al., 2008). Non enzymatic (Vitamins A, E and C) antioxidants are able to influence redox reactions. Vitamin A is a known antioxidant and is endowed with strong ROS-scavenging property. Vitamin C has been widely reported to have the capability of protecting cells from oxidative damage for neutralizing free radicals. Vitamin E is a known radical scavenger by trapping reactive oxyradicals and preserving membrane integrity (Le Prell et al., 2007; Wang et al., 2007; Chang et al., 2007; Sivaranjani et al., 2013).

Krebs cycle (tricarboxylic acid cycle), an aerobic metabolic pathway which is ubiquitously proceeded in mitochondria, is the main way to get energy in living organisms. Krebs cycle's enzymes, including isocitrate dehydrogenase (ICDH), alpha-ketoglutarate dehydrogenase (a-KDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and aconitase, catalyzed the associated metabolites to get more ATP. Reports have indicated that these enzymes were affected by BaP associated metabolites and ROS from mitochondrial function (Venkatraman et al., 2008; Anandakumar et al., 2008; Kamaraj et al., 2011; Bubber et al., 2011; Naveenkumar et al., 2013). However, the effects of BaP on mitochondrial enzymes have rarely been studied in the multiple organs such as liver, lungs, brain, stomach and kidneys.

Therefore, this study was aimed to assess the effects of BaP on LPO, antioxidant systems and non enzymatic antioxidant status, the Krebs cycle enzymes in mitochondria in multiple organs. We also looked for other parameters like body weight, weight of the organs under study as well as histopathological changes, to see the effects of PaB administration.

2. Materials and methods

2.1. Materials

Benzo(a) pyrene (purity >95%) was purchased from Sigma chemicals, St. Louis, USA. All other chemicals were of analytical grade and were obtained from local commercial sources.

2.2. Animals and housing

Healthy male ICR mice weighing 18–22 g were obtained from the Experimental Animal Center of Xi'an Jiaotong University (Shaanxi Province, China). Animals were housed in our departmental animal house in a cross-ventilated room at 22 ± 2 °C with relative humidity of 50–60% condition and a 12-h light–dark cycle. The mice were raised with conventional laboratory feed and water. They were acclimatized for a period of 1 week before the beginning of the experimental procedures. The experiments on animals were performed according to the guidelines of the Institutional Animal Ethics Committee (McEntee and Sandgren, 2007).

2.3. Experimental design

The experimental animals were divided into two groups, the intraperitoneal injection group and the oral gavage group. Each group contained five subgroups: control, vehicle, low-dose (2.5 mg/kg b.w.), middle-dose (5 mg/kg b.w.), and high-dose (10 mg/kg b.w.) subgroups. Each subgroup contained thirteen animals. The control subgroups were left untreated, and the vehicle subgroups received equal volumes of corn oil twice a week for 13 weeks. BaP was dissolved in corn oil shortly before the experiment. Mice received BaP at the corresponding dose for the low-, middle-, and high-dose subgroups, respectively, twice a week for 13 weeks.

At the end of the experimental period (13th week), all the animals were sacrificed by cervical decapitation under ether anesthesia and livers, lungs, brains, stomachs, kidneys were excised immediately and washed with ice-cold saline. One half of these tissues was then homogenized in 0.01 M Tris–HCl buffer (pH 7.4) and centrifuged at a speed of 12,000 g for 30 min in a refrigerated high-speed centrifuge at 4 °C for the following biochemical estimations in the supernatant as described previously (Gao et al., 2010).

2.4. Biochemical analysis

Mitochondria from liver, lung, brain, stomach and kidney were isolated by the method of Johnson and Lardy (1967) and the following parameters were assayed. Total protein was determined by the method of Lowry et al. (1951). Other biomarkers including SOD, CAT, GPx, GST, LPO, GSH, and Vitamin A, vitamin C, Vitamin E were estimated according to our previous protocol (Gao et al., 2010). GR was assayed by the way of Moron et al. (1979) method. The activities of ICDH, a-KDH, SDH, MDH and aconitase were assayed according to the method of the published reports (Gao et al., 2010; Bubber et al., 2011).

2.5. Examination of pathological variation

Hematoxylin and eosin (H&E) staining was used to observe pathological variation in routine histological tissues of liver, lung, brain, stomach and kidney. Briefly, one half of the liver, lung, brain, stomach and kidney tissues was fixed in 10% phosphate-buffered neutral formalin and then dehydrated in graded (50–100%) alcohol embedded in paraffin. The slices of 5-μm-thick were cut and stained with H&E for histological examination (Gao et al., 2011).

2.6. Statistical analysis

The data were analyzed using SPSS 13.0 software package. Values are presented as means ±SD with 10 animals/group. Analysis of variance was applied for comparison of different groups. Post hoc testing was performed for intergroup comparisons applying the least-significant difference. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of BaP on body-weight and relative weight of liver, lung, brain, stomach, kidney

There were significant increases ($P < 0.05$) in body-weight from week 3 in control, vehicle and 2.5 mg/kg BaP subgroups with oral

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