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Neonatal exposure to benzo[a]pyrene induces oxidative stress causing altered hippocampal cytomorphometry and behavior during early adolescence period of male Wistar rats

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ABSTRACT

Environmental neurotoxicants like benzo[a]pyrene (B[a]P) have been well documented regarding their potential to induce oxidative stress. However, neonatal exposure to B[a]P and its subsequent effect on anti-oxidant defence system and hippocampal cytomorphometry leading to behavioral changes have not been fully elucidated. We investigated the effect of acute exposure of B[a]P on five days old male Wistar pups administered with single dose of B[a]P (0.2 μg/kg BW) through intracisternal mode. Control group was administered with vehicle i.e., DMSO and a separate group of rats without any treatment was taken as naive group. Behavioral analysis showed anxiolytic-like behavior with significant increase in time spent in open arm in elevated plus maze. Further, significant reduction in fall off time during rotarod test showing B[a]P induced locomotor hyperactivity and impaired motor co-ordination in adolescent rats. B[a]P induced behavioral changes were further associated with altered anti-oxidant defence system involving significant reduction in the total ATPase, Na^+ K^+ ATPase, Mg^{2+} ATPase, GR and GPx activity with a significant elevation in the activity of catalase and GST as compared to naive and control groups. Cytomorphometry of hippocampus showed that the number of neurons and glia in B[a]P treated group were significantly reduced as compared to naive and control. Subsequent observation showed that the area and perimeter of hippocampus, hippocampal neurons and neuronal nucleus were significantly reduced in B[a]P treated group as compared to naive and control. The findings of the present study suggest that the alteration in hippocampal cytomorphometry and neuronal population associated with impaired antioxidant signaling and mood in B[a]P treated group could be an outcome of neuromorphological alteration leading to pyknotic cell death or impaired differential migration of neurons during early postnatal brain development.

1. Introduction

Pollutants, including persistent organic matters typically of anthropogenic origin, are known to adversely affect the physiological systems in all animal species. Polycyclic aromatic hydrocarbons (PAHs), typically produced by incomplete combustion of organic matter, are the most persistent organic pollutants commonly generated by anthropogenic activities (Hammond et al., 1976; Hecht et al., 1979; Larsson et al., 1983; Darby et al., 1986; Adams et al., 1987; Kaiserma and Rickert, 1992; Courter et al., 2007. Benzo[a]pyrene is a well addressed PAHs known for its neurotoxic potential established by several researchers in animal and human (Emre et al., 2007; Perera et al., 2006).

However, to know the mechanism of action and to understand the effects of these pollutants on animal models, there was a need to carry out a more specific mode of administration to determine its potential role in several neuropathological manifestations in early adolescence period. Further, oxidative damage poses a greater challenge to a developing brain since it is still at a morphologically, biochemically and functionally immature stage during early postnatal life. The developing brain is also subjected to high oxygen tension and lacks a functional blood brain antioxidant protection that is important during early development of the brain (Beiswanger et al., 1995; Dringen et al., 2000). B[a]P induced acute neurotoxicity is associated with oxidative stress accomplished through alteration in anti-oxidant scavenging system (Saunders et al., 2006). As the mammalian brain is known to

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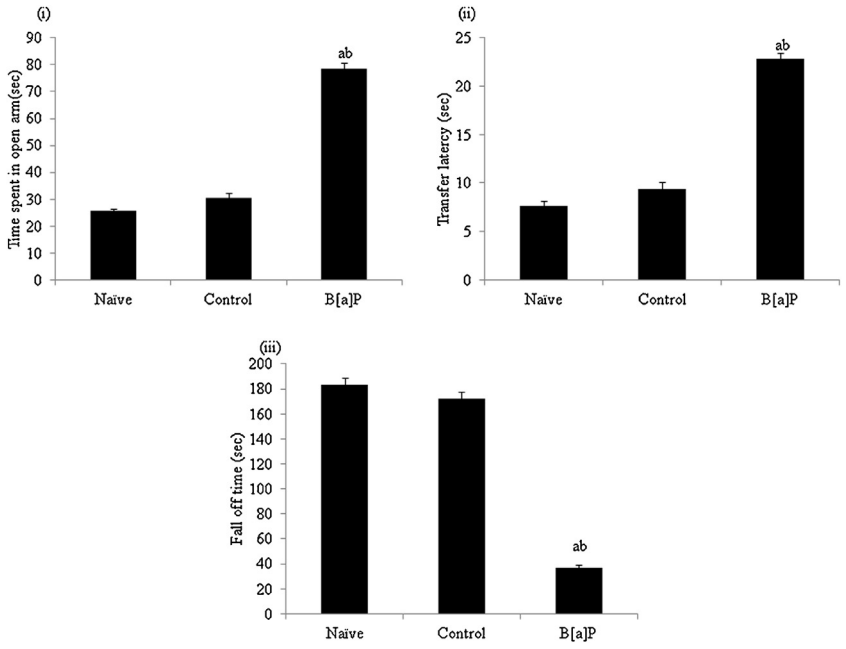

Fig. 1. Behavioral Tests.
Graphs representing changes in (i) stay time in open arm (sec), (ii) transfer latency (sec) in elevated plus maze test and (iii) fall off time (sec) in rota rod test in naïve, control (DMSO) and B[a]P (0.2 μg/kg BW) treated groups at PND 30. Values are expressed as mean ± SEM, n = 6. 'a' denotes $p < 0.05$ when compared to naïve group and 'b' denotes $p < 0.05$ when compared to control (DMSO) group.

be highly susceptible to toxic insults induced by various neurotoxicants, exposure to B[a]P in early phase of postnatal life and its subsequent effect on brain cytomorphology during adolescent period might provide a new insight into addressing its potential role in inducing neurological disorders.

Neurogenesis, formation of neuronal networks and synaptic connections are known to be formed in the initial phase of brain development. The young brain possesses the capacity to regenerate and repair the insults/injury caused by various toxicants to some extent, moreover neural progenitor cells also exists at this stage; however the injury or insult that occurs in this phase may result in abnormality leading to manifestation of neurological diseases (Bondy and Campbell, 2005). Furthermore, B[a]P can transfer transplacentally from mother to fetus, causing in utero injury to the developing brain, also the circulating B[a]P is able to penetrate the blood brain barrier (Das et al., 1985) and as a result, modifies the function of central nervous system (Konstandi et al., 1997). However, most of the in vivo toxicity studies involving B[a]P were conducted dermally, intraperitoneally or via inhalation with some studies done through the oral route (Godschalk et al., 2000; Tang et al., 2011; Liang et al., 2014).

Intracisternal mode of administration specifically gives a short and direct route to study the effects of B[a]P on neuromorphological changes (Patri et al., 2013). As blood brain barrier is the check point for metabolic transportation of compounds into the brain tissues, it is difficult to calculate approximately the amount of B[a]P injected peripherally that might reach the brain after crossing liver metabolism. This approach provides an opportunity to study the effects of B[a]P directly on brain development during early stage of postnatal life and subsequently causing mood and neuromorphological alteration thereof. With the ever increasing rate of industrialization and growth of human civilization, exposure to persistent anthropogenic genotoxicants like B[a]P during early stage of postnatal life might adversely affect the mood status and neuronal architecture leading to development of serious neurological problems in later phase of life.

Several studies on animal models have been conducted to study the effect of B[a]P on anti-oxidant defence system of sensitive brain regions (Saunders et al., 2006; Aktay et al., 2011; Murawska-Ciałowicz et al., 2011; Duan et al., 2013; Liang et al., 2014). Glutathione (GSH) is a tripeptide, composed of amino acid strings that are the basic building blocks of protein and is probably the most important cellular defence that allows the body to prevent and fight infections and diseases (Aoyama and Nakaki, 2012) whereas, glutathione-S-transferase (GST) comprises a multigene family of proteins that protects the cells against oxidative stress and is useful in monitoring cellular induction (Sarkar et al., 2010). It reacts with various xenobiotics to form GSH conjugation, leading to detoxification of the compounds and their excretion from the cell (Sohini and Rana, 2007; Tabrez and Ahmad, 2009). Intraperitonial exposure of B[a]P causes significant reduction of Na^+ K^+ and Mg^{2+} ATPase activity in brain hippocampus (Duan et al., 2013).

The present study was conducted to understand the direct and specific neurotoxic effects of B[a]P during early stage of postnatal brain development by considering intracisternal mode of administration. The purpose is to examine B[a]P induced homeostatic imbalance of anti-oxidant level and induction of oxidative stress causing cytomorphometrical changes in sensitive brain region during postnatal brain development.

2. Materials and methods

2.1. Ethics statement

All the protocols followed in the experiments were approved by the ethics committee of the institute (SOA University, Odisha, India) in accordance with the guidelines of the "committee for the purpose of control and supervision of experiments on animals (CPCSEA)" of Govt. of India. Highest care was taken to reduce suffering of animals during sampling.