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Effect of melatonin on PCB (Aroclor 1254) induced neuronal damage and changes in Cu/Zn superoxide dismutase and glutathione peroxidase-4 mRNA expression in cerebral cortex, cerebellum and hippocampus of adult rats

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ABSTRACT

Polychlorinated biphenyls (PCBs) are one of the environmental toxicants and neurotoxic compounds which induce the production of free radicals leading to oxidative stress. Free radicals represent a class of biologically generated species that pose a potential threat to neuronal survival. Cu/Zn superoxide dismutase (SOD) and glutathione peroxidase-4 (GPx-4) are the key cellular antioxidant enzymes by which neurons and other cells detoxify free radicals and protect themselves from damage. Melatonin, an indoleamine plays an important role in neurodegenerative diseases as an antioxidant and neuroprotector. The aim was to carry out to investigate the effect of melatonin on PCB (Aroclor 1254) induced changes in histomorphology and Cu/Zn SOD, GPx-4 mRNA expression in selected brain regions of adult rats. Group I: rats intraperitoneally (i.p.) administered with corn oil (vehicle) for 30 days. Group II: rats injected (i.p.) with Aroclor 1254 (PCB) at 2 mg/kg bw/day for 30 days. Groups III and IV: rats (i.p.) received melatonin (5 or 10 mg/kg bw/day) simultaneously with PCB for 30 days. Groups V and VI: rats (i.p.) received melatonin (5 or 10 mg/kg bw/day) alone for 30 days. After 30 days, rats were sacrificed and the brain regions were dissected to cerebral cortex, cerebellum and hippocampus. Activities of enzymatic antioxidants such as total SOD, Cu/Zn SOD, Mn SOD, glutathione peroxidase (GPx) were estimated. mRNA expressions of Cu/Zn SOD and GPx-4 were quantified by reverse transcriptase polymerase chain reaction (RT-PCR) method. Histological study was also observed. Specific activities of all antioxidant enzymes and mRNA expression of Cu/Zn SOD and GPx-4 were decreased in brain regions of PCB exposed animals. Neuronal damages were observed in all the brain regions. Exogenous melatonin supplementation retrieved all the parameters. These results suggest that melatonin protects PCB-induced oxidative stress and prevents neuronal damage in brain regions.

1. Introduction

Polychlorinated biphenyls (PCBs) are a family of halogenated aromatic hydrocarbons and one of the environmental pollutants which are widely used in electrical industries (Safe, 1994). PCBs are lipophilic and resistant to biological decomposition and can accumulate in higher tropic levels through the food chain (Kamrin and Ringer, 1994). Aroclor 1254 is a commercial mixture of polychlorinated biphenyls. PCBs are developmental neurotoxicants and it has been shown to produce neurochemical alterations in several experimental settings (Mariussen and Fonnum, 2001), as well as behavioral changes in learning, memory, motor activity and sexual behavior (Widholm et al., 2001).

Reactive oxygen species (ROS) are closely involved in several diseases of nervous system including Parkinson's disease, schizophrenia and Alzheimer's disease (Smythies, 1999). ROS-initiated oxidative stress can be regulated by cell defense mechanisms, which include enzymatic antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Halliwell and Gutteridge, 1999). The inherent biochemical and physiological characteristics of the brain, including high polyunsaturated fatty acids and energy requirements, make it particularly susceptible to free radicals mediated insult (Pajovic et al., 2003).

SOD catalyses the dismutation of $O_2^{\bullet -}$ to dioxygen and hydrogen peroxide. In the three SOD isoforms, Cu/Zn SOD is found in cytoplasm, whereas EC-SOD is extracellular. Both isoforms use

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copper and zinc as cofactors. Manganese is the cofactor for SOD2, which is found in mitochondria (Afonso et al., 2007). In the nervous system, as in other organs, Cu/Zn SOD is a key antioxidant enzyme involved in superoxide detoxification in normal cellular metabolism and after cell injury (Peluffo et al., 2005). In the adult central nervous system (CNS), Cu/Zn SOD is widely expressed in different neuronal populations; hippocampal CA pyramidal neurons and granual neurons of the dentate gyrus, cortical neurons (Kim et al., 2000). In most adult CNS injury models, the over expression of Cu/Zn SOD is thought to be neuroprotective (Peluffo et al., 2005). Phospholipid hydroperoxide glutathione peroxidase-4 (GPx-4/phGPx) is the selenium dependent enzyme in mammals with a pivotal role in brain development and function. GPx-4 shows a unique cellular distribution in brain compared to GPx-1 isoform. During postnatal development, GPx-4 mRNA is mainly distributed in cortex, hippocampus and cerebellum, indicating a neuronal rather than glial origin. In fully mature adult brain, GPx-4 is expressed in all neuronal cell layers and most prominently in the hippocampus (Savaskan et al., 2007).

Melatonin prevents oxidative stress both through its free radical scavenging effect and by directly increasing antioxidant activity (Tan et al., 1993), and different studies have demonstrated its protective role against oxidative damage induced by drugs and toxins (Reiter et al., 2007). Lowering circulating levels of melatonin also exaggerates the oxidative damage to tissues that are subjected to increased oxidative stress (Reiter et al., 1999). Earlier studies suggest that melatonin protects PCB (Aroclor 1254) induced ROS in rat brain regions (Venkataraman et al., 2008). Thus in the present study we compared the effects of melatonin on PCB (Aroclor 1254) induced alterations in mRNA expression of Cu/Zn SOD, GPx-4 and histological observations in cerebellum, cerebral cortex and hippocampus of rats.

2. Materials and methods

2.1. Animals

Healthy adult male albino rats of Wistar strain *Rattus norvegious* weighing 180–200 g (age 90 days) were housed in clean polypropylene cages and maintained in an air conditioned animal facility with constant 12 h/12 h dark and light cycle. All animal procedures were approved by our Institute of Ethical Committee. PCB treatment groups were as follows, with 6 rats per group. Group I: rats intraperitoneally (i.p.) administered with corn oil (vehicle) for 30 days. Group II: rats injected (i.p.) with Aroclor 1254 (PCB) at 2 mg/kg bw/day for 30 days. Groups III and IV: rats (i.p.) received melatonin (5 or 10 mg/kg bw/day) simultaneously with Aroclor 1254 for 30 days. Groups V and VI: rats (i.p.) received melatonin (5 or 10 mg/kg bw/day) alone for 30 days. The dosage and duration of PCB were selected according to our previous studies (Venkataraman et al., 2008). The dose level of melatonin was selected according to Gomez et al. (2005) and Feng and Zhang (2005). Aroclor 1254 was obtained from Chemservice, USA. Melatonin and all other chemicals were purchased from Sigma Aldrich chemicals Pvt. Ltd., USA and Sisco Research Laboratories (SRL), Mumbai, India. Primers for rat Cu/Zn SOD, GPx-4 and Ribosomal protein-19 (RPL-19) were purchased from Ocimum Biosolutions Ltd., Hyderabad, India. Melatonin was dissolved in 0.9% saline plus 2% ethanol, while Aroclor 1254 was dissolved in corn oil.

24 h after the last treatment, the animals were sacrificed by cervical decapitation. Brain was excised immediately; regions were separated and immersed in ice-cold physiological saline. Regions from each of the brain tissue were blotted, weighed accurately, and placed in chilled 0.1 mol/l Tris–HCl buffer, pH 7.4. The samples were homogenized using a Potter-Elvehjem homogenizer filled with Teflon pestle to produce 10% homogenates and used for determining the biochemical parameters described below. Protein concentrations of the homogenates were determined by Lowry et al. (1951), using bovine serum albumin as the standard. Estimations of total SOD, Mn SOD, Cu/Zn SOD and GPx were done.

2.2. Biochemical assays

2.2.1. Enzymatic and non-enzymatic antioxidant assay

Total SOD, Mn SOD and Cu/Zn SOD (EC 1.15.1.1) were determined from its ability to inhibit the auto-oxidation of pyrogallol according to the method of Del Maestro and Donald (1986). The enzyme activity was expressed as U/mg protein. GPx (EC 1.11.1.9) activity was determined by the method of Rotruck et al. (1973). The enzyme activity was expressed as U/mg protein (1 U is the amount of enzyme that converts 1 μmol GSH to GSSG in the presence of hydrogen peroxide/min).

2.3. Total RNA isolation and RT-PCR

Total RNA was purified from freshly isolated brain tissue using 1 ml of the Trizol reagent. The RNA purity and concentration were determined spectrophotometrically at A260/A280 nm. The purity of RNA obtained was 1.8–1.9. Two micrograms of total RNA were reverse transcribed by Qiagen One step RT-PCR kit according to the manufacture's instructions and further amplified by PCR. The details of the primers used, number of cycles and size of the PCR-amplified products are listed in Table 1. The reported sets of primers were selected based on earlier literature (Chang et al., 1988; Limaye et al., 2003; Nam et al., 2003) and synthesized according to the published cDNA sequence of rat Cu/Zn SOD, GPx-4 and RPL-19. Annealing temperature was at 55 °C. Ten microlitres of each PCR product were analyzed by gel electrophoresis on a 2% agarose gel. The bands were identified based on the product size using 100 bp ladder, photographed using a BioRad photo documentation system. The band intensification for Cu/Zn SOD and GPx-4 mRNA was normalized with that of the internal control RPL-19 using quantity one software method.

2.4. Histological studies

The same groups were maintained for histological study. Animals were sacrificed at the end of intended experiments by administering thiopental sodium (40 mg/kg body weight, i.p.). Then the animals were perfused transcardially. The entire blood was cleared from the circulation by flushing normal saline till the draining fluid becomes clear, then 10% formal saline was flushed for fixation. The head was separated and stored in 10% formal saline and after 2 or 3 days, the brain was removed. The olfactory bulb and the brain stem were removed and the cerebral hemisphere alone was stored. The cerebral hemisphere was cut into 3 coronal slices. Later, the tissue sections were processed for paraffin sectioning and tissue blocks were made in paraffin. The blocks were cut into 10 μ thickness using rotary microtome and the sections were stained using haematoxylin and eosin (Gurel et al., 2005). The cortical, cerebellar and hippocampal cellular morphology was analyzed and recorded by Nikon microscope (400×).

2.5. Statistical analysis

Data were statistically analyzed using analysis of variance. When the F ratio was statistically significant, the data were subjected to the Student–Newman–Keul's test. Values were considered significant at $P < 0.05$.

3. Results

3.1. Antioxidant enzymes activity

Table 2 shows the effect of melatonin on the activities of total SOD, Cu/Zn SOD and Mn SOD in cerebellum, cerebral cortex and hippocampus of PCB exposed adult rats. The specific activities of all the enzymes were found to be decreased significantly in all the

Table 1
Details of primers employed, number of cycles and expected size of the PCR-amplified cDNA.

	Sequence of the primer	Number of cycles	Products size (bp)
Cu/Zn SOD	Sense (nt. 58–77): 5′-GCAGAAGGCAAGCGGTGAAC-3′ Antisense (nt. 504–485): 5′-TAGCAGGACAGCAGATGAGT-3′	35	447
GPx-4	Sense (nt.265–285) 5′-ATGCACGAATTCTCAGCCAAG-3′ Antisense (nt.725–709) 5′-GGCAGGTCCTTCTCTTCTAT-3′	35	461
Ribosomal protein-19	Sense (nt. 401–421) 5′-CTGAAGGTCAAAGGGAATGTG-3′ Antisense (nt. 595–576) 5′-GGACAGAGTCTTGATGATCTC-3′	35	194