2,2',5,5'-TETRACHLOROBIPHENYL INDUCES OXIDATIVE STRESS-MEDIATED APOPTOSIS IN NEURONAL SK-N-MC CELLS

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Introduction

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants and have become distributed throughout the entire ecosystem[1]. Hepatic toxicity and carcinogenicity of PCBs have been investigated in some detail over the years. However, there is little information regarding the neurological effects of direct PCB exposure on primary neuronal cells as well as adults and developing animals. Several studies suggest that a critical underlying mechanism of PCB-mediated endothelial cell activation and dysfunction is an increase in cellular oxidative stress[2,3]. In the present study, we investigated whether 2,2',5'5'-tetrachlorobiphenyl (PCB 52) induces apoptosis and, if induced, whether the apoptosis is directly mediated by ROS generated during the oxidative stress in the neuronal SK-N-MC cells.

Materials and Methods

Human neuroblastoma SK-N-MC cell line was maintained in minimum essential medium (MEM) containing 2 mM L-glutamine, supplemented with 10 % fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM MEM nonessential amino acids, 100 units/ml penicillin, and 100 mg/ml streptomycin. Cell death was induced by treatment of PCB 52 into the cell cultures with 60 % of confluency for the indicated times and at the indicated concentrations. Apoptosis was determined by detection of nuclear DNA fragmentation[4] and poly (ADP-ribose) polymerase (PARP) cleavage[5]. Cell viability was assessed by trypan blue (0.2 %) exclusion. Generation of reactive oxygen species (ROS) was measured using the nonfluorescent probe 2',7'-dichlorofluorescin diacetate (DCFH-DA, Molecular Probes, USA) as described previously[6]. Protein concentrations were determined by the Bradford method using bovine serum albumin as the standard.

Results and Discussion

We previously examined the effect of PCB 52 on the induction of cell death in SK-N-MC cells and we found that PCB 52 at 15 mg/ml significantly induced cell death and at 12 h after the addition of PCB 52 to the culture medium[7]. At 36 h, nearly 80 % of the cells exhibited a loss of viability[7]. We also found that PCB 52 induced lipid peroxidation in SK-N-MC cells following exposure to PCB 52[7].

To determine whether cell death of SK-N-MC cells was accompanied by the induction of free radicals, quantitative changes in the concentrations of ROS using whole cell suspension were analyzed over a 120 min period after PCB 52 treatment (Fig. 1). Exposure to PCB 52 dose-dependently enhanced the level of ROS but not in control cultures. ROS level was increased after 15 min in all the concentrations applied and remained elevated for at least 90 min after stimulation. A maximum increase of ROS generation was observed at 45-min exposure, after then the increases were alleviated but still higher than that of control. After 120 min, the level had returned to baseline in 15 mg/ml of PCB 52. We also tested the influence of different ROS scavengers on the ROS production induced by

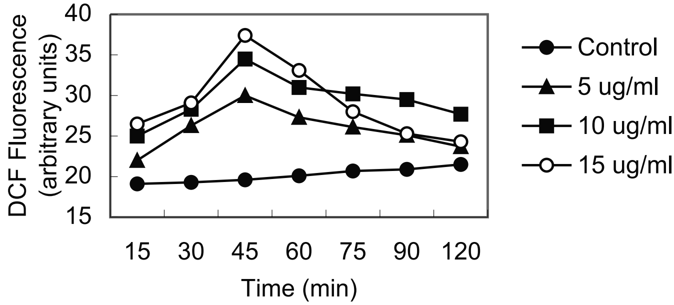

Figure 1. ROS generation in neuronal SK-N-MC cells by exposure to PCB 52.

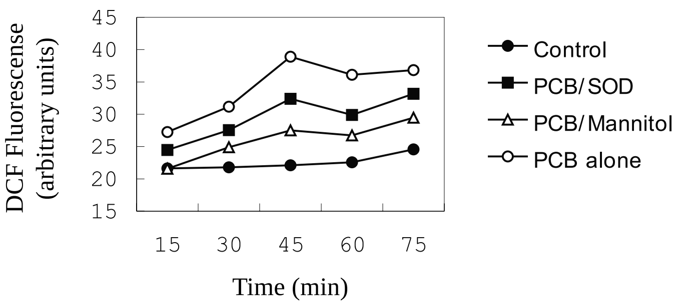

Figure 2. Effects of antioxidants on the inhibition of ROS generation in neuronal SK-N-MC cells by exposure to PCB 52. Cell suspensions were loaded with DCFH-DA for 20 min and then SOD (400 U/ml) or mannitol (5 mM) was loaded in the presence of 10 mg/ml of PCB 52. DCF fluorescence was monitored every 15 min over a 75 min period.

PCB 52 in SK-N-MC cells (Fig. 2). Both mannitol (5 mM) and SOD (400 U/ml) displayed strong scavenging capabilities against PCB 52-induced ROS formation, mannitol showing the stronger scavenging capacity. These results suggest that PCB 52 induce both oxygen free radicals and hydroxyl radicals in SK-N-MC cells, but to a more extent of hydroxyl radicals.

Through the experimental analysis of DNA fragmentation, we verified that death of neuronal SK-N-MC cells induced by PCB 52 was involved in their apoptosis[7]. To further verify the involvement of the death of neuronal cells in their apoptosis, we checked cleavage patterns of PARP and b-catenin, caspase substrates known to be cleaved in cells undergoing apoptosis[8,9]. We confirmed that PCB 52 induced concentration-dependently the cleavages of both PARP and b-catenin (Figs. 3 & 5). These results suggest that caspases responsible for the proteolysis of PARP and b-catenin are involved in the mechanism of apoptotic cell death induced by PCB 52. To determine whether the cleavage of caspase substrates (PARP and b-catenin) by PCB 52 in the neuronal SK-N-MC cells was accompanied by the generation of free radicals, the degradation of both caspase substrates induced by PCB 52 was analyzed in the presence of ROS scavengers (Figs. 4 & 6). Both mannitol and SOD protected the degradation of the caspase substrates, suggesting that ROS generated by PCB 52 is a direct source for the proteolyses of PARP and b-catenin in the neuronal SK-N-MC cells. Our results conclude that PCB 52 *in vitro* lead to an induction of oxidative stress, which subsequently promotes apoptotic cell death of neuronal SK-N-MC cells by the attack of ROS generated from PCB 52.

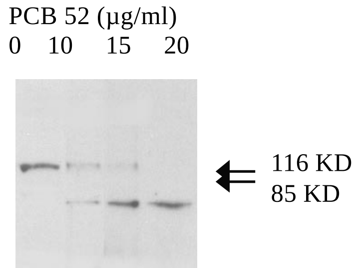

Figure 3. Proteolysis of PARP in apoptotic SK-N-MC cells by exposure to PCB 52.

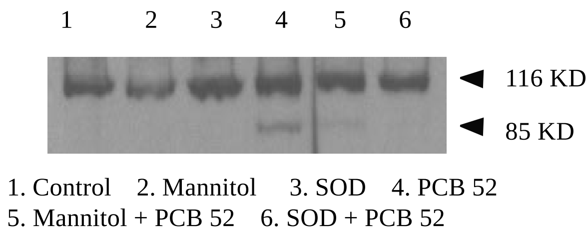

Figure 4. Protection effects of ROS scavengers neuronal against degradation of PARP by PCB 52.

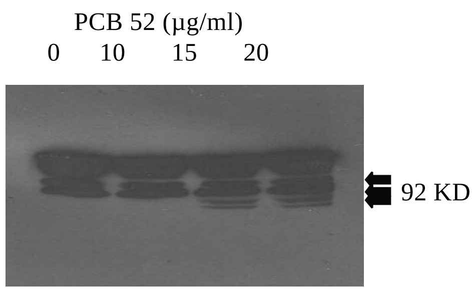

Figure 5. Proteolysis of b-catenin in apoptotic SK-N-MC cells by exposure to PCB 52.

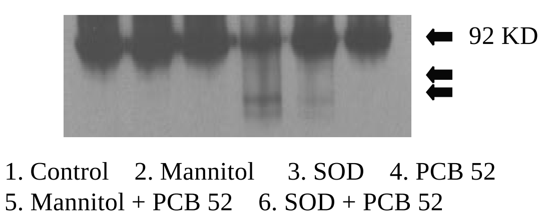

Figure 6. Protection effects of ROS scavengers neuronal against degradation of b-catenin by PCB 52.

Acknowledgments

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