EFFECT OF 2,2',5,5'-TETRACHLOROBIPHENYL ON THE ANTIOXIDANT ENZYMES IN NEURONAL SK-N-MC CELLS

Ji-Young Lee[1], Yong-Hoon Kim[3], Woo-Hong Joo[1,2], Yong-Kweon Cho[1,3], Ja-Young Moon[1,3]

[1]Institute of Genetic Engineering, Changwon National University, Changwon 641-773,
[2]Department of Biology; [3]Department of Biochemistry and Health Sciences, College of Natural Sciences, Changwon National University, Changwon, 641-773, Korea

Introduction

Recently there has been increased awareness of the possible adverse effects of environmental contaminants on human being. 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, little is known about mechanisms by which environmental chemicals alter the metabolism in neurons, and especially how PCB-mediated cell dysfunction can be prevented or blocked. There is evidence that the cellular antioxidant defense is depressed after exposure to PCBs[2]. However there is no report about the antioxidant defense mechanism against the PCB 52-mediated oxidative stress in human neuronal cells. PCBs are also known to cause alteration in the antioxidant defense of mammals. Therefore, in the present study, to determine whether similar detoxification processes are activated in response to PCB 52 exposure in the human neuronal cells, we investigated activities of antioxidant enzymes and glutathione status in the human SK-N-MC cells exposed to 2,2',5'5'-tetrachlorobiphenyl (PCB 52).

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. Cell viability was assessed by trypan blue (0.2 %) exclusion. Glutathione content was measured according to a modified method of Brwone and Armstrong[3]. Activities of superoxide dismutase (SOD)[4], catalase[5], glutathione peroxidase[6], and glutathione S-transferase (GST)[7] were measured as described previously. Protein concentrations were determined by the Bradford method using bovine serum albumin as the standard.

Results and Discussion

We have shown previously that PCB 52 induces apoptotic death in human neuronal cells[8]. Since thiol status is both an indicator of the oxidative stress response and an indicator of susceptibility to oxidative stress conditions, we evaluated the thiol status in the neuronal cell extracts following PCB 52 treatment. As shown in Fig. 1A, treatment with PCB 52 for 24 h resulted in a concentration-dependent decrease in the levels of glutathione (GSH). A significant decrease in GSH was detected after 6h of PCB 52 treatment (Fig. 1B). In the previous study[8], it was demonstrated that PCB 52 induces the generation of reactive oxygen species as well as lipid peroxidation in the neuronal SK-N-MC cells. From these results, we suggest that loss of GSH may result in impaired protection against lipid peroxidation.

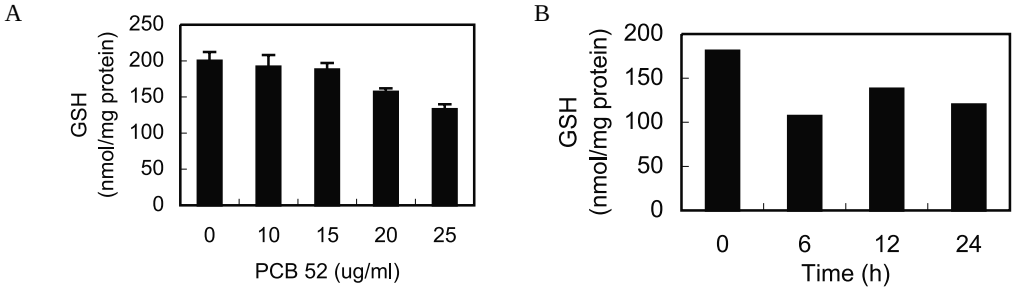

Figure 1. Effect of exposure to PCB 52 on the levels of GSH in human neuronal SK-N-MC cells. (A) Concentration-dependent changes of GSH (B) Time-dependent changes of GSH

Biological systems possess various defense mechanisms against the production of oxidative stress in response to exposure of environmental toxins. These mechanisms may include endogenous antioxidant enzymes. We also investigated the role of antioxidant enzymes in the neuronal SK-N-MC cells treated with PCB 52. To investigate the change of Cu/Zn-SOD enzyme activity after treatment PCB 52, SK-N-MC cells were treated with PCB 52 with different concentrations for 24h (Fig. 2). Fig. 2A shows that there is a significant increase in Cu/Zn-SOD activity in the neuronal cells treated with PCB 52 compared to that of untreated group. Treatment of PCB 52 also resulted in a concentration-dependent increase in the catalase activity (Fig. 2B). But the increases in catalase activity were smaller than the increases in Cu/Zn-SOD activities by PCB 52. The antioxidant enzyme Cu/Zn-SOD has been shown to accelerate dismutation of superoxide anion into hydrogen peroxide, which is then decomposed into water and oxygen by catalase. Data shown in the present study suggest the possibility that hydrogen peroxide may not be efficiently eliminated by catalase.

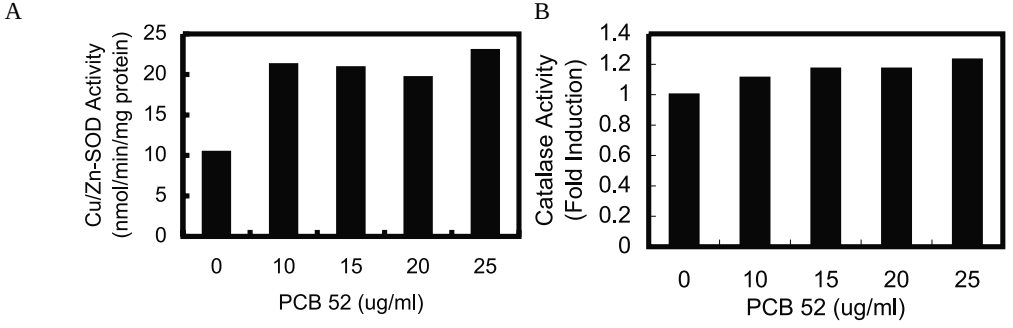

Figure 2. Effects of PCB 52 on the activities of antioxidant enzymes. (A) Cu/Zn-SOD (B) Catalase Neuronal SK-N-MC cells were treated with increasing concentrations of PCB 52 for 24h.

Treatment of PCB 52 increased GST activity after 24h of treatment (Fig. 3A), but the increase was not comparable to that of the Cu/Zn-SOD activity by PCB 52. In contrast, PCB 52 at 10 ug/ml had no effect on the activity of glutathione peroxidase (GSH-Px) in the neuronal cells (Fig. 3B). But at the higher concentrations (15, 20, and 25 ug/ml) its activity was rather significantly decreased. GSH-Px, which decomposes hydrogen peroxide, is a candidate for a critical enzyme in protecting against

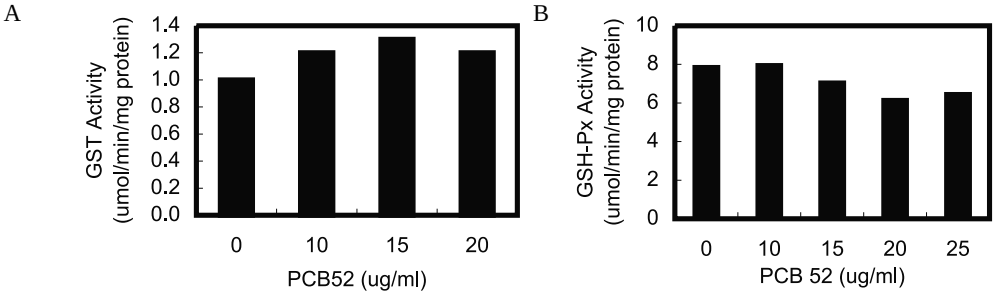

Figure 3. Effects of PCB 52 on the activities of antioxidant enzymes. (A) GST (B) GSH-Px Neuronal SK-N-MC cells were treated with increasing concentrations of PCB 52 for 24h.

hydrogen peroxide-induced oxidative stress. Our previous results have shown that PCB 52 induces largely hydroxyl radicals and superoxide radicals[8]. The present data show that PCB 52 differentially affects on the activities of antioxidant enzymes in the neuronal SK-N-MC cells. Taken together our previous and present data, we suggest the possibility that superoxide radicals generated by PCB 52 may efficiently degraded into hydrogen peroxide by the increased Cu/Zn-SOD activity, but the hydrogen peroxide produced by Cu/Zn-SOD may not efficiently eliminated by the decreased GSH-Px or catalase activity. Thus we also suggest the possibility that lipid peroxidation by PCB 52 may be due to largely hydroxyl radicals in the human neuronal SK-N-MC cells.

Acknowledgments

This work was supported by grant from Korea Research Foundation (KRF-99-005-D00016)

References

1. Safe, S.H. (1994) CRC Crit. Rev. Toxicol. 24, 87-149.
2. Hassoun, E. A., Li, F., Abushaban, A., and Stohs, S. J. (2000) Toxicology 145, 103-113.
3. Browne, R.W., and Armstrong, D. (1998) Free Radical & Antioxidant Protocols Edited by D. Armstrong, Humana Press 33, 347-352.
4. McCord, J.M., and Fridovich, I. (1969) J. Biol. Chem. 244, 6049-6055.
5. Aebi, H. (1984) Methods in Enzymol. 105, 121-126.
6. St Clair, D.K., and Chow, C.K., (1996) Free Radicals-A Practical Approach Edited by Punchard, N.A., and Kelly, F.J., IRL Press 227-231.
7. Habig, W.H., and Jakoby, W.B. (1981) Methods in Enzymol. 77, 398-405.
8. Hwang, S.G., Lee, H.C., Lee, D.W., Kim, Y.S., Joo, W.H., Cho, Y.K., and Moon, J.Y. (2001) Toxicology 165, 179-188.