ROLE OF CELL CYCLE REGULATORS IN NEUTOTOXIC EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN

Da Qing Jin[1], Yong Soo Lee[2], Keun Huh[1], Jung-Ae Kim[1]

[1]College of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea
[2]College of Pharmacy, Duksung Women's University, Seoul 132-714, Korea

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the best characterized environmental pollutants and is capable of causing a wide variety of toxicities including teratogenesis[1]. Recent reports have shown that the effects of TCDD are often contradictory at the cellular level. In rat hepatocytes TCDD has increased[2] and decreased[3,4] proliferation rates depending on the experimental conditions. In human keratinocytes TCDD has induced proliferation[5] as well as terminal differentiation[6,7]. Toxic effects of TCDD appear to be mediated through the activation of the arylhydrocarbon receptor (AhR), a ligand-activated transcription factor, leading to induction of genes possessing the dioxin response element (DRE) in various tissues[8]. TCDD-induced growth arrest in a rat hepatoma cell line has been shown to involve AhR-mediated induction of cell cycle inhibitor, p27[9]. Previously we have reported that TCDD reduced human neuronal cell proliferation without causing apoptosis. Thus, in the present study we investigated whether TCDD inhibits neuronal proliferation in an AhR-dependent manner and whether it is mediated through alteration of cell cycle regulators using SK-N-SH human neuroblastoma cells as a model human neuronal cellular system.

Methods and Materials

Human neuronal SK-N-SH cells were grown at 37ºC in a humidified incubator under 5% CO_2/95% air in an Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 200 IU/ml penicillin, 200 mg/ml of streptomycin and 1 mM sodium pyruvate. Culture medium was replaced every other day. After attaining confluence the cells were subcultured following trypsinization. Cell proliferation was determined by measuring [^{3}H] thymidine uptake according to the method of El-Metwally and Adrian[10]. For immunoblotting, nuclear extracts from cell lysate were fractionated on SDS-polyacrylamide gel and transferred to nitrocellulose membranes. To detect cell cycle regulators primary antibodies against p53, p21, p27 and pRB were used. The expression of mRNA levels was analyzed by RT-PCR.

Results and Discussion

In order to examine whether AhR mediates the effects of TCDD on cell proliferation, we first investigated the presence of AhR and arylhydrocarbon nuclear translocator (ARNT) in human neuronal cells. As shown in Fig. 1, SK-N-SH human neuronal cells normally expressed AhR and ARNT and TCDD did not alter the message levels. TCDD-induced inhibition of proliferation in SK-N-SH cells was significantly prevented by pretreatment with a-naphthoflavone (a-NF; 5 mM), a partial AhR antagonist, or 8-methoxypsoralen (MOP; 50 mM), a binding inhibitor of activated AhR to DRE, as shown in Fig. 2. These results clearly show that anti-proliferative action of TCDD against human neuronal cells may be mediated through AhR activation.

Figure 1. Effect of TCDD on the expression of AhR (A) and ARNT (B) mRNA in RT-PCR analysis.

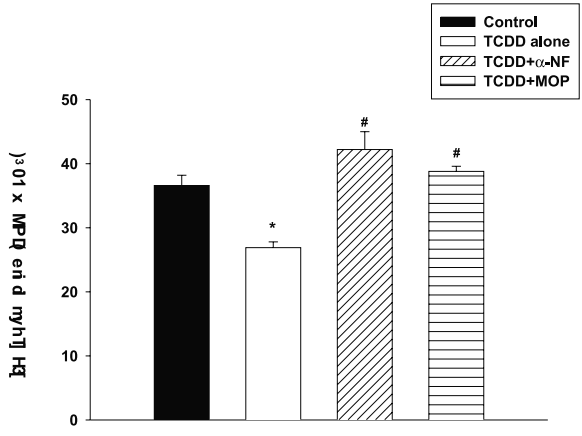

Figure 2. AhR inhibitors prevent the TCDD-induced inhibition of proliferation in SK-N-SH neuronal cells. *p<0.05 compared to control. #p<0.05 compared to TCDD alone.

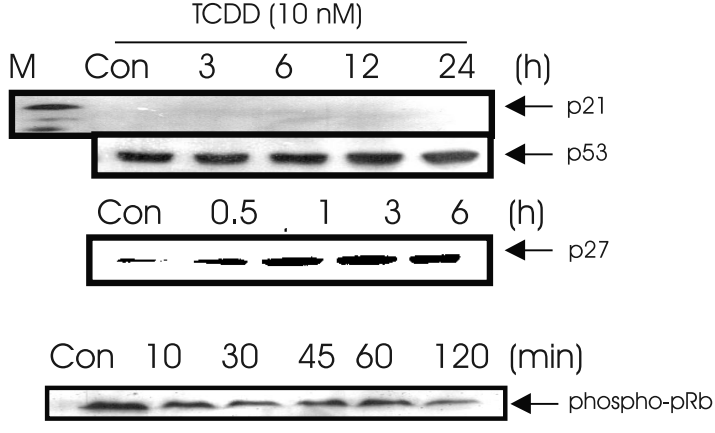

Figure 3. Effects of TCDD on the expression of p21, p53 and p27 and phosphorylation level of pRb in SK-N-SH human neuronal cells.

To further examine the role of cell cycle regulators in the TCDD-induced suppression of cell proliferation, we investigated whether TCDD can alter expression levels of p53, p21 and p27 as well as phosphorylation status of pRB. As shown in Fig. 3, whereas expression level of p53 was not changed and consequently p21 was not induced in the TCDD-treated cells, the level of p27 was significantly enhanced by TCDD. In addition, TCDD significantly reduced phosphorylation of pRB. Taken together, these results suggest that TCDD may induce p27 through the activation of AhR, and in turn, inhibit phosphorylation of pRB, culminating in arrested neuronal cell growth. These findings may contribute to the understanding of the mechanism by which TCDD induces developmental neurotoxicity.

Acknowledgments

This work was supported by NITR/Korea FDA Grant ED 2001-15.

References

1. Pohjanvirta R and Tuomisto J (1994) Pharmacol Rev 46, 483-549.
2. moolgavkar S, Luebeck EG, Buchmann A and Bock KW (1996) Toxicol Appl Pharmacol 138, 31-42.
3. Wiebel FJ, Klose U and Kiefer F (1991) Toxicol Lett 55, 161-169.
4. Wolfle D, Becker E and Schmutte C (1993) Cell Biol Toxicol 9, 15-31.
5. Milstone LM and LaVigne JF (1984) J Invest Dermatol 82, 532-534.
6. Choi EJ, Toscano DG, Ryan JA, Riedel N and Tocano WA Jr. (1991) J Biol Chem 266, 9591-9597.
7. Gaido KW and Maness SC (1994) Toxicol Appl Pharmacol 127, 199-208.
8. Nebert DW (1989) Crit Rev Toxicol 20, 153-174.
9. Kolluri SK, Weiss C, Koff A and Gottlicher M (1999) Genes Dev 13, 1742-1753.
10. El-Metwally TH and Adrian TE (1999) Biochem Biophys Res Commun 257, 596-603.