2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN INHIBITS CELL PROLIFERATION THROUGH REDUCED PRODUCTION OF REACTIVE OXYGEN SPECIES IN A HUMAN NEURONAL CELL LINE

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the best characterized dioxin congeners and the most toxic environmental pollutants[1]. Since TCDD is bioaccumulated in the food chain and efficiently transferred by lactation, a particular risk group may be newborn infants[2]. In fact, TCDD has been known as a developmental teratogen that causes cleft palate, craniofacial abnormalities, and especially developmental neurotoxicity such as brain asymmetry[3], decrease in neurotransmitters[4], neuronal calcium uptake[5] and death of neurons[6]. Although toxic effects of TCDD are mediated through the arylhydrocarbon receptor (AhR) in various tissues, the exact mechanism by which TCDD exerts neurotoxicity is mostly unknown. Several reports show that TCDD induces production of reactive oxygen species (ROS) and oxidative stress in brain tissue [7]. Recent studies have demonstrated that ROS act as an intracellular signaling molecule inducing cell proliferation as well as cell death[8]. Thus, in the present study we investigated whether ROS may play a role in TCDD-induced neurotoxicity using SK-N-SH human neuroblastoma cells as a model human neuronal cellular system.

Methods and Materials

Cell Culture. SK-N-SH cells were grown at 37°C 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 μg/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 Viability Assay. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) staining method[9].

Cell Proliferation Assay. Cell proliferation was determined by measuring [^{3}H] thymidine uptake according to the method of El-Metwally and Adrian[10].

Intracellular ROS Measurement. Relative changes in intracellular ROS were monitored using a fluorescent probe, 2',7'-dichlorofluorescin diacetate (DCFH-DA)[11].

Results and Discussion

TCDD reduced viability of SK-N-SH cells in a dose-dependent manner as shown in Fig. 1A. This effect of TCDD may be predominantly due to suppressed cell proliferation, rather than cell death, since [^{3}H] thymidine uptake was also decreased by TCDD in a concentration-dependent fashion as depicted in Fig. 1B. These results clearly show that TCDD has an anti-proliferative activity against human neuronal cells.

We investigated the possible involvement of ROS in the mechanism by which TCDD inhibits cell proliferation of SK-N-SH cells. TCDD suppressed the basal generation of ROS in a time- and concentration-dependent manner assessed using a fluorescent probe DCFH-DA as shown in Fig. 2. Previously, TCDD has been shown to increase the level of ROS in the all cell types tested[12]. However, we report here, for the first time, that TCDD has an inhibitory effect on the ROS production. Although we do not know the reason why this discrepancy between their and our studies occurred, TCDD may exert different responses depending on cell or tissue types.

Next, we examined whether the effects of TCDD on cell proliferation and ROS production result from its activation of AhR. The decrease in [^{3}H] thymidine uptake by TCDD (10 nM) was significantly prevented by pretreatment with either α-naphthoflavone (α-NF; 5 μM), a partial AhR antagonist, or 8-methoxypsoralen (MOP; 50 μM), a binding inhibitor of activated AhR to dioxin response element as shown in Fig. 3A. In addition, α-NF (5 μM) and MOP (50 μM) significantly reversed the TCDD (10 nM)-induced inhibition of production of ROS as depicted in Fig. 3B. These results imply that the anti-proliferation and inhibition of ROS production induced by TCDD may be mediated through the activation of AhR in SK-N-SH cells.

To further examine the relationship between ROS-reducing action of TCDD and decreased cell proliferation, we investigated whether exogenous application of an oxidant, H_2O_2 prevents suppressed neuronal proliferation. As shown in Fig. 4, the decreased [^{3}H] thymidine uptake by TCDD (10 nM) was significantly prevented by pretreatment with H_2O_2 (50 μM). Taken together, these results suggest that TCDD may reduce the basal generation of ROS through the activation of AhR, and in turn, inhibit neuronal cell proliferation. These findings may contribute the understanding of the mechanism by which TCDD induces developmental neurotoxicity.

Acknowledgments
This work was supported by Korea Food & Drug Administration Grant 2000.

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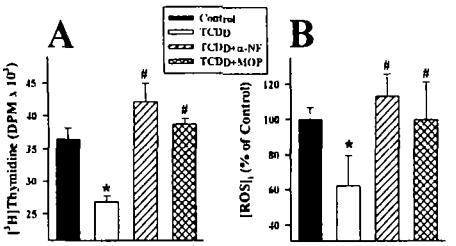

Fig. 1. TCDD decreases cell viability (A) and [^{3}H]thymidine uptake (B) in SK-N-SH cells. P<0.05 compared to control.

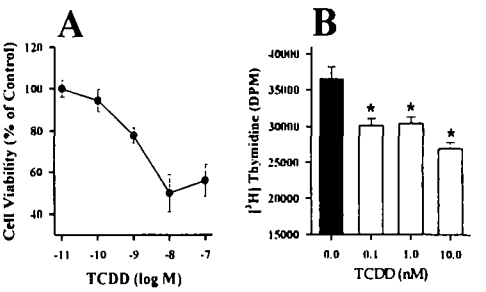

Fig. 3. AhR inhibitors prevent the TCDD-induced inhibition of [^{3}H]thymidine uptake (A) and ROS generation (B). *p<0.05 compared to control. #p<0.05 compared to TCDD alone.

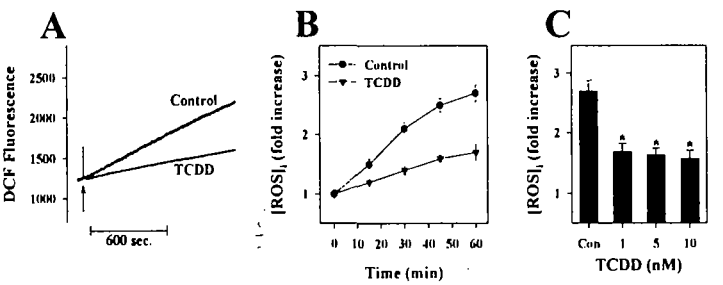

Fig. 2. TCDD suppresses the basal generation of ROS in a time- (A, B) and dose-dependent (C) manner. *P<0.05 compared to control.

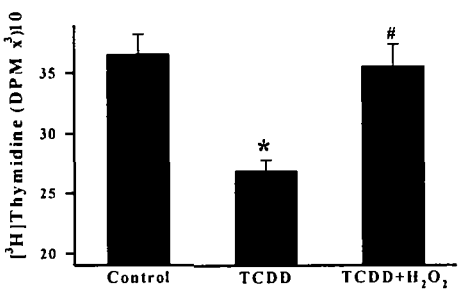

Fig. 4. Exogenous application of H_2O_2 prevents the TCDD-induced inhibition of [^{3}H]thymidine uptake. *p<0.05 compared to control. #p<0.05 compared to TCDD alone.