THE EFFECT OF COMBINED EXPOSURE OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (2,3,7,8-TCDD) AND TNF-ALPHA ON PROLIFERATION AND DIFFERENTIATION OF HUMAN MYELOBLASTIC LEUKEMIA ML-1 CELLS

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) causes a number of physiological effects including hematotoxicity. Tumor necrosis factor –alpha (TNF-α) is one of cytokines which is involved in cell death, proliferation, and differentiation. In this study, we investigated the effect of 2,3,7,8-TCDD without or with TNF-α on cell proliferation and differentiation of human myeloblastic leukemia ML-1 cells.

Methods and Materials

Cell Culture and treatments

Human myeloblastic leukemia ML-1 cells were cultured in RPMI1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.292 mg/L L-glutamine in a humidified 5% CO_2 atmosphere at 37°C. 1×10^6 cells were seeded into 10-cm dishes containing fresh media. 24 hours later, cells were exposed to the test chemicals. Solutions of the test chemicals dissolved in DMSO were added to the medium as a final DMSO concentration of 0.1% (v/v).

Cell proliferation assay

Inhibitory effect of 2,3,7,8-TCDD without or with TNF-α on ML-1 cells was estimated by trypan blue dye exclusion. Living cell numbers were counted with a hemocytometer and compensated by multiplying the cell counts by the dilution factor.

Cell differentiation assay

Cell differentiation was assayed by the nitroblue tetrazolium (NBT) test and morphological change.

NBT reduction was determined by a modification of the procedure described by Takuma et al[1]. 3×10^5 cells/mL were incubated in 96-well flat-bottom plates for 60 min at 37 °C in the presence of 1 mg/mL NBT and 100 ng/mL 12-*O*-tetra-decanoylphorbol-13-acetate (TPA). After incubation, the reaction was stopped by adding 2 N hydrochloric acid, the

supernatant solution was removed by pipette, and the cells were dried overnight at 37°C. Formazan was dissolved in DMSO, and its absorbance at 540 nm was measured by an autoreader.

For assessing the morphological change, ML-1 cells were fixed and stained with Wright-Giemsa.

RT-PCR

Total RNA was isolated from the ML-1 cells by using RNeasy Mini Kit (QIAGEN, Hilden, Germany). cDNA synthesis and quantitative competitive PCR for CYP1A1 was carried out by using human cytochrome P450 competitive RT-PCR set (Takara, Shiga, Japan) and RNA PCR kit (AMV) Ver.2.1 (Takara, Shiga, Japan). For AhR, Arnt, TNFR1, p53 and p21, cDNA synthesis and PCR was carried by using RNA PCR kit (AMV) and these genes were quantified by using working curve of CYP1A1 and compensated by length of PCR products.

Results and Discussion

2,3,7,8-TCDD alone didn't affect the cell proliferation (Fig. 1-A) and differentiation (Fig. 1-B). However, combination of 2,3,7,8-TCDD and TNF-α resulted in strong inhibition of cell proliferation in a dose-dependent manner (Fig. 2-A) and approximately half of the cells were dead (data not shown). In this case, 2,3,7,8-TCDD enhanced TNF-α -induced cell differentiation (Fig. 2-B).

The cells treated with 2,3,7,8-TCDD and TNF-α showed an increased cytoplasma with polygonal nuclei (Fig. 3).

Alterations of gene expression which are involved in drug metabolism and TNF-α-induced apoptosis were shown in Fig. 4.

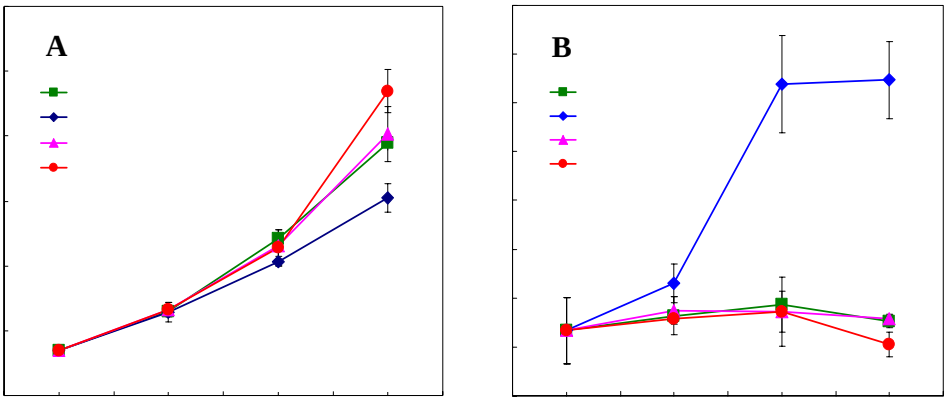

Fig. 1 The Effect of 2,3,7,8-TCDD without TNF-α on cell proliferation (A) and differentiation (B) of ML-1 cells. Values are given as mean±S.D. of triplicate samples.

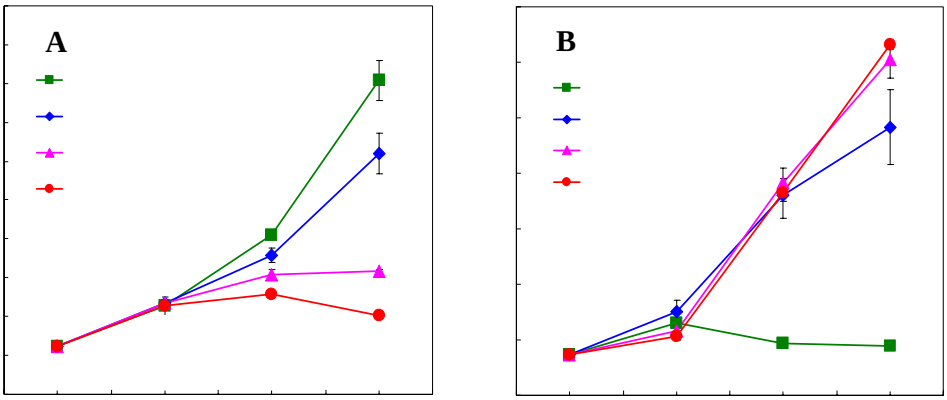

Fig. 2 The Effect of 2,3,7,8-TCDD in combination with TNF-α on cell proliferation (A) and differentiation (B) of ML-1 cells. Values are given as mean±S.D. of triplicate samples.

RT-PCR revealed that CYP1A1, TNF receptor 1 (TNFR1), p21 were up-regulated approximately 320, 1.5, 2 times than control, respectively. AhR was down-regulated 5.1 times. AhR nuclear translocator (Arnt) and p53 were highly expressed in control and their mRNA expression levels did not change significantly.

TNF-α is produced in activated macrophages, natural killer cells, some T-lymphocyte subpopulation and some tumor cell lines[2]. Therefore, when dioxins reach bone marrow where TNF-α is expressed, there is much possibility that abnormal inhibition

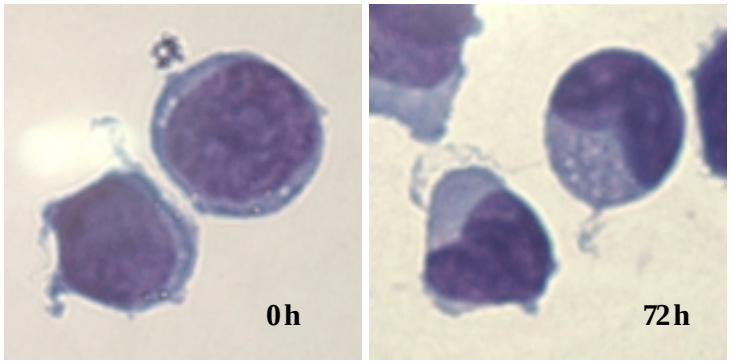

Fig. 3 Morphological change of ML-1 cells treated with 2,3,7,8-TCDD 10 nM and TNF-α 10 ng/mL.

of hematopoietic stem cell proliferation happens. We hypothesized this is one of the mechanisms for damages to immune function, especially hematotoxicity caused by dioxins. Investigation of gene expressions confirmed p21, which plays important role in apoptosis, was induced by 2,3,7,8-TCDD exposure. Induction of TNFR1 could be also one of the reasons, but so far not so critical. Further study should be made to elucidate the mechanism for this inhibition of ML-1 cells proliferation caused by 2,3,7,8-TCDD

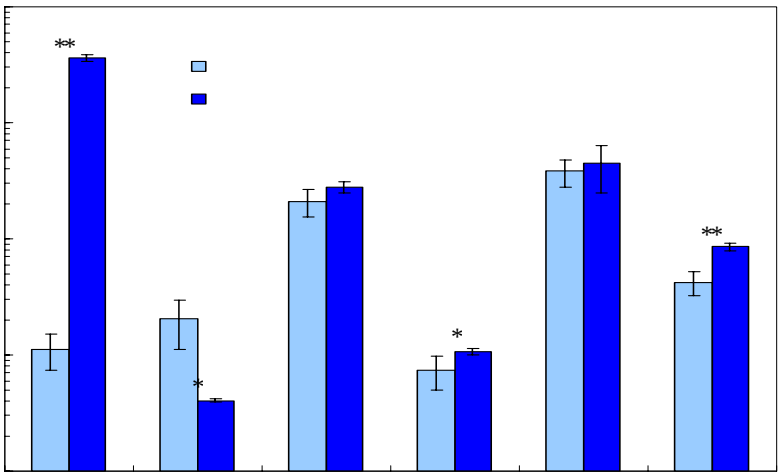

Fig. 4 Induction / repression of genes caused by 2,3,7,8-TCDD involved in drug metabolism and TNF-α-induced apoptosis. Values are given as mean±S.D. of triplicate samples. *P<0.05, **P<0.01.

and TNF-α.

Conclusions

2,3,7,8-TCDD affected human myeloblastic leukemia ML-1 cells proliferation and differentiation as follows:

- 2,3,7,8-TCDD alone didn't affect ML-1 cells proliferation and differentiation.
- When combined with TNF-α, 2,3,7,8-TCDD strongly inhibited ML-1 cells proliferation and enhanced TNF-α-induced differentiation.

References

1. Takuma T., Takeda K., and Konno K. (1987) Biochem. Biophy. Res. Commun. 145, 514
2. Fiers W. (1991) FEBS lett. 285, 1991