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Differential regulation of phospho-p38 and phospho-ERK by TCDD

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Introduction

The contamination of the environment with pollutants is one of the main problems of modern life, and the levels pollution in industrialized regions are giving raise to increased public concern. The mitogen-activated protein kinase (MAP kinase) are play a pivotal role in the regulation of important cellular functions by activation of specific signal transduction pathways from cell the surface to the nuclei. Three major subgroups of MAP kinases have been identified, and these comprise the extracellular signal-regulated kinase (ERK), the c-Jun amino-terminal kinase (JNK), and the p38 MAP kinases [1-3]. Herein, we investigated the effect of regulation of phospho-JNK (p-JNK), phospho-p38 (p-p38) and phospho-ERK (p-ERK) by TCDD.

Materials and Methods

Immunoblot analysis: RAW 264.7 cells were cultured in the plate in the presence or absence of TCDD. Cells were lysed in the lysis buffer and were sonicated by sonicator. The protocol on electrophoresis, transfer to membrane, incubation with antibodies were carried out according to the previously detailed method [4]. The primary antibodies form Santa Cruz Biotechnology were detected using alkaline phosphate (AP)-conjugated anti-mouse, rabbit or goat IgG and visualized by NBT/BCIP substrate after extensive washing of the membranes.

MTT Assay: The colorimetric MTT assay was performed as described by Hanelt *et al.* [5] to assess proliferation/viability in RAW 264.7 cell cultures. The significance of the results was analyzed by one-way ANOVA; $p<0.05$ was considered significant. DNA agarose gel electrophoresis: Isolation of apoptotic DNA fragment was carried out according to the previously detailed method [4].

Result

MAP kinases activation in RAW 264.7 cells: The level of activation of ERK (44KDa) was increased within 4h after TCDD treatment and the level of activation of p38 was elevatecd within 1h, and these activation were maintained for 48 hrs in macrophages. In contrast, JNK MAP kinase activities (Fig. 1) was unchanged after treatment. Also Caspase-3 slightly activated through time 30 min to 48 h.

Effects on DNA fragmentation: We isolated DNA from RAW 264.7 cells exposed 10nM of TCDD. Apoptotic fragment was significantly shown from TCDD treated time on 1 h through 48 h. Distinct 180-bp DNA fragments were detected on the TCDD-treated cells (Fig. 2).

MTT Asssy: A significant cell death were shown on Figure 3. As shown Figure 3, approximately 17%, 20%, 23%, and 35% of Raw 264.7 cells died under 10nM TCDD from 8 h through 48 h.

Discussion and Conclusion

Dioxin and its structural analogs are widespread environmental contaminants and highly toxic. In our study, we investigated the effect of regulation of phospho-JNK (p-JNK), phospho-p38 (p-p38) and phospho-ERK (p-ERK) by TCDD. P-p38 was activated by TCDD within 1h and p-ERK was activated by TCDD within 4 h after treatment and these activation were maintained for 48 h in macrophages. And caspase-8activity was slightly induced through the experimental time course. On the other hand, c-Jun N-terminal protein kinase (JNK) remained unchanged. These data suggest that TCDD may regulate the cellular functions through ERK and p38 cascades.

References

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