2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Induces Adseverin Gene Expression in Mouse Thymus – a Protein Possibly Important in TCDD Induced Immunotoxicity.

Camilla Svensson and Katarina Lundberg

Department of Pharmaceutical Biosciences, Division of Toxicology, Uppsala University, P.O. Box 594, SE-751 24, Sweden

Introduction

Thymus atrophy and suppression of the humoral and cell-mediated immune-responses are striking signs of 2, 3, 7 ,8 -tetrachlorodibenzo-*p*-dioxin exposure in experimental animals. In the thymus TCDD causes a rapid but transient decrease in cell proliferation and a subsequent reduction of mainly immature thymocytes. Although the proliferation returns to normal within a few days, the cell number in the thymus remains low for a long period of time. (1,2)
The mechanisms behind the TCDD induced thymus atrophy are poorly understood. However, effects on the epithelium, the thymocytes or the prothymocytes have been proposed.
Most, if not all, toxic effects of TCDD are believed to be the result of Ah-receptor mediated gene regulation. So far only a few TCDD regulated genes (mostly metabolic enzymes like CYP1A1) have been identified and none of these can explain the toxicity caused by TCDD(3).
In order to identify genes that are involved in TCDD induced immunotoxicity we have performed differential display RT-PCR on mouse thymus, a technique identifying differentially expressed mRNAs in two cell populations.

Materials and methods

Female C 57 Bl/6 mice, six weeks of age, were exposed to TCDD (0.5, 2.5, 5, 10 or 50μg/kg i.p.) or dexamethasone (1 mg/kg) and sacrificed at different time points, from three hours up to two weeks thereafter. Total RNA from whole thymus, isolated thymocytes and liver were extracted according to the acid guanidinium-isothiocyanate-phenol-chloroform method (4) and subjected to differential display RT-PCR (5). The PCR products from controls and TCDD treated samples were runned side by side on a polyacrylamide gel followed by autoradiography. Differentially expressed bands were identified by cloning and sequencing according to standard procedures.
RT-PCR was performed with adseverin and CYP 1A1 specific primers according to a modified version of a protocol published by Lai et al (6). Radioactive PCR products were separated on a 1,5 % agarose gel and autoradiographed. The autoradiograms were scanned and the density of the resulting bands were calculated with the house-keeping gene hypoxanthine phosphoribosyltransferase as an internal standard.

Results and discussion

Differential display PCR revealed a TCDD induced band identified as a gene coding for adseverin, an actin binding protein. RT-PCR with adseverin specific primers showed a dose and time dependent increase in adseverin gene expression after exposure to TCDD in both whole thymus as well as in isolated thymocytes. However, no increased adseverin gene expression was observed in the liver. In contrast, CYP 1A1 gene expression, was induced both in liver and thymus. Interestingly, adseverin gene expression was not induced by dexamethasone, an agent with immunotoxic effects similar to TCDD.

This indicates that upregulated adseverin gene expression is a primary, immune-specific, possibly Ah-receptor mediated event and not a consequence of general toxic effects or changes in cell number.

Adseverin is a calcium dependent protein whose main function is to bind to and sever actin filaments, thereby facilitating cell migration, division and exocytosis (7, 8). Thus, adseverin is of decisive importance for normal cell functions. The role of adseverin in TCDD induced immunotoxicity still remains to be investigated. However, one interesting fact is that adseverin interacts with phosphatidylinositol 4,5-bisphosphate which links adseverin to important signal transduction pathways in the cell.

References

1. Lundberg, K., Grönvik, K-O., Goldschmidt, T. J., Klareskog, L and Dencker, L.; *Chem.-Biol. Interactions* , **1990**, 74, 179
2. Lundberg, K.; *Biochem. Biophys. Res. Comm.* **1991**, 178, 16
3. Greenlee, W. F., Sutter, T. R. and Marcus, C.; *Prog. Clin. Biol. Res.*, **1994**, 387, 47
4. Chomczynski, P. and Sacchi, N.; *Anal. Biochem.*, **1987**, 262, 156
5. Diachenko, L., Ledesma, J., Chenchik, A. A. and Siebert, P. D.; *Biochem. Biophys. Res. Comm.* **1996**, 219, 824
6. Lai, Z.-W., Hundeiker, C. Gleichmann, E and Esser, C.; Mol Pharmacol, **1997**, 52, 30
7. Schafer, D. A. and Cooper, J. A.; *Annu. Rev. Cell dev.*, **1995**, 11,497
8. Rodrígues Del Castillo, A., Vitale M. L. and Trifaró, J.-M.; *J. of Cell Biol.*, **1992**, 119, 797