TCDD-Mediated Alterations of Tyrosine Phosphorylation in Human and Rat Cell Lines

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Introduction:

Numerous studies have suggested that TCDD alterations in kinase activity and protein phosphorylation may lead to alterations in cell growth and/or cell differentiation (reviewed by Matsumura [1]). It is generally accepted that TCDD-mediated effects result from alterations in gene expression through enhancer activity of the liganded Ah receptor in association with the Arnt protein. Alterations of protein kinase activities and protein phosphorylation may result from AhR regulation of gene transcription, although direct alterations of kinase activity have not been eliminated as a potential pathway. In this respect, alterations of kinase activity in murine B lymphocytes occur within minutes of TCDD exposure suggesting that some of the effects may not be transcriptional [2]. It will be important to disect how altered kinase activity contributes to altered cell growth and/or differentiation caused by exposure to TCDD.

Evidence indicates that ovarian hormones play a role in the hepatocarcinogenic activity of TCDD in the female rat [3]. Reproductive tissues have also been shown to be a target for TCDD's effects [4]. This suggests that ovary and liver as estrogen responsive tissues may be a target organ for TCDD-mediated effects. We have been investigating *in vitro* models that may help to dissect the various contributions of cell types and biochemical pathways to the hepatocarcinogenic effects of TCDD. One cellular model for hepatocytes and liver as a target tissue is the WB cell, a non-transformed rat liver epithelial cell that expresses a normal functioning EGF receptor system [5][6][7]. Another cellular model we are investigating is the human ovarian carcinoma cell line, BG-1, which has been shown to have a functional estrogen receptor system as well as a normal functioning EGF receptor system [8][9]. In the current study we investigated the effects of *in vitro* exposure to various concentrations of TCDD on alterations of tyrosine protein phosphorylation in WB and BG-1 cells.

Materials and Methods:

WB cells were kindly provided by Dr. Joseph W. Grisham, University of North Carolina, Chapel Hill. The WB cells were grown in Richter's improved minimal essential media with 0.1 µM insulin in 35 mm tissue culture plates with the addition of 10% fetal calf serum at 37C in a humidified incubator with 95% air/5% CO2. All experiments with WB cells were performed with cells that had just reached confluence and media was replaced at treatment with fresh media and different concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin in DMSO or vehicle (final concentration of DMSO did not exceed 0.05%) were added to the cells and grown for 5 days. The BG-1 cells were provided by Dr. Jeff Boyd. BG-1 cells were grown in DMEM/F-12 media containing 10% fetal calf serum to approximately 60% confluence and treated with TCDD or DMSO and grown for 18h.

To terminate treatment, cells were washed with Hanks balanced salt solution and cell lysis was accomplished by addition of lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 2.5 mM EDTA, 500 µM NaVn, 1% Triton X-100, 1% deoxychloate, 0.1%SDS, 10 mg/ml PNPF, 20 µg/ml aprotinin, and 40 µg/ml APMSF) to the tissue culture plates and rocking for 5 minutes on ice. The cell lysate was transferred to a centrifuge tube, sonicated and clarification of the lysate by centrifugation at 14,000 x g for 5 minutes. Protein was assayed using the BCA method on an aliquot of these lysates. A 200 µl aliquot of the lysates was immediately treated with 200 µl of 2xSDS buffer and heated to 95C for 5 min and frozen until electrophoresis and western blotting.

Equivalent amounts of protein from each treatment were produced by dilution in SDS buffer and loaded (20 µg/lane) onto 8% Novex SDS polyacrylamide gels and electrophoresed. Western blotting for phosphotyrosine was done as described [10] using a mouse monoclonal antibody. Quantification of intensity of bands was done by scanning laser densitometry with an LKB UltrascanXL.

Results:

In the human ovarian carcinoma, BG-1, TCDD caused a dose-dependent increase in cell proliferation as indicated by increased cell number following an 18 hour exposure . A similar dose-dependent induction of CYP1A1 mRNA and protein occurred with TCDD treatment. The EC50 for cell proliferation and induction of CYP1A1 activity was approximately 200 pM TCDD. The results demonstrate that the BG-1 cell line is extremely sensitive to the effects of TCDD, and therefore possesses a functional Ah receptor system. Treatment of BG-1 with various concentrations of TCDD showed a dose-dependent increase in tyrosine phosphorylation of proteins with an estimated molecular mass of 181, 162, 154, 123, 114, 107, and 87 kDa while a dose-dependent decrease was detected in a 49 kDa protein. Shown in figure 1 are TCDD-mediated dose response relationships for alterations of protein phosphorylation for these proteins. The increases in the phosphoryaltion of the 181 kDa protein did not appear to be associated with the epidermal growth factor receptor since no apparent changes in receptor expression occurred in BG-1 at this exposure time.

Table 1. Alterations of Protein Phosphorylation* by TCDD in BG-1 Cells.

kDa pTyr protein	Control	30 pM TCDD	300 pM TCDD	3000 pM TCDD
181	1.54 ± 0.05	2.08 ± 0.38	2.44 ± 1.20	3.29 ± 1.72
162	1.55 ± 0.10	1.50 ± 0.58	2.07 ± 1.34	2.79 ± 1.26
154	2.63 ± 0.18	2.27 ± 0.66	2.72 ± 1.34	3.72 ± 1.23
123	4.00 ± 0.68	4.70 ± 1.32	6.81 ± 0.68	8.40 ± 2.37
114	4.90 ± 2.00	5.90 ± 2.95	9.31 ± 3.63	10.73 ± 5.45
107	4.44 ± 2.37	5.13 ± 2.26	9.22 ± 2.86	10.20 ± 4.34
87	4.00 ± 0.47	4.23 ± 0.80	6.68 ± 1.20	7.86 ± 1.60
49	12.74 ± 1.19	11.14 ± 0.35	9.12 ± 2.26	6.92 ± 1.30

* Numbers represent density as determined by scanning laser densitometry. Mean band density plus or minus standard deviation for 3 replicate dose response experiments is shown.

Exposure of WB cells induced cell proliferation and CYP1A1 associated enzyme activity. The EC50 for enzyme induction in this cell line was estimated to be approximately 50 pM TCDD while the EC50 for increased cell proliferation was estimated to be approximately 10 pM TCDD. This suggests that the WB cell is exquisitely sensitive to TCDD's effects. Increases in proterin phosphorylation were observed in proteins of molecular mass of 120, 68 and 66 kDa while decreases were observed at 170 kDa in WB cells.

Discussion:

TCDD-mediated effects on tyrosine phosphorylation in BG-1 and WB cells occur with alterations in cell proliferation and differentiation. It will be interesting to determine the cause and effect relationships between these alterations of protein phosphorylation and resulting changes in cell proliferation. The identity of the majority of these phosphoproteins is unknown at this time. In WB cells the decrease in protein phosphorylation of the 170 kDa protein has been established to be due to a decrease in the synthesis of the epidermal growth factor receptor which results in an apparent decrease in protein phosphorylation [7]. BG-1 has been shown to express functional receptor systems for estrogen and epidermal growth factor [8][9]. Therefore, this cell line may be useful as a model system to investigate the interactions of various receptor systems that control cell growth and differentiation and interactions with the Ah receptor.

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