Induction of Protein Synthesis by Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin in Primary Cultured Rat Liver Parenchymal Cells and Rat Livers

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is proposed to bind to an intracellular arylhydrocarbon receptor, which is then translocated to the cell nucleus where it associates with specific responsive elements on the genome. One consequence of this association which has been well described is the induction of cytochrome P-450 and other drug metabolizing enzyme activities. However, the effect of TCDD on the expression of other genes has not been extensively studied. Although unknown gene products induced by TCDD are also possibly responsible for its carcinogenicity, teratogenicity and other biological activities, TCDD inducible gene products other than cytochrome P-450 and related enzymes have yet to be identified. We demonstrate here the induction of several proteins which have not been previously documented following *in vitro* and *in vivo* exposure to TCDD. Proteins with molecular weights (Mr) of 26000 and 39000 were expressed by TCDD *in vitro* in primary cultured rat liver parenchymal cells. *In vivo* TCDD administration to rats resulted in the induction of five proteins in the liver.

Primary cultured rat liver parenchymal cells were incubated in modified William's E medium containing 1 nM TCDD for 23 h and 47 h. After exposure to TCDD, newly-synthesized proteins were labeled with [³⁵S] methionine for 1 h. The cell lysates prepared from TCDD exposed and control cells were separated by SDS/polyacrylamide gel electrophoresis and [³⁵S] labeled proteins were detected by fluorography. Two

proteins with Mr of 26000 and 54000 dalton (26k-P and 54k-P, respectively) were markedly induced by TCDD exposure for 23 h. Prolonged exposure to TCDD (47 h) caused induction of not only 26k-P and 54k-P but an additional protein with Mr of 39000 (39k-P).

In order to further analyze the alteration of gene expression by TCDD precisely, the cell lysate from the cells exposed to TCDD for 47 h was subjected to 2D gel electrophoresis. Synthesis of four proteins was significantly increased in the TCDD exposed cells. Judging from their Mr, three of these proteins should be 26k-P, 39k-P, and 54k-P. On the other hand, synthesis of three proteins with Mr of 24000, 25000 and 29000 (24k-P, 25k-P and 29k-P, respectively) was inhibited by TCDD exposure. Reduced synthesis of certain proteins by TCDD has not been reported.

In vivo effect of TCDD on the relative amounts of proteins in rat liver was also examined. TCDD was administered perorally to rats at a dose of 100 µg/kg body weight. On day 14 after administration, rats were sacrificed. Crude extracts prepared from a TCDD administered and a control rat liver were separated by 2D gel electrophoresis. Amounts of seven proteins whose Mr were similar to 26k-P, 39k-P and 54k-P (two proteins with Mr of 26000, one of 36000, two of 39000 and two of 54000) were increased in a TCDD administered rat. 54k-P migrated to the same positions on 2D gel as one of the two proteins with Mr of 54000. However, the positions of 26k-P and 39k-P were not identical but close to those of the proteins increased in TCDD treated rat liver.

Our investigations demonstrate that TCDD can regulate the expression of several genes (induction of 26k-P and 39k-P, and inhibition of synthesis of 24k-P, 25k-P and 29k-P) in rat liver parenchymal cells. These alterations in gene expression may be related to the varied biological activities caused by TCDD. However, the precise functions of 26k-P and 39k-P are still unknown and remain to be further studied.