TOXICITY OF TCDD IN RAT HEPATOMA CELLS: DEPENDENCY ON THE AH RECEPTOR

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ABSTRACT

TCDD inhibits growth of 5L rat hepatoma cells, which possess a high level of Ah receptor, but not the growth of receptor deficient 5L variant cells. The Ah receptor ligands benz[a]anthracene and 3,3',4,4'-tetrachlorobiphenyl (TCB) exert toxic effects which resemble those of TCDD, whereas the non-binding 2,2',4,4'-TCB does not elicit toxic responses. The present results strongly suggest that interaction of TCDD with the Ah receptor is a necessary link in the chain of events leading to the toxic response in 5L cells.

INTRODUCTION

The extremely high toxicity of TCDD and related compounds has been the subject of intensive studies (1,2), but the mechanism of action is still unknown. One of the best characterized properties of TCDD is its high affinity binding to a specific cytosolic protein complex, the Ah receptor (3), which regulates the expression of a number of genes, notably cytochrome P450IA1 (4). A number of studies have suggested that the Ah receptor plays an important role in the toxicity of the dioxin (1, 5, 6). However, there is evidence that binding proteins other than the Ah receptor might be involved in mediating the toxic responses to TCDD (7, 8, 9).

In the past, studies on the mechanism of action of TCDD have been severely hampered by the lack of cellular test systems, particularly continuous cell lines (10) which are amenable to selection and genetic engineering. Recently, we have shown that toxic effects of TCDD can readily be detected in the rat hepatoma line 5L (Wiebel, Klose, Kiefer, in preparation). In the present study we i) investigated the effect of TCDD on growth and vitality of the 5L cells and ii) tested the role of the Ah receptor in mediating the toxic effects.

EXPERIMENTAL PART

Dedifferentiated 5L Reuber hepatoma cells (11) were cultured using standard techniques. Chemicals were added dissolved in DMSO or acetone. The parameters for cell growth and vitality were analysed in cultures on 96-well microtiterplates. The amount of DNA per culture was determined fluorimetrically according to Labarca and Paigen (12), the amount of protein was measured using the Kenacid Blue staining (13) and the uptake of *neutral red* was determined as described by Borenfreund and Puerner (14). Cytochrome P450IA1 activity was measured as the hydroxylation of benzo[a]pyrene (15). The cytosolic levels of the Ah receptor were determined in ligand binding assays as described previously (16).

RESULTS AND DISCUSSION

Effect of TCDD on cell growth and vitality: Treatment of 5L cells with 1 nM TCDD markedly inhibits the increase in cell number and the amount of DNA per culture (Figure 1). Cell vitality, as indicated by the uptake of neutral red, and increase in the amount of protein are less strongly affected (Figure 1). In consequence, this leads to an increase in the protein to DNA ratio, i.e. cellular 'hypertrophy'. The amount of DNA per cell is decreased by 34 %, indicating an accumulation of cells in the G_1-phase of the growth cycle. Thus, inhibition of growth by the dioxin appears to be attributable to a delay in the progression of cells through the G_1-phase of the growth cycle. TCDD does not significantly affect the pattern of cellular proteins suggesting that the dioxin does not substantially alter the status of cell differentiation.

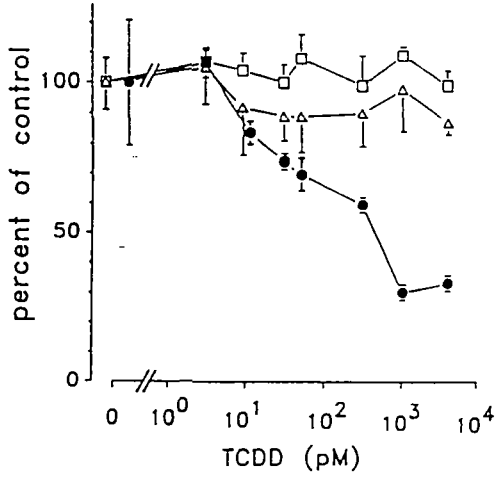

Figure 1: Effect of TCDD on DNA content, total protein and uptake of neutral red in 5L cells. Cells grown on 96 well microtiter plates were exposed to various concentrations of TCDD for 48 h. DNA content/well (●——●), amount of total protein (□——□) and neutral red uptake (Δ——Δ) were determined as percent of the solvent-treated controls.

Role of the Ah receptor in the toxicity of TCDD: The involvement of the Ah receptor in the toxic response of the 5L cells to TCDD is suggested by 3 findings. First, TCDD affects growth of wild type 5L cells possessing the Ah receptor but not growth of their receptor deficient variants (Table I). Second, TCDD exerts toxic effects at concentrations similar to those inducing P450IA1 activity, which has been shown to be dependent on the interaction of the inducer with the Ah receptor (17). Third, the Ah receptor ligands 3,3',4,4'-tetrachlorobiphenyl (TCB) and benz[a]anthracene, but not the non-binding 2,2',4,4'-TCB, elicit toxic responses which resemble those of TCDD.

Table I: Sensitivity to TCDD, AHH inducibility and Ah receptor content in 5L wild type cells and variant clones

Cell	Cell growth[a,b] % of control	AHH activity (pmol/mg x min)[c]		Ah receptor (fmol/mg)[d]
		control	induced[a]	
5L wild type	57 ± 4	3.4 ± 0.6	106 ± 3	84 ± 19
5L-rB[a]P-8	113 ± 9	< 0.1	< 0.1	< 3
5L-rB[a]P-12	83 ± 7	< 0.1	< 0.1	< 3
5L-rDNP-1	97 ±15	< 0.1	< 0.1	< 3
5L-rDNP-3	97 ± 8	< 0.1	< 0.1	< 3

[a] Cells were treated for 24 h with 1 nM TCDD.
[b] Cell growth was determined by counting cells in TCDD- versus solvent-treated cultures (mean±SD).
[c] The AHH activity is related to total protein content of cell homogenates (mean±SD).
[d] The amount of the Ah receptor is calculated per mg of cytosolic protein (mean±SD).

The presence of the Ah receptor, although necessary, is not sufficient to render cells susceptible to TCDD toxicity. Thus, there are many cell lines including hepatoma lines related to the 5L cells which possess the receptor protein but are insensitive to the toxicity of TCDD (10 and Wiebel, Klose, Kiefer, in preparation). Clearly, additional conditions, e.g. a particular status of regulation, have to be met for cells to become sensitive to TCDD in analogy to the differential response of cells to hormones or growth factors (18).

In conclusion, the interaction of TCDD with the Ah receptor appears to be the first link in a chain of events leading to toxic responses. Cell lines such as 5L may offer a powerful system for further dissecting the molecular mechanism of dioxin toxicity.

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