Inhibition of Gap-Junctional Intercellular Communication by 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD): Possible Role of the Ah Receptor.

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Abstract:

Inhibition of gap junctional intercellular communication (GJIC) was used as an *in vitro* system for analysis of potential tumor promotor activity of 2,3,7,8-TCDD in a hamster lung fibroblast cell line (V79) and a mouse hepatoma cell line (Hepa-1c1c7). In both cell types GJIC was significantly inhibited. However a more drastic and sustained inhibition of GJIC (up to 70%) was observed in Hepa-1c1c7 cells. GJIC appeared to be affected after short exposure times, even before ethoxyresorufin-*O*-deethylase (EROD) activity could be established. α-Naphthoflavone significantly reduced 2,3,7,8-TCDD induced inhibition of GJIC, which may suggest at least partial involvement of the Ah receptor.

Keywords: Gap-Junctional-Intercellular-Communication; Tumor Promotion; Cytochrome P450IA1; Ah receptor; 2,3,7,8-TCDD.

Introduction:

Polychlorinated dibenzo-*p*-dioxins (PCDD's) and related compounds are ubiquitous environmental pollutants with similar toxicological properties. Firm evidence exists for a central role of the aryl hydrocarbon (Ah) receptor in most of the toxic effects[1] induced by these compounds. Structure dependent correlations between Ah receptor mediated P450IA1 induction and other toxic responses (e.g. immunotoxicity, hepatotoxicity) have been established[2] for many of the PCDD, PCDF's and coplanar PCB congeners. There is however some dispute on the possible role of the Ah receptor in carcinogenic events induced by 2,3,7,8-TCDD and related congeners.
In a two stage model of carcinogenesis PCB's and dioxins increase the incidence of

carcinomas in skin[3], altered hepatic foci[4] *in vivo* and of focus forming in cells[5], indicating that these compounds are strong tumor promotors.
Tumor promoting stimuli induce clonal proliferation of initiated cells that would otherwise remain pathologically dormant. In maintaining this growth restraint gap-junctional intercellular communication (GJIC) with surrounding normal cells is thought to be an important feature. Indeed a great number of tumor promotors has already been shown to inhibit GJIC in vitro[6] in different cell types. 2,3,7,8-TCDD however failed to inhibit GJIC in V79 cells[7] in spite of being two (*in vivo*) to four (*in vitro*) orders of magnitude more potent than 12-*O*-tetradecanoylphorbol-13- acetate (TPA) in promotion assays other than GJIC.
In this "extended abstract" we show effects on GJIC of 2,3,7,8-TCDD in two cell types namely chinese hamster V79 cells in which the Ah receptor has not been detected[8] and Hepa-1c1c7 cells that possess an Ah receptor and inducible P450IA1 activity. In Hepa-1c1c7 cells we also measured EROD in order to correlate an Ah receptor mediated effect with inhibition of GJIC. Moreover we used α-naphthoflavone (α-NF), a notorious competitive inhibitor of Ah receptor mediated responses[9], to investigate potential involvement of this receptor protein in inhibition of GJIC.

Material and methods:

I Cell Culture.
Cells were cultured in Ham's F10 (V79 cells) and α-MEM (Hepa-1c1c7 cells) supplemented with antibiotics (gentamicin, 50 μg/ml) and FCS (10%). TCDD (99% pure, Schmidt, The Netherlands) was dissolved in DMSO (Merck, Germany). Exposure of cells at indicated concentrations took place in culture media for 24 hours unless otherwise indicated. The concentration of DMSO never exceeded 0.5 % wich was used in control incubation.

II Measurement of GJIC.
Intercellular communication was measured using a dye transfer technique[10]. Lucifer yellow was injected in a single cell in monolayer culture, and the number of surrounding dye recieving cells was counted. For each concentrations 6 different dishes were tested and in every dish at least 30 injections were done. Significance was tested using the Student-*t*-test.

III Measurement of Ethoxyresorufin-*O*-deethylase (EROD).
Cells were seeded (4.10^5 cells per well) in 6 well dishes. After 48 hours cells were exposed for 24 hours unless otherwise indicated. Cells were harvested in Tris/sucrose buffer (pH 7.8), protein was determined using the BioRad protein assay[12]. For each 2,3,7,8-TCDD concentration tested 6 wells were measured in duplo. EROD was determined using a method adapted from the procedure described by Pohl and Fouts [11] The reaction took place in Hepes buffer containing BSA (Sigma Chemical Co, USA), NADPH and NADH (Boehringer, Germany) (0.7 mg/ml) and magnesiumsulphate (Sigma)(1.4 mg/ml) at 37°C. Reaction started by addition of ethoxyresorufin (50 μl from 200 μM stock in ethanol), and stopped after 15 minutes with 3 ml methanol. After centrifugation fluorescence was measured at a wavelength 550 nm (excitation) and 585 (emission) slitwidths were 5 nm an 10 nm respectively.

Results

2,3,7,8-TCDD inhibited GJIC in a dose dependent manner in both V79 and in Hepa-1c1c7 cells (data not shown). Differences were observed in the temporal effects of 2,3,7,8-TCDD in V79 cells as compared to Hepa-1c1c7 cells (fig 1). In V79 cells only at very early time points after exposure to 2,3,7,8-TCDD a drastic inhibition of GJIC (up to 60%) was observed, which diminished to 40% inhibition at 24 hours after exposure. In Hepa-1c1c7 cells inhibition was 80% maximum and persisted throughout the 48 hour time span of exposure. In figure 2 a comparison between the induction of EROD activity and the inhibition of GJIC in Hepa-1c1c7 cells by 2,3,7,8-TCDD treatment is shown. GJIC appeared to be a very rapid event and was affected significantly already after 30 min. of exposure. A significant EROD induction was observed at 3-5 hours after exposure in the same Hepa-1c1c7 cells.

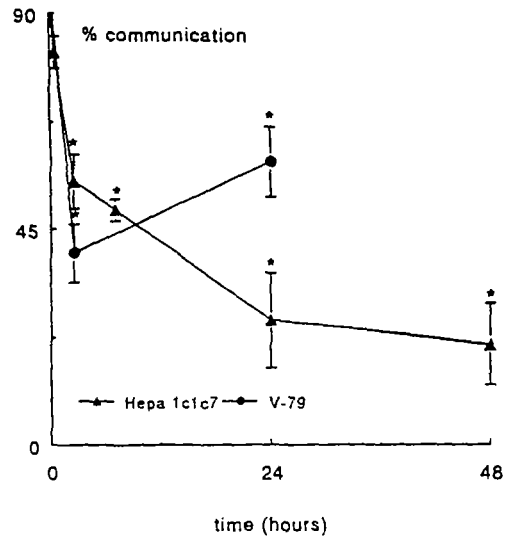

Figure 1: Inhibition of GJIC in Hepa-1c1c7 (•) and V79 (▲) cells by 1nM 2,3,7,8-TCDD. Values are mean ± sd. Asterisks indicate significancy from control ($p \leq 0.005$).

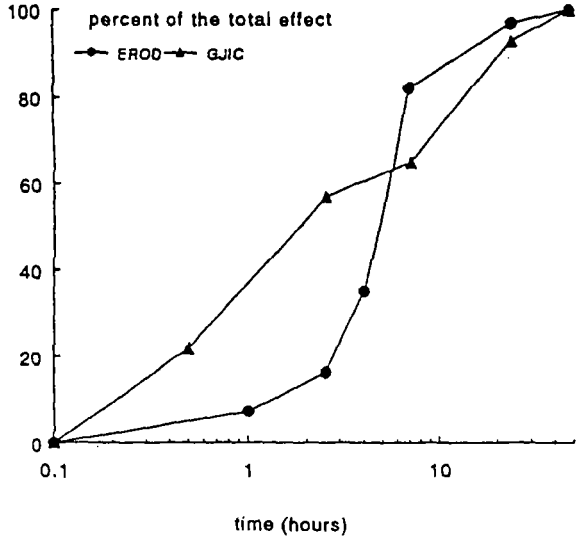

Figure 2: Inhibition of GJIC (▲) compared to induction of P450IA1 (•) in Hepa-1c1c7 cells to 2,3,7,8-TCDD (1nM) for different periods of time. Values are percentages of the maximum effect.

Coadministration of 2,3,7,8-TCDD and α-NF, at a concentration that blocks 2,3,7,8-TCDD binding to the Ah receptor, resulted in a significant reduction of the inhibition of GJIC by 2,3,7,8-TCDD in Hepa-1c1c7 cells (tabel 1).

Tabel 1: Percentage of GJIC inhibition in Hepa-1c1c7 cells after exposure to different concentrations of 2,3,7,8-TCDD and after co-treatment with 2,3,7,8-TCDD and α-NF (1μM) for 24 hours.

Exposure	-αNF	+αNF
2,3,7,8-TCDD 0.25 nM	52*	23
2,3,7,8-TCDD 0.50 nM	57**	22
2,3,7,8-TCDD 1nM	59**	53*
DMSO 0.5%		0

*: significantly different from control ($p \leq 0.01$)
**: significantly different from control ($p \leq 0.005$)

Discussion

In contrast to other reports 2,3,7,8-TCDD did inhibit GJIC in a dose dependent manner in our system in both V79 and Hepa-1c1c7 cells. Inhibition was more pronounced and sustained in Hepa-1c1c7 cells as compared to V79 cells. From these data it is obvious that when investigating tumor promotion capacity of these compounds the choice of cell type and exposure time is very important. The reason for these differences may be several, such as different culture media, or the fact that these cells are not only originating from different species (hamster versus mouse) but also from different tissues (lung versus liver). However, it is also possible that discrepancies in effects on GJIC are caused by differences in presence and levels of the Ah receptor. V79 cells are thought to be deficient in inducible P450IA1 activity and may thus not possess an intact Ah receptor-mediated transcription-enhancer system. Hepa-1c1c7 cells on the other hand are well-known to possess the Ah receptor[13]. Possible involvement, at least in part, of the Ah receptor in GJIC inhibition is further strengthened by the observation that α-NF co-treatment significantly abolished the GJIC inhibitiory effect of 2,3,7,8-TCDD. α-NF is known to competitively bind to the Ah receptor but does not elicit a transcriptional enhancer signal for the CYPIA1 gene[9]. Further research will be focused on the role of the Ah receptor and other mechanisms involved in GJIC.

In conclusion, 2,3,7,8-TCDD was found to be a very potent inhibitor of GJIC in both V79 and Hepa-1c1c7 cell lines. There are indications that inhibition of GJIC may be partly mediated by the Ah receptor.

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