Interaction between the Heme Biosynthesis and Induction of EROD Activity in Chicken Embryo Hepatocytes Exposed to 2,3,7,8-TCDD and PCBs

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1. Introduction

The mechanisms of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds have been extensively studied and the most important has been found to consist of a receptor mediated mechanism via the aryl hydrocarbon (Ah) receptor protein, resulting, for example, in the induced gene transcription of cytochrome P4501A1 and 1A2 hemoproteins and their associated microsomal monooxygenase activitites, which include aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin-*O*-deethylase (EROD) activities[1]. Further, there are indications that cytochrome P450 monooxygenase activities are involved in this deregulation of the heme biosynthesis resulting in an increase of liver uroporphyrins as well as other forms of porphyrins [2,3].

In the following experiments with chicken hepatocytes, two biochemical effects, induction of EROD activity and porphyrin accumulation, will show different structure-activity relationships for non-dioxin like PCBs and dioxin-like PCBs and TCDD. In addition, it is shown that interactions between both responses can strongly influence the induction of EROD activity. The observed inhibitory effects on the EROD activity could have a significant impact on the value of EROD activity as a biomarker in the present methods of risk assessment for these compounds.

2. Materials and Methods

The induction of EROD activity and accumulation of total cellular porphyrins were investigated in primary chicken embryo hepatocytes *in vitro* as described by Kennedy *et al.* [4]. Primary chicken hepatocytes were cultured in 48-well cell culture plates. The EROD assays and the measurement of porphyrin concentrations were carried out in the wells. Each dose was in triplicates and the complete experiment was repeated three times.The presented dose-response curves are based on the means of the triplicates. The two biochemical responses were measured after 24 hours of exposure to dilution series of 2,3,7,8-TCDD, and twenty PCB congeners. The selection of PCBs was based on a multivariate physico-chemical characterization of all tetra- through hepta-chlorinated congeners [5] and resulted in

twenty structurally different congeners representative for the whole chemical class. The congeners were selected by combining principal component analysis with a factorial design[5]. The following PCB congeners were tested in the chicken embryo hepatocyte bioassay (numbering according to IUPAC): 2,2',3,4-TeCB (PCB#41), 2,2',4,6'-TeCB (PCB#51), 2,3,3',5'-TeCB (PCB#58), 2,3,4,4'-TeCB (PCB#60), 2,3',4,5'-TeCB (PCB#68), 3,3',4,5-TeCB (PCB#78), 2,2',3,4',6-PeCB (PCB#91), 2,2',4,4',5-PeCB (PCB#99), 2,2',4,6,6'-PeCB (PCB#104), 2,3,3',5,6-PeCB (PCB#112), 2,3,4,4',6-PeCB (PCB#115), 3,3',4,4',5-PeCB (PCB#126), 2,2',3,4,5,6'-HxCB (PCB#143), 2,2',4,4',5,5'-HxCB (PCB#153), 3,3',4,4',5,5'-HxCB (PCB#169), 2,2',3,3',4,5,6-HpCB (PCB#173), 2,2',3,4,4',6,6'-HpCB (PCB#184), 2,2',3,4',5,6,6'-HpCB (PCB#188), 2,3,3',4,4',5,6-HpCB (PCB#190), and 2,3,3',4',5,5',6-HpCB (PCB#193).

3. Results and Discussion

Figur 1A shows the EROD activity and porphyrin dose-response curves for chicken embryo hepatocytes exposed to TCDD. Increased EROD activity can be seen at low doses, reaching a maximal induction level at approximately 1nM. Noteworthy is the decrease of EROD activity at higher concentrations of TCDD. This decrease has been recognized in numerous studies, including studies of mammals, birds, fish, *in vivo* as well as *in vitro*[6,7]. Until recently, this phenomenon was either ignored or regarded as "cytotoxicity". However, there are several studies showing that cytotoxicity is not responsible for this effect[8,9].

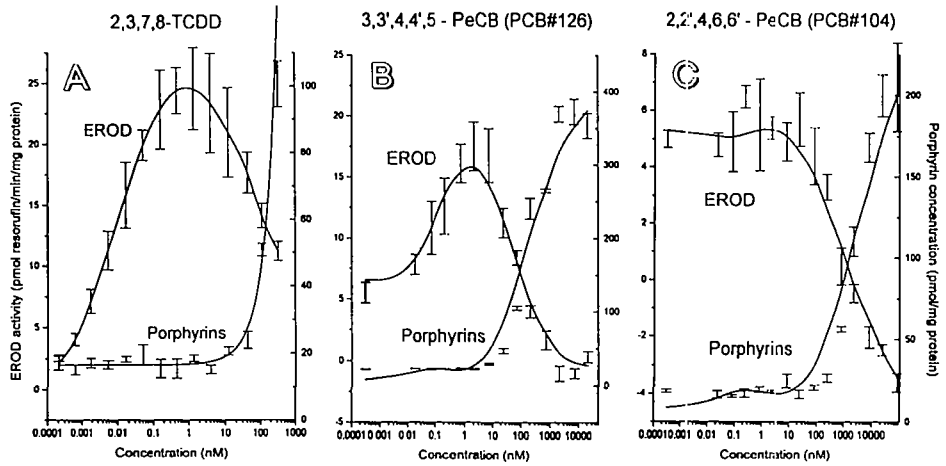

Figure 1. The effects of 2,3,7,8-TCDD (A), PCB#126 (B), and PCB#104(C) on EROD and accumulation of porphyrins in chicken embryo hepatocytes.

The accumulation of porphyrins occurs at higher doses of TCDD, and concurs with a decrease of EROD activity. The EROD activity and porphyrin accumulation for a major environmental contaminant like PCB#126, show a similar pattern as that found for TCDD, see Fig. 1B. Having no chlorine atoms in the ortho positions, PCB#126 is also structurally similar to TCDD and the biochemical responses are "dioxin-like". One example of a non-dioxin-like PCB, viz. 2,2',4,6,6'-PeCB (PCB#104), is shown in Fig. 1C. This tetra-orthochlorinated congener will not act via an Ah-receptor mediated mechanism, due to low Ah-receptor binding affinity, which is confirmed by the lack of EROD induction. However, exposure of the

chicken hepatocytes to PCB#104 results in a significant accumulation of porphyrins which concurs with a decrease in "background" EROD activity. The structure-activity relationships for EROD activity and porphyrin accumulation for all 20 PCBs are shown i Fig. 2.

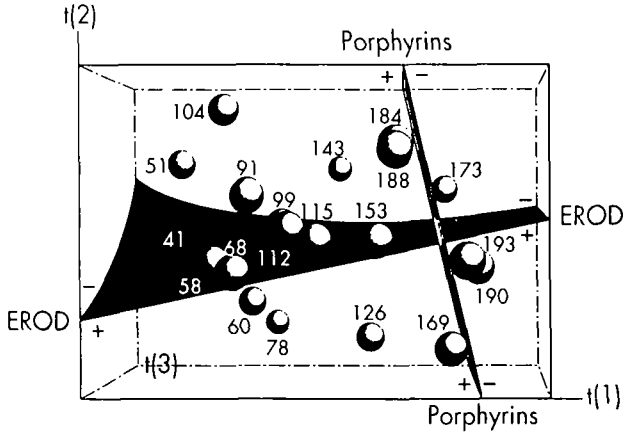

Figure 2. The distribution of the selected PCB congeners in the chemical domain (based on principal properties) of the PCBs. The sub-parts of the domain in which EROD activity and accumulation of porphyrins were measured are indicated with two planes. EROD activity is seen for congeners in the lower part of the plot and porphyrin accumulation is seen for congeners to the left in the plot, as indicated with + and - signs.

These results clearly indicate that there seems to be an inhibition of EROD activity which appear to be related with porphyrin accumulation. If this inhibition can be seen for a single dioxin or PCB, it can also be expected from mixtures. In order to study this possible interaction, chicken hepatocytes were dosed to mixtures of 2,3,7,8-TCDD and two ortho-substituted PCBs not showing any EROD activity in the previous experiments. These included PCB#153, which is present in high amounts in the foodchain, and PCB#104. TCDD was dosed at 0.02, 0.2, and 1nM, resulting in an increased EROD activity, followed by a constant dose of 1 nM (Fig. 3). Additionally, a complete dilution series of PCB#153 was

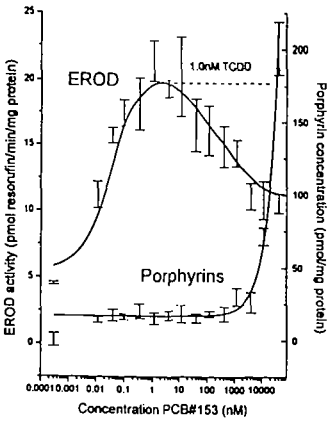

Figure 3. The effect of 2,3,7,8-TCDD in combination with increasing concentrations of PCB#153 on EROD activity and accumulation of porphyrins in chicken embryo hepatocytes. The dashed line shows the EROD activity caused by 2,3,7,8-TCDD alone.

dosed to the same hepatocyte cultures. At 1 nM, the EROD activity was found to be constant and, unsurprisingly, no direct inductive effects were observed from the PCB#153.

Nevertheless, as seen in Fig. 3, the combination of 2,3,7,8-TCDD and PCB#153 has a clear inhibitory effect on TCDD-induced EROD activity at dose levels which caused porphyrin accumulation. Earlier experiments had shown that at these dose levels TCDD itself did not cause porphyrin accumulation (Fig.1A). Inhibition of EROD activity caused by PCB#104 was similar to PCB#153 (data not shown).

4. Conclusions

Our results support the conclusion that there are nonadditive interactions between nondioxin-like PCBs and dioxin-like compounds[1]. The interaction between Cytochrome P450 activity and the heme biosynthesis makes the prerequisite of additivity in the TEF concept for environmental mixtures highly spurious. In our experiments porphyrin accumulation in chicken hepatocytes leads to an inhibition of EROD activity, a commonly used biochemical marker for Ah-receptor mediated dioxin-like activity. This inhibition of EROD activity could lead to a change in the dose-response curve, thereby influencing the EC_{50}-value, which is used as a sum parameter for dioxin-like activity of complex environmental mixtures. Thus, if ranking of biochemical activity of dioxin-like compounds is only based on *in vitro* EROD induction data, this could lead to misinterpretations from a toxicological point of view. Our experiments show that EROD induction and porphyrin accumulation are apparently governed by two classes of PCBs with distinct different structure-activity relationships. It should be recognized that both identified classes of PCBs are present in the environment and accumulate through the foodchain. Thus, the observed inhibition of EROD activity in present of nondioxin-like PCBs, which cause porphyrin accumulation, is of direct importance for the present risk assessment of these compounds, when this enzyme activity is used as a biomarker.

5. References

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