AHR RESPONSE TO TCDD IN HUMANS

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Introduction

2,3,7,8, tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) is the most potent toxic contaminant in the environment and induces in both humans and experimental animal models chloracne, wasting sindrome, altered sex hormon levels and affects developmental outcomes, thyroid function, immune function, epidermal growth factor receptor signaling, cellular growth and differentiation, cancer and induces drug-metabolizing enzymes (1,2). Most if not all of biological response elicited by dioxin are mediated by aryl hydrocarbons receptor (AHR) that binds specifically TCDD with high affinity. Following ligand binding, the AHR complex undergoes transformation, during which it is released from proteins to which it is associated including at least two molecules of a heat shock protein of 90 kDa (hsp90) and an additional 37 kDa AHR interacting protein. Liganded AHR complexes subsequently translocate into the nucleus and following their association with the ARNT (AHR nuclear translocator) and possibly other factors, the AHR complex is converted into its high affinity DNA binding form (3).
In animals TCDD exposure upregulates AHR expression (4).
In this work Ahr expression has been studied in humans accidentally exposed to TCDD in 1976 in Seveso to understand if AHR regulation can explain the reason why humans are possibly less sensitive than some animals for some acute toxic effects. Our preliminary results show the correlation between AHR mRNA levels and serum TCDD values in 1992 in people exposed in 1976.

Materials and Methods

Blood samples were collected in 1992 from 52 exposed subjects and 28 controls and mononuclear cells isolated by step gradient centrifugation on Histopaque. Total RNA was extracted according to the method of Chomczynski and Sacchi (5). Competitive RT-PCR for AHR and β-Actin were performed as described (6). For each RNA sample 6 equal aliquots (100 ng) were prepared, and a dilution series of the recombinant RNA internal standard was added to these aliquots. Following the first cDNA synthesis (reverse transcription) of the combined sample of RNA and rcRNA internal standard, a mix containing PCR reagents was added and 35 cycles of PCR were performed. Aliquots of the PCR were electrophoresed on 2% agarose gels and fragments visualized with ethidium bromide staining (Fig. 1). A photografic negative was prepared and densitometry was carried out and used to calculate the amounts of mRNA for Ahr and β-Actin.
AHR mRNA levels were standardized with β-Actin mRNA levels and expressed as multiple of median of control subjects (MoM).
TCDD serum levels were measured by GM-MS at Centers for Disease Control and Prevention 7(CDC) Atlanta, GA,USA and expressed as lipid adjusted part per trillion (ppt) (7).

Analysis using univariate statistic and Wilcoxon Rank test were performed with the use of SAS software. A p value less than 0.05 was used to indicate a statistically significant difference.

Results and Discussion

Exposed subjects showed two types of response: 45 of them had a reduction of AHR mRNA levels that was negatively correlated with TCDD serum levels in 1992 (p=0.01). On the contrary 7 subjects among exposed people had AHR mRNA levels higher than controls (p=0.0001) (Fig. 2).
The observed reduction in the 45 exposed subjects showed a dose-response effect with TCDD serum levels. For people with TCDD serum levels higher than 200 ppt, AHR mRNA levels are reduced to 70% of controls. As far as the expression in mononuclear cells can represent the response to TCDD exposure in liver also our results are in contrast with reduction observed in rodents. This different behavior may represent a defense mechanism against acute toxic effects of TCDD in humans (8,9). It will be of interest to estimate if AHR dependent induction of CYP1A1, CYP1A2 and CYP1B1 observed in animal models will be still induced in humans where AHR mRNA is down-regulated.
The higher AHR mRNA levels found in 7 exposed subjects with TCDD serum levels lower than 250 ppt may indicate a different AHR regulation mechanism in response to TCDD. Investigation on TCDD effects in these subjects will be useful to verify their AHR dependence.

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Figures

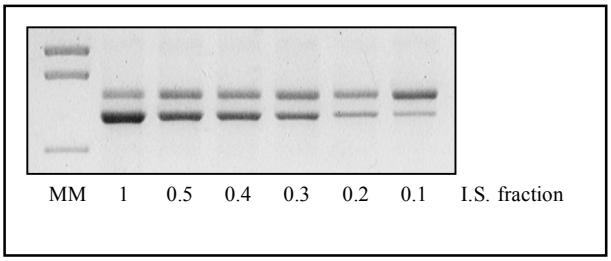

Fig.1: Ethidium bromide stained agarose gel showing quantitation of AHR mRNA in human mononuclear cells.

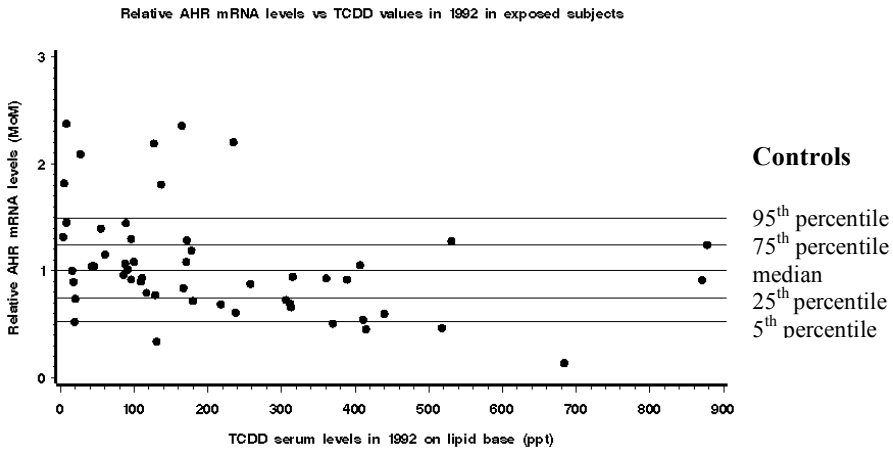

Fig.2: TCDD exposed subjects. Correlation between AHR mRNA levels and TCDD in 1992 serum samples levels on lipid base (ppt).