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Effects of PCB mixtures on recognized targets of endocrine disrupters exposure: search for early biomarkers

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Abstract

Polychlorinated Biphenyls (PCBs) are Endocrine Disrupters (EDs) relevant to human dietary exposure. Since liver is the main organ involved in metabolism, a human hepatoblastoma cell line (HuH6) was used as in vitro model. A panel of nuclear receptors (NRs) was selected as early markers of PCBs exposure and analysed by qPCR. PCB congeners, grouped in three mixtures according to similarities in the modes of action, were used at the concentrations derived from previous data on human internal exposure. Preliminary results indicated that PCB mixtures exert different NRs modulations in HuH6.

Introduction

PCBs are a class of EDs highly relevant to environmental contamination due to the high lipophilicity and stability; thus, PCBs bioaccumulate in the lipid fraction of animal tissues. Human exposure occurs mainly through diet to a mixture of different congeners [1,2]. Liver represents one of the PCBs target organ, highly responsive to endocrine regulation since it exhibits a high expression of NRs. PCBs display interaction with a wide range of NRs shown to be target for EDs and selected as panel of possible early biomarkers of effect: Estrogen Receptors (ERα, ERβ), Aryl Hydrocarbon Receptor (AhR), Costitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) involved in detoxifying phase I and II metabolic pathways. Moreover, PCBs interfere with Testosterone-Androgen Receptor (AR) binding, Peroxisome proliferator-activated receptor γ (PPARγ) and Thyroid Hormone Receptor α (THR-α) signalling and interact with NRF2, a transcription factor implied in the regulation of different antioxidative genes. The aim of the present study is to investigate the effects exerted by PCB mixtures on human hepatoblastoma cell line (HuH6), selected as *in vitro* model, in order to assess possible effects following the three PCBs mixtures exposure on the selected panel of NRs. We grouped 21 relevant PCB congeners in three mixtures on the basis of similarities in the modes of action [3]: one featuring DL-PCBs (Mix2) and two featuring NDL-PCBs (Mix1; Mix3). Preliminary results on ERα, ERβ AhR AR and PPARγ gene modulation were reported.

Materials and methods

PCB congeners were mixed to obtain 3 different mixtures (tab.1). Concentrations of PCBs in each mixture has been derived from previous analysis on human adipose tissues [4].

Mix 1	
Congener	**pg/ml medium**
44	0.759
49	0.602
52	1.280
101	2.233
174	1.184
177	14.607
187	53.201
201	18.564

Mix 2	
Congener	**pg/ml medium**
77	0.407
81	0.226
105	14.776
114	3.383
118	68.309
126	0.256
169	0.132

Mix 3	
Congener	**pg/ml medium**
99	41.848
153	288.947
180	202.199
183	29.570
196	22.887
203	

Table 1. The PCB congeners composition of the three mixtures and final concentrations in cell culture medium.

In order to exclude any cytotoxic effect of PCB mixtures at the experimental concentrations on HuH6, an MTS assay was performed.
Cells were treated with the three PCB mixtures or medium alone as control for 72h at 37 °C. Afterwards, cell monostrates were extracted for their total RNA content. RNA were quantified and retrotranscribed to cDNA. qPCR reactions were performed for each gene of interest. Data were analysed for their statistical significance by t-test.

Results

None of the PCB concentration tested resulted cytotoxic for HuH6. HuH6 liver cells resulted affected by the three PCBs mixtures treatments displaying an altered gene expression modulation for almost all the NRs analysed as shown in fig.1.

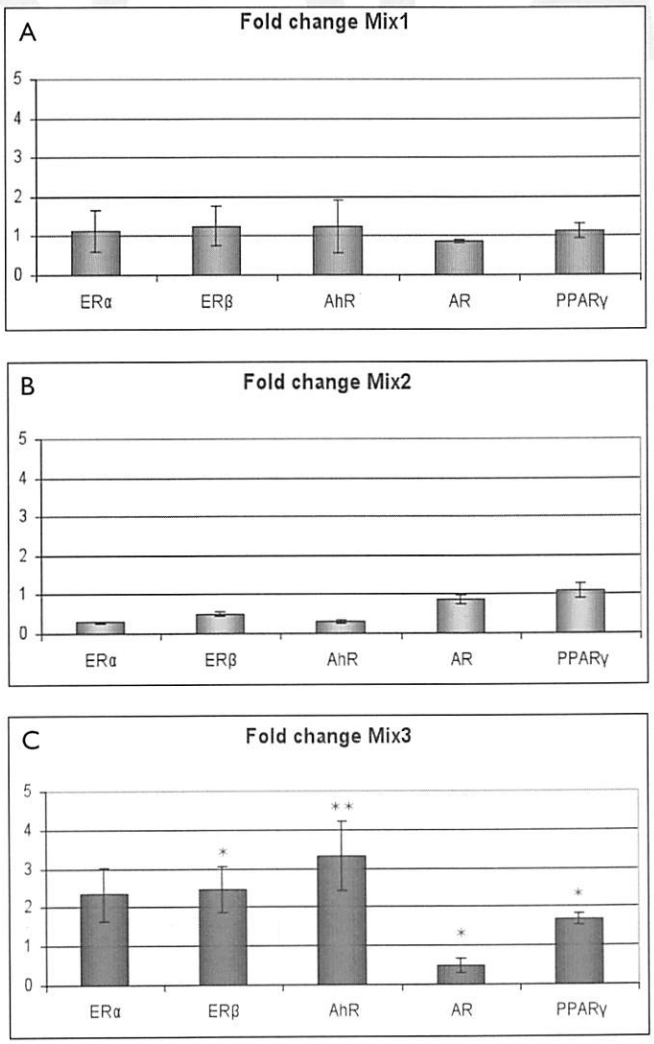

Figure 1. Gene expression values of NRs analyzed expressed as Fold change ± SEM (control value = 1), with GAPDH as reference gene. Statistical significance between treated samples and control is indicated by asterisks (=p-value <0.05; **<0.01; ***<0.001)*

In particular, the three mixtures evoked different gene expression patterns. Mix3, one of the NDL mixture, exerted the higher magnitude in modulation: ERα, ERβ, AR, PPARγ resulted significantly up-regulated whereas AR resulted significantly down-regulated (fig.1C). Although not significant a down-regulation of all NRs was observed in Mix2-treated HuH6 cells. In Mix 1 gene modulation was similar to control.

Discussion

HuH6 hepatic cells demonstrated to be a suitable and sensitive *in vitro* model to highlight gene expression modulations following treatment with PCBs at real human exposure concentrations. The selected NRs displayed to be a reliable panel of biomarkers of effects to determine potential differences exerted by different PCB mixtures treatments (fig.1). The three PCB mixtures evoked different responses in HuH6 with Mix3 displaying the higher magnitude in gene modulation (fig.1C). It is interesting to note that Mix1 and Mix3 show different pattern although being both NDL-PCB mixtures (fig. 1A, 1C). Such results support the PCBs congeners sub-division in three groups which allow to highlight distinct patterns of modulation, especially among the NDL-PCB mixtures, thus confirming the previously hypothesized different modes of action [3].

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