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LONG-TERM EFFECTS OF TCDD EXPOSURE ON HUMAN ARYL HYDROCARBON RECEPTOR (AHR) MEDIATED SIGNALING PATHWAY

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INTRODUCTION

The toxic action of the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) varies greatly, depending on the species, the type of tissue, age and sex, and on the timing of exposure (1). It has been classified as a human carcinogen (2-4) and it has the potential to disrupt multiple endocrine pathways (5-7). Most of the biological effects of dioxins are mediated by the aryl hydrocarbon receptor (AHR) that regulate the expression of a number of genes either by traditional transcription-dependent mechanisms or by other pathways including promoter methylation. (1,8-13) .

The AHR repressor (AHRR) plays an important role in the regulation of AHR-mediated gene expression by Polycyclic Aromatic Hydrocarbons (PAHs) (14, 15). Quantification of the regulation of AHR and related genes could be useful to monitoring the individual response to TCDD and PAHs exposure (16-19). We present data regarding the long-term effects of TCDD exposure on the expression of the AHR-dependent pathway genes (AHR, AHRR, ARNT, CYP1B1) studied in peripheral blood mononuclear cells of 49 patients exposed to different concentrations (extremely high to low) of TCDD after an accident in 1976 at Seveso, who did or did not develop chloracne. The main goal of the study was to verify whether serum levels of TCDD, measured 16-20 years from the exposure, were associated to modification of the expression of the AHR-dependent pathway genes.

MATERIALS AND METHODS

Study population

a) People exposed to TCDD in 1976 in Seveso: On July 10, 1976, an explosion in a trichlorophenol-manufacturing plant (ICMESA) close to Seveso (Italy) released into the atmosphere probably up to 30 Kg of TCDD that contaminated thousands of people of both sexes and various ages (20). As part of a health assessment, blood samples from Seveso residents were collected soon after the explosion for clinical chemical testing; the remaining portion of each serum sample was stored at –20°C. We studied 49 subjects (19 males and 30 females) from 17.0 to 70.5 years of age (mean 38.9) with available 1976 and 1992-96 serum TCDD concentrations, measured by high resolution GC-MS (21) at the Centers for Diseases Control and Prevention (Atlanta, GA, USA). TCDD serum concentrations in 1976 ranged from 84 to 56,000 ppt with a median of 6,054 ppt (mean 2,520 ppt) (22, 23), while 1992-96 values ranged from undetectable to 878 ppt with a median of 169 ppt (mean 229 ppt). For non-detectable values, a value of one-half of the detection limit for that sample was assigned (23, 24). Among these patients 29 (21 females and 8 males) had developed chloracne in 1976: 13 of type 1 and 2 (light chloracne) and 16 of type 3 and 4 (severe chloracne) (25).

b) Comparison group: 60 healthy subjects (31 females and 29 males, mean age 34.6 years , range 19.6-64.6) matched for age and smoke habit were recruited as comparison from people living in the Seveso surrounding non-contaminated area. Mean TCDD serum concentration in people of the comparison group was 4.2 ppt (17).

Gene Expression

Total RNA was extracted (26) from peripheral blood mononuclear cells (PBMC) isolated from fresh whole blood within two hours after collection, frozen with an automatic apparatus and thereafter stored in liquid nitrogen. Quantitative analysis of mRNA expression of AHR, AHRR, ARNT, CYP1B1 genes and ß-actin gene (as reference gene) was measured by Real-time Reverse Transcription PCR using the TaqMan technology on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Relative fold changes in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method (27), where $2^{-\Delta\Delta Ct}$ represents the amount of target, normalized to an endogenous reference and relative to untreated controls.

RESULTS AND DISCUSSION

AHR: In the 49 Seveso exposed subjects were detected higher values of AHR gene expression (range 0.80-4.64, median =1.60) than respective controls (n=60, range 0.49-2.09, median =0.99). This different expression can be observed both in males (n=19, median 1.57) and in females exposed (n=30, median 1.68) compared to respective controls (median 0.92 and 1.02 respectively) ($p<0.001$). No difference in expression can be detected in relation to gender or smoke habit both among exposed and comparisons (Fig.1A).

AHR-R: In the exposed subjects we did not observe a statistically significant difference of AHR-R gene expression (n=49, range 0.29-4.77, median = 0.76) compared to controls (n=60, range 0.34-6.65, median = 0.90). Males have higher values both in exposed and comparisons (range 0.41-4.77, median= 1.26 and range 0.34-6.65, median= 1.21 respectively) than females (range 0.29-1.99, median = 0.63 and range 0.36-2.69, median= 0.64) ($p=0.048$). Smoke habit induces the expression of AHR-R gene both in Seveso exposed (Female: smokers median=0.99, non-smokers= 0.59; Males: smokers median=2.05, non-smokers= 0.79) and controls (Female: smokers median=1.46, non-smokers= 0.58; Males: smokers median=2.22, non-smokers= 0.80) (Fig.1B-C).

CYP1B1: The results of CYP1B1 gene expression are reported in Fig. 1D. Increased levels of CYP1B1 gene expression can be observed in males older than 10 years at exposure to TCDD (median= 1.41) compared to controls (median= 0.86, $p=0.008$). (Fig.1E). These results differs from previous data reporting no differences on CYP1B1 expression in uncultured lymphocytes (17). In females there is no difference between exposed and controls; but the levels of CYP1B1 mRNA are inversely related to the age at the exposure to TCDD in 1976 with the youngest subjects (n=11, <10 years) showing lower expression of CYP1B1 than those older than 10 years in 1976 (n=19) (median= 0.92 and median= 1.38 respectively, $p=0.01$) (Fig.1F). No correlation of CYP1B1 expression with age has been observed in comparisons, either males or females.

ARNT: ARNT gene expression was not modified in exposed subjects compared to controls (data not shown). This result is in agreement with data from animal studies where ARNT gene expression appears not to be modified after TCDD exposure (28,29)

The long-term or permanent effects, in the Seveso exposed people, consist in a significant increase of the AHR gene expression both in males and in females, but not related neither to 1976 nor 1992 TCDD serum concentration (about one third of exposed patients with TCDD serum concentration between 100 and 1,000 ppt) with no induction of AHRR. In a previously published study on 62 Seveso people AHR mRNA levels, measured by quantitative competitive RT-PCR in uncultured lymphocytes, were negatively associated with plasma TCDD concentration being lower in exposed people (10.7 copies x 105/μg) than in controls (13.7 copies x 105/μg) (17). It has to be noted that these subject had lower TCDD serum levels (ranging from 3.5 to 90 ppt) than our group of exposed (range: from undetectable to 878 ppt, mean 229 ppt, median 169 ppt).

CYP1B1 is induced only in males exposed to TCDD after 10 years of age; CYP1B1 is slightly increased in females when exposed to TCDD after 10 years of age, and slightly decreased when exposed before 10 years of age. This can be the result of different effects of TCDD depending on sex, and the age at the time of exposure, being CYP1B1 in adults acutely induced and then induction tapers with time, while in children after 16 –20 years from the exposure the expression of CYP1B1 is slightly depressed. Age of exposure can influence the response to TCDD, as reported for other effects of TCDD in humans such as sex ratio modification (6).

Altogether, these data provide a picture of the response of AHR battery to TCDD in humans on a very wide period (almost 20 years). In conclusion, AHR gene still remains induced after many years from exposure to TCDD, differently from AHRR which response cannot be detected in the long-term period. Age of exposure influences the long-term response of CYP1B1 gene to TCDD.

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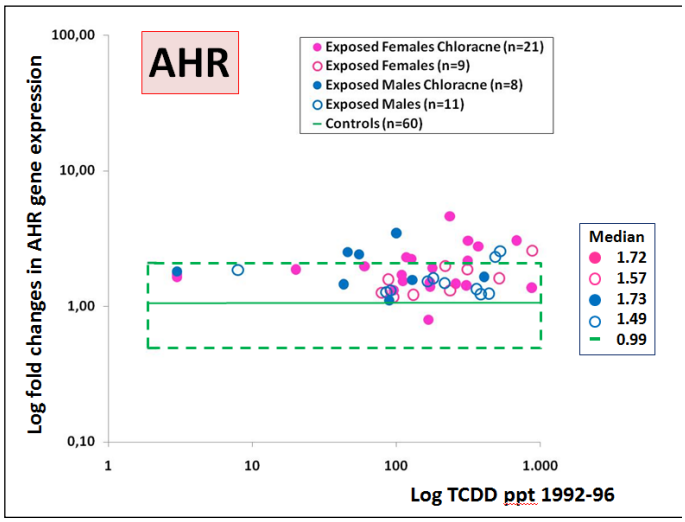
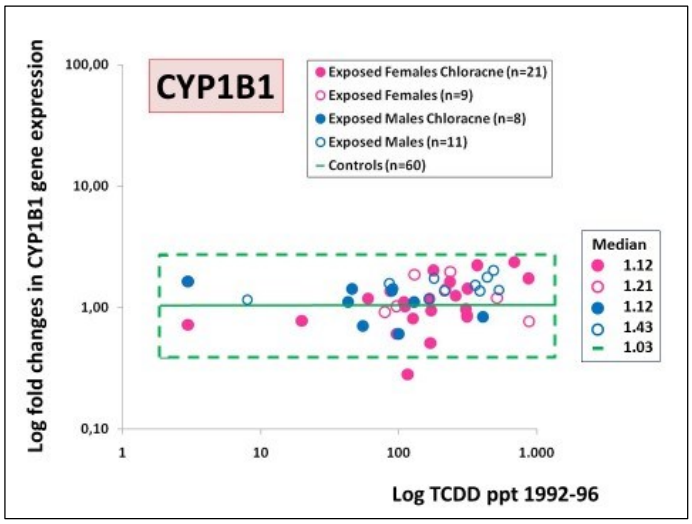


Fig.1A



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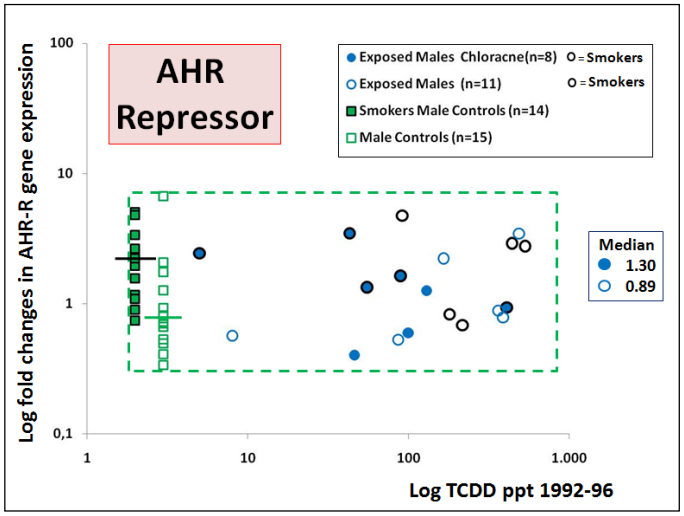

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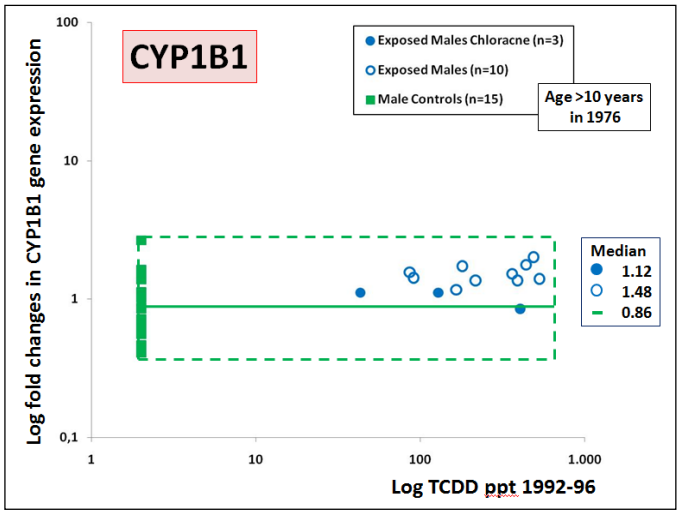

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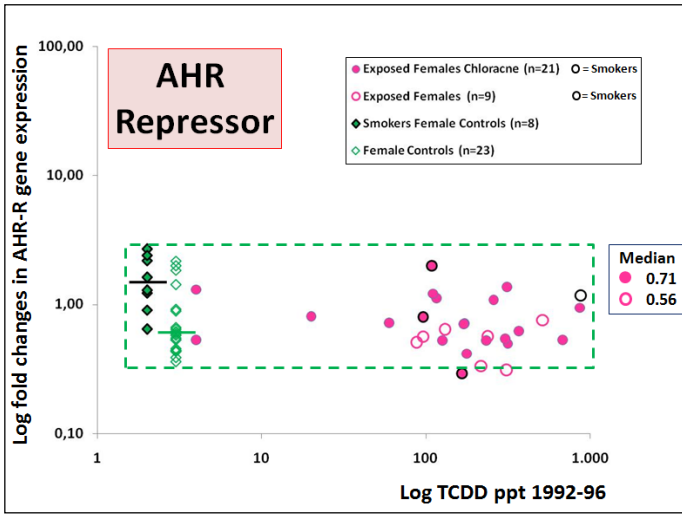

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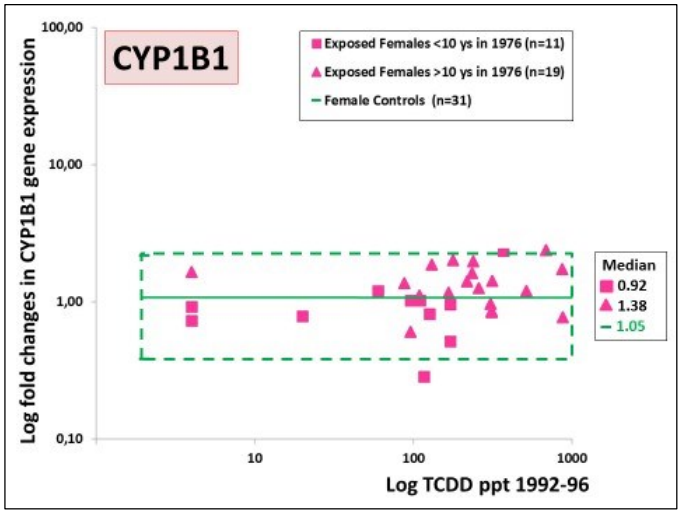

Fig.1F