HIGH EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN: HEPATIC CYP INDUCTION AND IMMUNE FUNCTION IN COMPARISON TO A CONTROL GROUP

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Introduction

In autumn 1997, several employers of a textile research institute in Vienna were exposed to 2378-tetrachlorodibenzo-p-dioxon (TCDD) in unknown manner. After development of choracne in a 30- and a 26-year-old woman, first TCDD measurements were performed in spring 1998, revealing the highest concentrations ever measured in adults (subject 1: 144,000 ppt; subject 2: 26,000 ppt in blood fat). Consequently, all employees of the institute were investigated for TCDD, showing the highest value in a healthy 26-year-old man (subject 3: 865 ppt in blood fat)[1,2]. We here report on measurements of hepatic cytochrome P450 (CYP) induction and of parameters of the immune system in these three subjects in comparison to a control group.

Material and Methods

Ah-receptor mediated transcriptional activation of cytochrome P450 1A2 (CYP1A2) was measured using caffeine as specific test substrate. 3-methyl-^{13}C-caffeine was applied orally at a dosage of 3 mg/kg body weight in the morning before breakfast. Metabolism was measured by the breath test in the following 4 hours (duplicate samples of expired air were taken every 15 minutes during the first 2 hours, and every 30 minutes thereafter). Analysis was by gas isotope ratio mass spectrometry. Results were evaluated as % cumulative ^{13}C exhalation (% of applied total ^{13}C dose). Additionally, caffeine and paraxanthine were measured in serum (before application and 30, 120 and 240 minutes later, using HPLC). Furthermore, caffeine and its metabolites AFMU, 1U, 1X and 17U were measured in urine collected quantitatively during 3 hours following the application. Methods were reported in detail elsewhere[3].

Blood was also sampled for the measurement of several parameters of the immune system, including blood count, immunoglobulins, IgG subclasses, specific tetanus antibodies, lymphocyte subpopulations (three colour FACS analysis using MABs), lymphocyte proliferation (^{3}H-thymidin incorporation following mitogen and antigen stimulation), intracellular cytokine production and cytokine secretion of PBMCs, as well as granulocyte function (chemiluminescence).

The three TCDD exposed subjects 1, 2 and 3 were investigated in October 2000, when TCDD concentrations in blood fat were 30,300, 10,100 and 420 ppt, respectively. They all are non-smokers. As a control, an age-matched group of 50 healthy volunteers was investigated between August and November 2000, using the same methods. They had to be either non-smokers (n=30, for at least one year) or heavy smokers (n=20, at least 20 cigarettes, mean 28 cigarettes per day).

Results and Discussion

Hepatic CYP1A2 induction

We here report on 6 parameters of caffeine metabolism, as compiled in the Table. Compared to the mean of non-smokers, 8-10 times higher values were observed in the highly exposed subjects 1 and 2 for 5 parameters. Due to this high induction, values of the cumulative 120 min. % dose of the breath test were already less different from controls. In subject 3, results were non consistent: values of the breath test and the urine metabolic ratio were next to the 95th percentile of non-smokers, whereas those evaluated from serum measurements were next to the mean of non-smokers. The effect of cigarette smoking on hepatic CYP1A2 induction was confirmed, but found to be much lower than that of high TCDD exposure.

	non-smokers	smokers	subject		
Parameter	mean ± SD, n=30	mean ± SD, n=20	1	2	3
Breath test (cum. 15 min % dose)	0.28±0.15	0.51±0.17	2.82	3.12	0.42
Breath test (cum. 120 min % dose)	5.89±2.36	9.61±2.49	28.7	33.1	9.8
Serum ratio paraxanth./caffeine (30 min.)	0.10±0.06	0.15±0.05	1.00	1.06	0.13
Serum ratio paraxanth./caffeine (120 min.)	0.24±0.09	0.38±0.13	1.97	2.25	0.32
Serum caffeine clearance (L/h*kg)	0.07±0.02	0.12±0.03	0.52	0.63	0.07
Urine ratio (AFMU+1U+1X)/17U	3.65±1.35	6.55±2.81	24.2	34.5	12.3

Taking our results and those of other studies in exposed people together, TCDD concentrations in blood fat higher than about 1000 ppt seem to be necessary to induce a significant effect detectable in epidemiological studies. This concentration level, however, may already cause chloracne in sensitive people. Therefore, direct measurement of dioxins is more sensitive to indicate the exposure than measurement of the biological indicator CYP1A2.

Immune system

In contrast to results of the CYP1A2 activity, no evident change of immunological parameters could be observed in association with the TCDD exposure (164 parameters evaluated). However, interpretation of the values measured in subject 1 was limited due to the chronic skin infection which required systemic therapy with corticosteroids (causing e.g. leucocytosis) and additional drugs. Regarding an effect of cigarette smoking on the immune system, 34/164 (21%) of the parameters were significantly different ($p<0.05$) in non-smokers and smokers.

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