BIOMARKERS IN THREE SUBJECTS FROM VIENNA HIGHLY EXPOSED TO 2378-TCDD: CYP INDUCTION AND IMMUNE FUNCTION

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Introduction

In autumn 1997, several subjects were exposed to 2378-TCDD in unknown manner in a textile research institute in Vienna. After development of choracne in a 30- and a 26-year-old woman (one severe, one mild form), first 2378-TCDD measurements were performed in spring 1998, revealing the highest concentrations ever measured in adults (144 000 ppt and 26 000 ppt in blood fat, respectively). Consequently, all employees of the institute were investigated for 2378-TCDD, showing the highest value in a healthy 26-year-old man (865 ppt in blood fat). For details, see other reports in this issue (A. Geusau et al).
Here, we report on measurements of cytochrome P450 induction in liver and lymphocytes and parameters of immune function in these three subjects.

Material and Methods

Ah-receptor mediated transcriptional activation of cytochrome P450 1A2 (CYP1A2) was measured with caffeine as specific test substrate. 3-methyl-^{13}C-caffeine (isotopic enrichment 99% according to the manufacturer Cambridge Isotope Laboratories, USA) was applied orally at a dosage of 3 mg/kg body weight in the morning before breakfast. Metabolism was measured by breath test in the following 6 hours by collection of expired air (containing the ^{13}C-CO_2) in 10 ml vials using a straw (duplicate samples every 15 minutes during the first 2 hours, every 30 minutes in the following 2 hours, every hour thereafter). Analysis was by gas isotope ratio mass spectrometry. Results were evaluated as % cumulative ^{13}C exhalation over two hours (% of applied total ^{13}C dose). This parameter is a well-established indicator of CYP1A2 mediated caffeine demethylation.
In order to compare different methods for measurement of caffeine metabolism, the half-life of the parent compound was also measured in blood serum (before application and 30, 60, 120 und 240 minutes later), using High Pressure Liquid Chromatography (HPLC). Additionally, caffeine metabolites were measured in urine collected quantitatively during 6 hours following application.

Additionally, CYP1A1 induction was measured as mRNA in lymphocytes. Heparin blood was collected by venipuncture in the morning before breakfast and was transferred to Berlin within 3 ½ hours. Peripheral blood mononuclear cells (PBMCs) were isolated using ficoll separation. After

washing, cells were cryoconserved and stored in the vapour phase of liquid nitrogen. After thawing, CYP1A1 mRNA was quantified by competitive RT-PCR as described earlier (1,2), with the modification that fluoreceine labeled primers were used and the products were quantified by polyacrylamide gel electrophoresis on an ABI 373 sequencer (Perkin Elmer/Applied Biosystems) using the genescan software.

Blood was also sampled for the measurement of several functions 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), cytokine production of PBMCs following stimulation with phytohemagglutinin (PHA), and granulocyte function (chemiluminescence).

Results and Discussion

Caffeine breath test (liver CYP1A2)
Results of the ^{13}C-Caffeine breath test are compiled in Table 1, including the results of an investigation of smokers and non-smokers using the same methods (3). In subjects 1 and 2, two tests were performed (in December 1998 and March 1999), showing excellent reproducibility. As result of liver CYP1A2 induction, metabolism of the applied caffeine was much faster compared to non-smokers and even heavy smokers (fortunately, the three subjects from Vienna are non-smokers). For the % cumulative 2h dose usually presented as measure for the metabolic rate, values were up to 4.9 and 6.3 times higher in subjects 1 and 2, respectively, compared to the mean of 11 non-smokers (mean age 26 years). Compared to the mean of the % cumulative 2h dose

Table 1 Results of the ^{13}C-Caffeine breath test, evaluated as % cumulative 2h dose exhaled (percentage of the applied total ^{13}C dose), and results of the CYP1A1 mRNA measurements in lymphocytes, evaluated as molecules per 50 ng RNA. For comparison, historical investigations in heavy smokers and non-smokers using the same methods are also presented for the breath test (3) and the CYP1A1 mRNA measurements (4).

Subject No.	initial 2378-TCCD conc. (pg/g blood fat)	time of invest.	cum. 2h dose (%)	CYP1A1 mRNA (molecules/50 ng RNA)
1	144 000	12/98 3/99	23.0 26.9	201 000 analysis not completed
2	26 000	12/98 3/99	34.7 33.7	325 000 analysis not completed
3	865	3/99	11.7	analysis not completed
heavy smokers (without filter), n=10			9.3 ± 3.5*	not performed
non-smokers, n=11			5.5 ± 2.8*	not performed
heavy smokers, n=17			not performed	58 000** (<LD–1 340 000)***
non-smokers, n=11			not performed	<LD** (<LD – 81 000)***

* mean ± standard deviation, ** median, LD = limit of detection, *** range

in 10 heavy smokers using no filters (mean age 28 years, more than 20 cigarettes per day, average consumption 32 cigarettes per day), values were up to 2.9 and 3.7 times higher in subjects 1 and 2, respectively. Subject 1 with the highest TCDD concentration in blood fat did show a slower metabolism than subject 2. This may be due to biological variation. In subject 3, the % cumulative 2h dose was 2.1 times higher compared to the non-smokers. The value was beyond the + 2σ value (11.1%), proving an induction of CYP1A2 at a 2378-TCDD concentration in blood fat of approximately 800 ppt.

Compared to other investigations of liver CYP1A2 induction in humans following exposure to PCDDs, PCDFs or PCBs, the values of subjects 1 and 2 are among the highest reported. In a cohort of 10 adult subjects from Yu-Cheng exposed to high dosages of PCDFs and PCBs, the highest % cumulative 2h dose was reported to be 23.6 % (5).
Due to the fast caffeine metabolism in subjects 1 and 2, measurements of the parent compound in serum 6 hours after application showed very low concentrations near to the limit of detection (first test, December 1998). Therefore, blood was sampled earlier and more often after application (see methods) during the second caffeine breath test in March 1999. Analysis was not yet completed in May 1999. Caffeine metabolites measured in urine collected quantitatively during 6 hours following the application did not seem to be a good measure for the increased liver CYP1A2 activity (first test, December 1998). This may be due to the relatively long collection period of 6 hours, as usually used for unexposed subjects. Therefore, urine was collected during a shorter period (3 hours) during the test in March 1999. Analysis was not yet completed in May 1999.

CYP1A1 mRNA in lymphocytes
Results of CYP1A1 activity measured ex-vivo as mRNA in lymphocytes are also compiled in Table 1 (for the blood sampling in March 1999, measurements are not completed). For non-smokers, values were above the limit of detection in two cases only, lower than those in subjects 1 and 2. The median of 17 heavy smokers (more than 20 cigarettes per day, average consumption 26 cigarettes per day) was also found to be lower than their values. However, these smokers were found to have a very high interindividual variability for CYP1A1 mRNA values in lymphocytes, and the values of subjects 1 and 2 were found to be within the range of these smokers.

With blood drawing, human cells are easily available (in contrast to human tissue samples), and the ex-vivo measurement of CYP1A1 mRNA in lymphocytes seemed to be an elegant method to look for a CYP induction due to PCDD/PCDF/PCB exposure (6). But results of our heavily 2378-TCDD exposed subjects 1 and 2 were disappointing: CYP1A1 mRNA measurements in lymphocytes seem to be a less sensitive and less specific biomarker for PCDD/PCDF/PCB exposure than the indirect liver CYP1A2 measurement via caffeine metabolism (breath test and decrease of serum concentrations). For long term follow-up and monitoring of induced biological changes due to high 2378-TCDD exposure, these minimal invasive methods seem to be the most suitable ones.

Immune function
In order to get substantial information on possible effects on the immune system of the three 2378-TCDD exposed subjects, an extensive program was applied (see methods). With minor

modifications, this program is identical with that applied for a study in 11-month-old breast-fed infants (K. Abraham et al, this issue). In the 3 subjects in question, no evident change of immunological parameters could be observed in association with the 2378-TCDD exposure. However, interpretation is limited due to two points: Firstly, the severe choracne led to a chronic skin infection with elevated markers of inflammation (e.g. C-reactive protein) in subject 1 who required a systemic therapy with corticosteroids (causing e.g. leucocytosis) and additional drugs which possibly also influence the immune system. Secondly, for interpretation of some of the immunological parameters measured (e.g. cytokine production), an age-matched control group has to be investigated (planned for the next months).

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