CYP1A1 AND CYP1B1 IN HUMAN LYMPHOCYTES AS BIOMARKER OF EXPOSURE: EFFECT OF DIOXIN EXPOSURE AND POLYMORPHISMS

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Introduction

Cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) are phase I enzymes that can be found in many tissues including peripheral blood lymphocytes. They are involved in the metabolic activation of many poly aromatic hydrocarbons (PAHs) but also in the oxidative metabolism of the estrogens to potentially genotoxic catechol estrogens [1]. CYP1A1 and CYP1B1 expression is regulated through the aryl hydrocarbon receptor (AhR)-mediated pathway. Several environmental contaminants including PAHs and persistent organochlorine pollutants such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins (PCDDs), are AhR agonists and can affect the expression of CYP1A1 and CYP1B1.

There are several known genetic polymorphisms of the *CYP1A1* and *CYP1B1* genes. A polymorphism in the 3'-untranslated region of the *CYP1A1* gene (CYP1A1 MspI or CYP1A1 m1) is often studied in relation with breast or lung cancer, but little is known about the functional effect of this polymorphism. An amino acid substitution in codon 432 (Val to Leu) of the *CYP1B1* gene is associated with a lower catalytic activity of the enzyme [2,3]. However, the involvement of these polymorphisms on the inducibility of CYP1A1 and CYP1B1 gene expression is unclear.

CYP1A1 and CYP1B1 mRNA expression levels can be determined in peripheral blood lymphocytes. This makes them potential candidates for use as biomarker of exposure to environmental compounds. Interindividual variations in mRNA expression patterns, catalytic activity and polymorphisms are very important factors when CYP1A1 and CYP1B1 expression patterns are used as biomarker of exposure, but little is known about it. Spencer et al. showed a concentration-dependent increase of CYP1B1 mRNA in lymphocytes upon exposure *in vitro* to 2,3,7,8-tetrachloro-p-dibenzodioxin (TCDD), the most potent dioxin [4]. Yet, only a few studies describe the *in vivo* correlation between polymorphisms, mRNA expression level and exposure to environmental factors. In this study, we wanted to obtain a better insight in the CYP1A1 and CYP1B1 mRNA expression and enzyme activity in human lymphocytes. We determined the constitutive CYP1A1 and CYP1B1 mRNA expression in lymphocytes of ten healthy volunteers and the variability in sensitivity toward enzyme induction by TCDD. Further, the CYP1A1 m1 and CYP1B1 Val432Leu polymorphisms were determined.

Materials and Methods

All individuals (N=10) were female non-smokers currently living in Utrecht (The Netherlands) with an average age of 26.4 years (range 24.1-28.7 yr.). Lymphocytes were isolated from fresh blood samples within 1 hour of collection using Ficoll-Paque isolation according to the manufacturer's instructions (Amersham Biosciences Corp, Piscataway, NJ, USA). The lymphocytes were suspended in culture medium consisting of phenol red-free RPMI 1640 supplemented with penicillin, streptomycin, phytohaemagglutinin and fetal bovine serum. Lymphocyte concentrations were determined using a coulter counter and cells were plated onto 12-wells plates. Then, culture medium containing the desired concentration of TCDD or the solvent vehicle (final concentration of 0.1% v/v DMSO) was added. Cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C. After 72 hours, the culture medium was replaced with Tris-buffer (0.5 mM, pH 7.8) containing 7-ethoxyresorufin for EROD activity determination and fluorescence (excitation wavelength of 530 nm, emission wavelength of 590 nm) was measured every 3 minutes for half an hour.

RNA isolation was perfomed using the phenol-chloroform method. RT-PCR conditions, primers and amplification parameters are as described previously [5]. RT-PCR products were run on a 2% agarose gel and bands were stained with ethidium bromide. Intensity of the ethidium bromide staining was quantified using a FuorImager (Molecular Dynamics, USA).

Genomic DNA was isolated from whole blood with a DNA isolation kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. Genotype analysis for CYP1A1 MspI and CYP1B1 Val432Leu polymorphisms was performed by PCR-RFLP, adapted after Kawajiri et al. and Tang et al. [6,7].

Results and Discussion

Constitutive EROD activity was low, but detectable in all individuals and the activity was concentration-dependently increased by TCDD (figure 1). There were differences in the maximum EROD activity among the individuals. However, when the activity was scaled to a % of the maximum activity, no differences were found in concentration-response curves. There were no significant differences found in EC50 values, which varied between 0.7 and 1.1 nM, indicating that the potency of TCDD was not different between individuals.

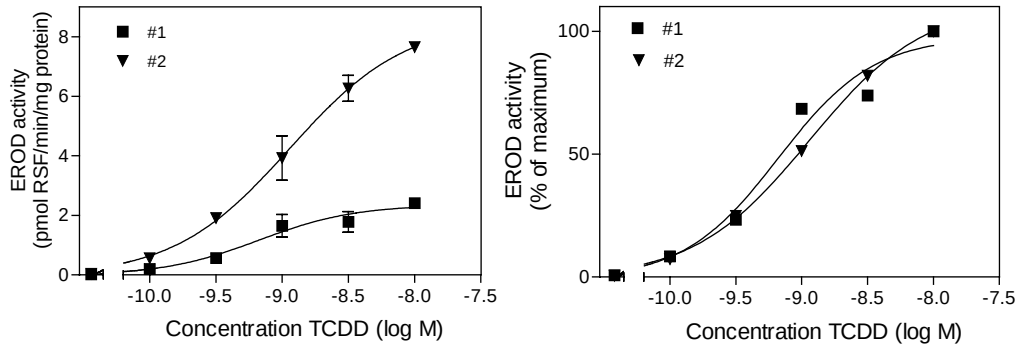

FIG 1. EROD activity in cultured lymphocytes of two individuals. Left, EROD activity is represented as pmol resorufin (RSF)/min; right, EROD activity is represented as % of the maximum activity. Data are represented as means of two determinations and the range.

In most samples, CYP1A1 expression was too low to be detected. CYP1B1 on the other hand, showed a high constitutive expression and was induced by TCDD. The differences in maximum EROD activity was reflected by the CYP1B1 mRNA levels; individuals with a higher maximum EROD activity showed a higher induction of CYP1B1 expression.

CYP1A1 m1 and CYP1B1 Val432Leu genotypes were determined of all individuals. The allele frequencies were 0.89 for CYP1A1 m1 and 0.4 for CYP1B1 432Val. These frequencies are similar to other observed frequencies in various healthy Caucasian populations. EROD activity can largely be attributed to CYP1A1, but CYP1B1 also exerts some activity. In our study, there was no apparent influence of the CYP1A1 and CYP1B1 polymorphisms on EROD activity. Both individuals shown in figure 1 have the CYP1A1 m1/m1 and CYP1B1 Val/Leu genotype.

This study shows *in vitro* exposure to TCDD resulted in a concentration-dependent increase of EROD activity and an increase of CYP1B1 expression in human lymphocytes. These effects were comparable among the individuals and the genetic polymorphisms appeared to have no effect in this study. However, the absolute mRNA expression levels and EROD activity can vary substantially among individuals. In addition, the EC10 values for TCDD observed *in vitro*, are only about a 10-fold higher than the background concentrations (TEQs) found in human plasma, which makes it not unlikely that the plasma TEQs affect CYP1A1 and CYP1B1 expression and activity in lymphocytes. This complicates the use of CYP1A1 and CYP1B1 as biomarker of exposure. Although the number of study individuals was small in this study, we think these findings may provide some insight in interindividiual differences and responses upon exposure to dioxin and dioxin-like compounds. Epidemiological studies investigating the correlation between exposure to

environmental compounds, mRNA expression, catalytic activity and polymorphisms of CYP1A1 and CYP1B1 should be performed to confirm these findings.

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