A PROPOSED PHYSIOLOGICAL ROLE OF THE ARYL HYDROCARBON RECEPTOR

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Introduction

Dioxins and dioxin-like chemicals are probably the most studied class of chemicals in the field of toxicology. TCDD is known to cause progressive anorexia and death in animals at low doses via its binding to a cytosolic receptor protein, the aryl hydrocarbon receptor (AHR) (for a recent update see [1]). For a long time the AHR was not considered to have an important physiological role and was thought only to induce xenobiotica metabolising enzymes [2]. Today, it is known that the binding of TCDD to the AHR starts, in a cell- and time-dependent manner, a transcriptional up-regulation of a large battery of genes involved in cell cycle regulation (i.e. apoptosis, proliferation and differentiation), morphogenesis, oxidative stress response and immune suppression. In spite of the massive research on chemicals that bind to and activate AHR, or which are metabolised through AHR-regulated biotransformation enzymes into potent carcinogenic metabolites, the physiologic role(s) of the AHR is not known.

We have introduced a new concept in dioxin research, which suggests that the TCDD binding protein, the AHR, has a physiological function in neuro-endocrine signalling of light. In several publications we have suggested that the AHR is a sensor for the illuminated environment through the binding of oxidized tryptophan derivatives, which may function as light hormones. This hypothesis is based on our original findings of; i) very high AHR binding affinity of certain tryptophan photoproducts [3], ii) the dependency on tryptophan for induction of AHR-regulated gene expression by UV irradiation of cells *in vitro* [4], iii) diurnal and seasonal rhythms in AHR-regulated gene expression [5,6], and iv) high CYP1A1 induction in the pituitary and activation of the proopiomelanocortin (POMC) gene by TCDD [7]. Several unexplained observations have been published with regard to the expression and activity of AHR regulated enzymes in the absence of exogenous ligands. One such is the description by Paigen and coworkers [8] of seasonal variation in aryl hydrocarbon hydroxylase (AHH) capacity and inducibility in human lymphocytes. Illuminated medium containing tryptophan and riboflavin induces AHH, as was shown already in 1976 by Paine [9]. UV light causes cutaneous and hepatic induction of AHH and ethoxyresorufin deethylase (EROD) activity in rats and mice [10,11] as well as cutaneous mRNA and protein induction of CYP1A1 and CYP1B1 in humans [12]. The formation of endogenous ligands for AHR as a result of photo-oxidation of L-tryptophan could explain these results. The photoproducts, which we have structurally identified as formylindolo[3,2-*b*]carbazoles, bind to AHR with very high affinity and induce CYP1A1 at pM concentrations [3,13]. Recently, we have shown that formylindolo[3,2-*b*]carbazoles are formed in cell culture medium exposed to light in the presence of riboflavin [14,15].

In the present study we quantified mRNA levels of CYP1B1 in uncultured peripheral blood mononuclear cells (PBMC) collected every second month and related the levels of basal CYP1B1

expression to exposure to polycyclic aromatic hydrocarbons (PAH) and to sunlight. For comparisons, the CYP1B1 mRNA levels in PBMC cells cultured for 3 hours with or without addition of the tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole (FICZ) were analyzed.

Methods and Materials

Blood samples were collected from 16 healthy volunteers of both sexes every second month in 1999 and 2000. The monocyte content in PBMC was determined using a fluorescence activated cell sorter. Information about environmental PAH exposure and solar radiation was obtained through questionnaires and solar radiation records, respectively. Average solar radiation during 28, 21 and 14 days before each blood sampling was calculated from daily solar exposure records using solar radiation data. For quantitation of CYP1B1 quantitative, competitive PCR with additions of artificial dsDNA competitive templates (CT) at four dilutions with known concentrations was performed. The linearity of the quantitative RT-PCR assay was investigated by titration of CYP1B1-CT in a concentration range of 1 330-170 000 molecules (0.00221-0.282 aM) and β-actin-CT in a concentration range of 18 700–4 780 000 molecules (0.0311-7.94 aM) against a pooled cDNA template corresponding to 0.1 μg of total RNA. The assay was found to be linear over this concentration range. The CYP1B1 expression was calculated per 10^6 β-actin mRNA molecules to compensate for differences in the amount of RNA and for differences in the cDNA synthesis efficiency. Both quantitative PCR reactions were performed in the same tube (multiplex PCR).

FICZ, which is an efficient AHR ligand and potent inducer of CYP1A1, was used to study the induction of CYP1B1 in PBMC *in vitro*. The cells, prepared from one individual, were grown in RPMI 1640 medium supplemented with 10 % fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine for 3 hours in the presence of 0-100 nM FICZ dissolved in DMSO.

Results and Discussion

The data revealed high levels of CYP1B1 mRNA and pronounced intra- and interindividual variations in CYP1B1 expression (Figure 1). The maximum range of CYP1B1 expression across different blood collection time points and subjects was 25-fold (1 670-41 300 CYP1B1 mRNA molecules/10^6 β-actin mRNA molecules). The individual average levels of expression ranged from 6 730 to 17 990 mRNA molecules/10^6 mRNA molecules (2.7-fold variation).

No significant correlation between monocyte content in PBMC and CYP1B1 mRNA expression was found (r_s=0.03, P=0.79). None of the genetic polymorphisms in *CYP1A1* (T6235C and I462V) or *CYP1B1* (R48G, A119S, L432V, N453S) affected the mRNA levels of *CYP1B1* in PBMC. In the questionnaires, sunbathing was reported in 12.9 % of all answers and PAH exposure (smoking or dietary PAH) in 26.8 %.

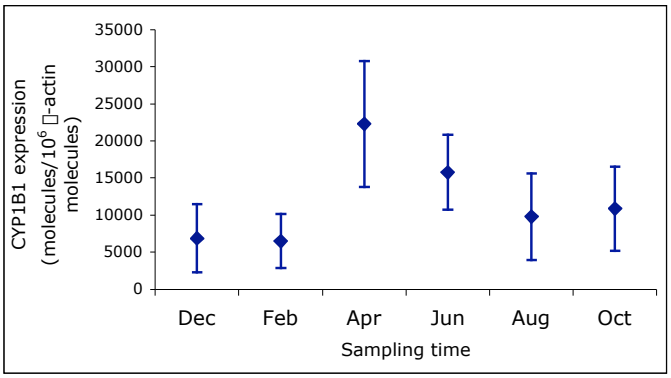

Figure 1: Constitutive *CYP1B1* mRNA expression in peripheral blood mononuclear cells from the study subjects at different blood sampling time points. Average values ± SD are shown.

No significant correlation between any of these factors and *CYP1B1* mRNA levels was noted. However, a significant correlation between solar radiation records and CYP1B1 expression was found (r_s=0.30, P=0.012).

The inducibility of CYP1B1 in PBMC *in vitro* was tested using the tryptophan photo-oxidation product FICZ. A dose-dependent increase in CYP1B1 mRNA, with maximal induction by 10 nM FICZ (5.6 fold induction), was observed after 3 h of incubation (Figure 2). CYP1B1 expression in the cultured, untreated sample (431 200 molecules/10^6 □-actin mRNA molecules) was 34-fold higher than the corresponding average level in uncultured cells (12 700 molecules/10^6 □-actin mRNA molecules). CYP1B1 was also induced 4.1 fold by 6 □M □-naphtoflavone. This medium-dependent 34-fold increase in basal CYP1B1 mRNA expression is probably caused by the presence of tryptophan (and tryptophan photoproducts) in the medium as has been shown in numerous studies since the original observation published by Paine [9]. FICZ has previously been shown to transiently induce CYP1A1 by an AHR-dependent mechanism [16]. The transience depends to a large extent on the efficient metabolism of indolo[3,2-*b*]carbazoles via the induced biotransformation enzymes [17,18]. We are currently investigating the metabolism of FICZ, which shows a very interesting metabolic pattern involving several AHR-regulated biotransformation enzymes [19,20].

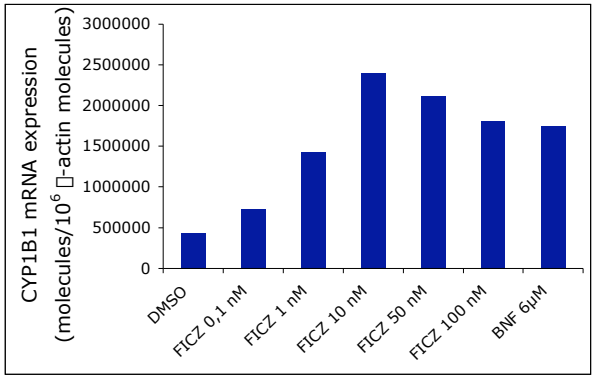

Figure 2: CYP1B1 mRNA expression in cultured peripheral blood mononuclear cells from one subject. 6-Formylindolo[3,2-*b*]carbazole (FICZ) and □-naphtoflavone (BNF) was added dissolved in dimethyl sulphoxide (DMSO).

Conclusions

The result from this and many earlier studies showing seasonal variation in AHH, EROD and CYP1A1/1B1 activity can be explained by the formation of tryptophan photoproducts, which may act as chemical messengers of light. These are carried by the blood from the site of formation to a specific receptor protein - the AHR - to signal changes in the environment and to trigger cell-specific responses. Like many lipid-soluble hormones these ligands are able to cross the plasma membrane and interact with a cytosolic receptor protein. The resulting hormone-receptor complex binds to transcription-control regions in DNA and affects expression of specific genes. In accordance with what is known about hormone signalling, the presence of the photoproducts seems to be carefully regulated and the target cells degrade them via the produced biotransformation enzymes (phase I and phase II enzymes) to terminate the response.

In contrast to the photoproducts, the environmental contaminant TCDD and related molecules of the halogenated aromatic hydrocarbon type bind with high affinity to the AHR but are not metabolically degraded and thereby the normal feed back regulation of the AHR signalling is disturbed. The persistent xenobiotic ligands also attenuate the responsiveness of the AHR to its endogenous ligand.

Light of different wavelengths seem to have the potential to modify the neuro-endocrine systems that regulate feeding behaviour, reproduction, pigmentation, fur growth, immune response and cardiovascular and hemodynamic parameters. We suggest that inappropriate activation of the AHR could potentially manifest itself as a disturbance of such light-regulated physiological processes.

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