INFLUENCE OF LIGHT ON THE *CYP1A1* ACTIVITY IN RAT HEPATOMA CELLS

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Introduction

The physiological roles and the endogenous ligands for the Ah-receptor (AhR) are still unknown. Among the candidates for the endogenous ligands, derivatives of indolocarbazole formed from the amino acid tryptophan by photooxidation, are among the most potent [1]. Their relevance *in vivo* e.g. as indicators of oxidation and their biological properties need to be characterized further both *in vivo* and *in vitro*. There are several reports in the literature on AhR dependent CYP1A1 induction that can be explained by light exposure of medium components [2,3,4]. It has been reported that changing medium gives rise to a transient induction of CYP1A1. Rannug et al. have in several studies shown that a specific photoproduct of tryptophan, 6-formylindolo[3,2-*b*]carbazole (FICZ), can bind to and activate the AhR with a very high specificity [5,6,7] (Figure 1). The aim of this project was to study and quantify the impact of light in combination with tryptophan and riboflavin, a well known photosensitiser, on the CYP1A1 induction *in vitro*. In particular we aimed at a quantitative comparison between photoproducts of tryptophan and light exposed medium. Since the AhR is a cytosolic transcription factor that is involved in the regulation of hundreds of genes and is involved in cell regulation processes such as proliferation and differentiation, control of its basal activity is important in experimental systems.

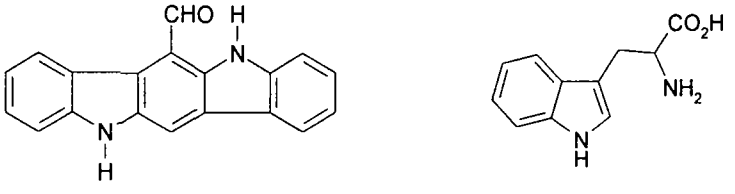

Figure 1. 6-Formylindolo[3,2-b]carbazole (FICZ) and tryptophan

Methods and Materials

All experiments were performed using an EROD-assay essentially as described earlier [8]. CYP1A1 activity was measured as 7-ethoxyresorufin-*o*-deethylase (EROD) activity in a rat hepatoma cell line (MH1C1). The cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10 % foetal bovine serum (Life technologies, Germany), sodium pyruvate (0.6 mM) and L-glutamate (3.8 mM) at 37°C in a humidified air/carbon dioxide (95/10 %) atmosphere. The concentration of tryptophan and riboflavin in the medium were 16 and 0.1 mg/litre, respectively. Cells were seeded into 96-well plates, at a density of 20,000 cells per well, in 0.2 ml of medium. After 24 hours, the plates were treated with light exposed medium or FICZ at concentrations from 0.0001 to 100 nM. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) at concentrations from 0.0001 to 0.16 nM was used as a positive control and internal standard. After exposure, each well was washed and the plates were stored at -80°C until further analysis. After thawing, the formation of

resorufin from ethoxyresorufin was determined fluorimetrically (544/590 exc/em). The values of relative fluorescence units from the EROD measurements were fitted to a logistic dose response curvature using the software TableCurve 2D™. In the first experiment the background activity of CYP1A1 in cells grown in medium exposed to normal laboratory hood light (2x36 W fluorescent tubes, 50 cm distance) for 0, 2 and 13 h was compared. The experiment was also performed with light exposed medium which was placed in darkness for 24 hours before it was used for exposure of cells. In a second experiment, dose-effect curves for FICZ were compared with TCDD after 3, 6 12 and 24 h of exposure.

Results and Discussion

The first experiment showed a clear and dose-dependent induction of CYP1A1 activity after treatment with normal medium exposed to visual light in the laboratory (Figure 2). The same effect was seen also when the medium was first exposed to light and then placed in darkness before the cell treatment (data not shown). A comparison between the light exposed medium and protected medium showed that the background CYP1A1 activity could be decreased more than 60 percent by minimising the light exposure.

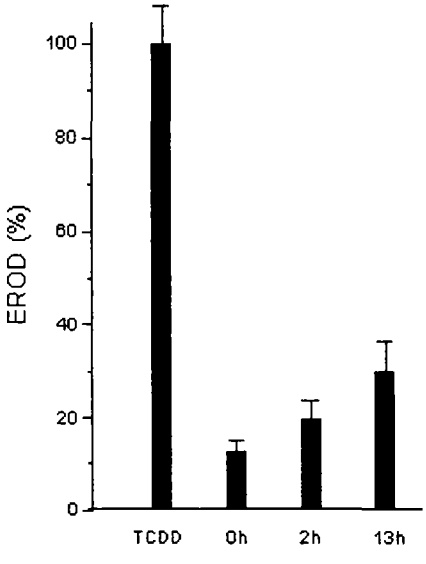

Figure 2. Influence on EROD induction by medium exposed to visual light in the laboratory, measured in the rat hepatoma cell line MH1C1. TCDD (0.0781 nM) giving max-induction.

The second experiment confirmed the reported potency of FICZ (Table 1). It has a potency to induce the AhR related CYP1A1 activity that is comparable to the potency of TCDD, but the induction is transient. Between 3 and 24 hours the EC50-value increased from 0.034 to 0.83 nM, while EC50 for TCDD was stable at about 0.016 nM. In previous studies a very short half-life of FICZ has been indicated which was shown to be caused by metabolism via the induced CYP1A1 enzyme [7].

Assuming that FICZ is the only inducing ligand that is formed in the first experiment one can calculate the amount of FICZ formed per hour of light exposure, using the dose-response curve for FICZ (12 h) and the EROD induction shown i figure 2. This model predicts a concentration increase of FICZ of about 0.002 nM per hour, which is a very small portion of the total tryptophan concentration (0.079 mM).

In conclusion the results point to the importance of careful protection of cell culture medium to light during experiments where the EROD activity is used as an endpoint. The results also indictate that the photoproducts of tryptophan of the formylindolocarbazole type could be the active AhR ligands formed under normal cell culturing conditions. To further study the "dioxin-like" light we plan to use a 2^3 full factorial design to enable the quantification of impact of factors

like concentrations of tryptophan and riboflavin in medium and light exposure. This information may then be used to optimise in vitro systems, where control of AhR basal activity is necessary.

Table 1. EROD induction in the rat hepatoma cell line MH1C1. EC50, maximal induction (Rel. Ymax) and relative potency value (REP) for FICZ relative to TCDD at four different times of exposure. *

		3 h	6 h	12 h	24 h
TCDD	EC50 (nM)	0.016	0.013	0.020	0.016
FICZ	EC50 (nM)	0.034	0.12	0.37	0.83
	Rel. Ymax	1.26	1.36	1.09	1.35
	REP	0.5	0.1	0.05	0.02

* Eight doses (5 wells/dose) were fitted to the equation $y = a+b/(1+(x/c)^d$, where c = EC50

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