The Ah receptor agonist 6-formylindolo[3,2-*b*]carbazole functions as a chemical messenger of light

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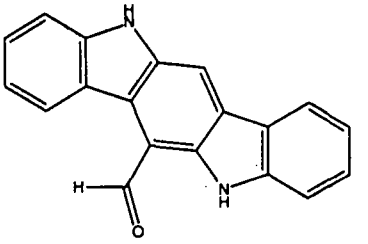
Introduction

The physiological function of the Ah receptor has until now escaped detection. Partly, this may be a consequence of the focusing on the model ligand TCDD, which is not metabolized by the cytochrome P-4501A1 (CYP1A1) protein and thus blocks the autoregulatory loop involving the *CYP1A1* gene and its product.

UV-irradiation of tryptophan results in the formation of a number of stable photoproducts that are ligands for the Ah receptor (1-5). Among these, 6-formylindolo[3,2-*b*]carbazole (FICZ) has been shown to possess remarkably high Ah receptor affinity and *CYP1A1* inducing capacity (1, 6, 7). The induced *CYP1A1* gene expression by FICZ is rapid, appearing in less than 30 minutes after start of exposure. In contrast to TCDD, the induced gene expression by FICZ is transient in nature (7). In the present study, we characterized the effect of UV light on *CYP1A1* gene expression in human and mouse cells and the relationship between UV and the compound FICZ.



6-formylindolo[3,2-*b*]carbazole (FICZ)

We have also investigated whether tryptophan derived compounds are responsible for the high basal expression of the *CYP1A1* gene that is found in some cell lines.

Tryptophan is the precursor of many important signal substances like the plant growth hormone indole-3-acetic acid and the human neuroendocrine hormones serotonin and melatonin. We now suggest that the tryptophan derived formylindolo[3,2-*b*]carbazole compounds belong to a new class of signal substances that may function as chemical messengers of light.

Materials and Methods

We have studied the induction of *CYP1A1* mRNA by FICZ treatment and by UV irradiation of cultured human keratinocytes (HaCaT), mouse Hepa-1 wild-type (Hepa-1-wt) and Ah receptor deficient (Hepa-1-c12) cell lines as well as primary human blood lymphocytes. The cells were exposed to UV light, delivered by a bank of 6 Philips TL20/12RS sun lamps emitting primarily in the UVB range, in the absence and presence of tryptophan. DMSO and β-naphthoflavone were used as a vehicle control and positive control, respectively.

Also, the Hepa-1 cell line, c37, that expresses the *CYP1A1* gene at an elevated basal level, as compared to wild-type cells, was used to investigate the source of the high basal expression. Cells were grown in medium with different tryptophan concentrations and the up- and downregulation of gene expression was followed over time.

A semiquantitative RT-PCR was used for analysis of gene expression in the treated cells. Total mRNA was isolated, reverse transcribed, amplified by PCR, electrophoresed and the products were visualized with ethidium bromide staining. Densitometrical quantitation of the intensity of the RT-PCR products was performed using a volume analysis technique and the levels of *CYP1A1* mRNA were related to the levels of mRNA of the house keeping gene *β-actin*.

Results and Discussion

A dose dependent gene induction, starting at a 10^{-10} M concentration, is seen with FICZ treatment of mammalian cells in *vitro*. The results from UV-treatments showed that the *CYP1A1* mRNA level induced in the presence of tryptophan was higher than that induced by UV alone in both HaCaT cells and lymphocytes. The induction occurred at low doses of UV (2.5 to 40 mJ/cm^2). Hepa-1-wt cells were inducible both by FICZ and UV-irradiation but very low or undetectable levels were observed in the Ah receptor deficient Hepa-1-c12 cells.

Growth medium containing higher concentrations of tryptophan caused elevated *CYP1A1* gene expression. Our data thus indicate that the basal expression of the *CYP1A1* gene is tryptophan dependent. The mutant Hepa-1-c37 cells, that have deficient CYP1A1 enzyme activity, could

not rapidly downregulate the high *CYP1A1* gene expression when transferred into a medium without tryptophan. The high basal expression in these cells might be explained by higher constitutive levels of tryptophan derived Ah receptor ligands of the suggested formylindolo[3,2-*b*]carbazole type.

Induction of *CYP1A1* gene expression by UV is Ah receptor dependent and the UV-induced expression occurs via oxidation of tryptophan. Thus, the identified tryptophan photoproducts with their extremely high affinity for the Ah receptor are suggested to be mediators of light and as such could have a role in light-regulated biological rhythms (8).

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