Ah receptor-mediated luciferase expression: a tool for monitoring dioxin-like toxicity.

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Abstract

A bioassay for Ah-receptor-mediated toxicity was developed using recombinant mouse Hepa-1c1c7 cells showing Ah receptor-controlled luciferase expression. The assay was found to reflect the relative toxic potential of several polyhalogenated aromatic hydrocarbons and allowed less than 1 pmole of TCDD to be detected.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are widespread environmental pollutants. Risk assessment of exposure to these compounds is hampered by the large number and the interaction of congeners, the low levels of compound to be detected and by species differences in the induced effects.

Most of the toxic and biochemical responses induced by PCDDs, PCBs and related compounds are mediated by the aryl hydrocarbon (Ah) receptor. Several biomarker assays for PCDDs/PCDFs have already been developed based on the Ah receptor-mediated transcriptional activation of genes such as the *cyp1A1* gene, which results in the induction of ethoxyresorufin-O-deethylase (EROD) activity associated with the *cyp1A1* gene product, cytochrome P450IA1. The induction of EROD activity in the H4IIE cell line is most widely used for the purpose of monitoring potential dioxin-like toxicity[1,2].

In this presentation, we report on the development of another approach, using recombinant vectors containing the luciferase gene under transcriptional control of dioxin-responsive enhancers to construct a cell line which shows Ah receptor-mediated luciferase gene expression. The reliability of luciferase induction in this cell line as a reporter of Ah-mediated toxicity was evaluated and a comparison was made with the commonly used ethoxyresorufin-O-deethylase (EROD) induction assay.

Materials and Methods

Cell lines
Mouse Hepa-1c1c7 and rat H-4-II-E hepatoma cell lines were cultured in α-MEM supplemented with 10% (v/v) fetal calf serum.

Luciferase reporter gene plasmid
The luciferase reporter gene plasmid, pGudluc1.1, was prepared by subcloning an 1810 bp fragment (containing the mouse mammary tumor virus (MMTV) promotor under Ah-receptor mediated inducible control of several dioxin-responsive elements (DREs)) immediately upstream of the firefly (*Photinus pyralis*) luciferase gene in the plasmid pGL2-basic (Promega Corporation, USA). The DRE's confer PCDD/PCB-responsiveness upon the luciferase gene. We have described construction of an analogous PCDD-responsive reporter plasmid in detail[3].

Transfection
Hepa-1c1c7 cells were cotransfected with pGudluc1.1 and pSV2neo according to a protocol described by Kawai and Nishizawa[4]. Stable transfectants were selected in 500 μg/ml G418 sulphate (Geneticin, GIBCO) and the transfectant showing the highest luciferase expression upon exposure to 1 nM 2,3,7,8-TCDD was chosen for further analysis.

Luciferase induction assay
pGudluc1.1-transfected Hepa-1c1c7 cells were grown in 6-well plates (Costar, 30 mm well diameter) in 3 ml of medium until confluent ($\pm 1.4 \cdot 10^6$ cells). Compounds were tested in triplicate and added to the culture medium in 3 μl DMSO (Janssen, Cat. No. 16.785.04, Beersse, Belgium). After 24 hours of exposure, luciferase activity was assayed using the Promega luciferase assay system, essentially according to the instructions of the manufacturer. Cells were harvested in 250 μl of Promega cell lysis reagent per well. Luminescence produced by 20 μl of sample (equivalent to about 10^5 cells) was quantified as the average counts per minute (cpm) detected during 10 subsequent 0.5 min measurements in a Canberra Packard 1600TR scintillation counter, operated in the single photon counting mode. When the linear response range of the counter was exceeded, the sample was diluted with cell lysis reagent (Promega) containing 1 mg/ml bovine serum albumin.

EROD induction assay
Cells were seeded ($4 \cdot 10^5$ cells per well) in 6 well dishes. After 48 hours cells were exposed to 2,3,7,8-TCDD for 24 hours. Cells were harvested in Tris/sucrose buffer (pH 7.8). For each concentration tested 6 wells were measured in duplo. EROD was determined using a method adapted from the procedure described by Pohl and Fouts[5].

Protein assay
Protein was determined using the BioRad protein assay[6].

Results

Among the Hepa-1c1c7 clones stably transfected with pGudluc1.1, the clone showing the highest dioxin-activated luciferase expression was selected and exposed to a number of polyhalogenated aromatic hydrocarbons of known toxic potential (Table 1).

Table 1. EC_{50} values and relative potencies of some halogenated aromatic hydrocarbon compounds to induce luciferase expression in Hepa-1c1c7 cells stably transfected with pGudluc1.1.

compound	EC_{50}	relative potency (EC_{50} TCDD / EC_{50})	TEF value (Safe, 1990)[7]
2,3,7,8-TCDD	10 pM	1	1
PCB77	7 nM	0.0014	0.01
PCB128	3 µM	0.0000033	0.00002
PCB52	>25 µM	<0.0000004	0.00002

Luciferase induction by 2,3,7,8-TCDD was dose-dependent and saturated at concentrations above 100 pM (Fig. 1). The detection limit ($p<0.05$) for 2,3,7,8-TCDD was found less than 1 pmole (≈1 pg). Ethoxy-resorufin-O-deethylase (EROD) induction by 2,3,7,8-TCDD in Hepa-1c1c7 cells showed a pronounced decline, in contrast to luciferase induction, at concentrations above 1000 pM (Fig. 1). Polychlorinated biphenyl congeners tested so far induced luciferase expression in accordance with their reported TEF values (Table 1).

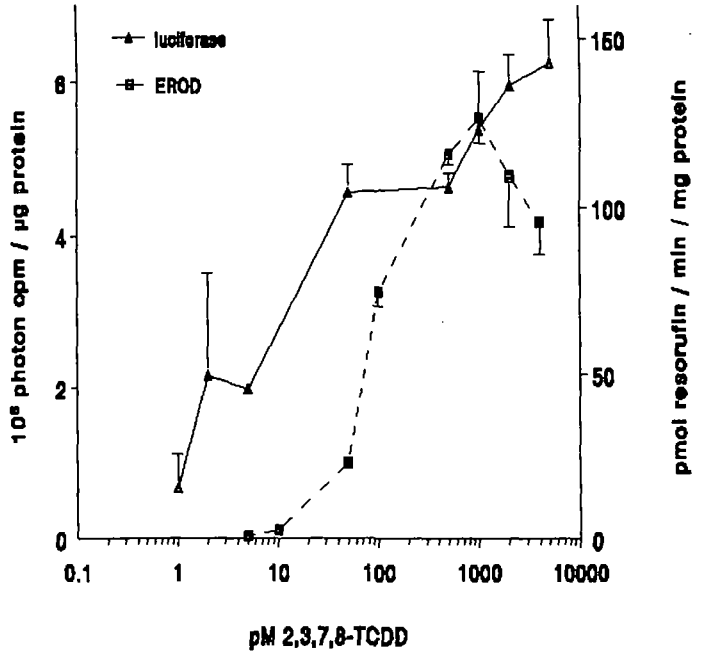

Fig. 1. Response observed in the luciferase and EROD induction assays in relation to dose of 2,3,7,8-TCDD.

Discussion

The presented luciferase induction assay was found at least ten times more sensitive than EROD induction in H4IIE cells. In the present set-up of the assay, 1 pg of 2,3,7,8-TCDD (3 ml of 1 pM) was significantly detected using only 10^5 cells. This is comparable to the detection-limit attained in the sensitive EROD induction assay using primary chicken hepatocytes which was recently described by Kennedy *et al.*[8]. Down-scaling of the luciferase induction assay is likely to decrease the detection limit still further. Moreover, luciferase activity is not influenced by competitive inhibition of the compound tested, allowing unambiguous determination of the toxic potential of the sample under investigation. In addition, the relevant upstream regulating nucleotide sequences could be manipulated to improve sensitivity with relative ease, since the luciferase induction assay is not based on an endogeneous gene but on a reporter gene constructed *in vitro*.

Acknowledgements

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