CHARACTERIZATION OF THE CALUX AND GRAB BIOASSAYS FOR SENSITIVITY AND SPECIFICITY IN DETECTION OF PHARMACOLOGICAL AGENTS THAT ACTIVATE THE Ah RECEPTOR SIGNALING SYSTEM

George C. Clark[1], David J. Brown[1], Shawn. D. Seidel[2] Dorothy Phelan[2] and Michael S. Denison[2]

[1]Xenobiotic Detection Systems Inc., Durham NC, and
[2]Department of Environmental Toxicology, University of California, Davis, CA.

Introduction:

The Ah receptor (AhR) is a ligand dependent transcription factor that mediates the majority of the biochemical and toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related chemicals. The understanding of the mechanistic basis by which these chemicals express their toxic effects presents the opportunity to develop rapid screening methods for these environmental toxins.

We and others have used various aspects of the AhR-dependent mechanism of action to develop rapid and sensitive bioassays for the detection of HAHs and other AhR ligands. Two AhR-based assays which have gained widespread use include measurement of the chemical-dependent activation of: 1) gene expression in cells that have an intact and complete Ahr signaling pathway ("in cello") and 2) AhR binding and the resulting DNA binding that occurs in a cell free system (in vitro). The two variations of these assays investigated in the present studies include the Chemically Activated LUciferase eXpression (CALUX bioassay) using a cell based system, and the Gel Retardation of AhR DNA Binding (GRAB bioassay) using an in vitro cell free system. Activation of these bioassay systems occurs in a time-, dose-, chemical- and AhR-dependent manner. Given the potential utilization of these AhR-based bioassays for large scale chemical and environmental screening for TCDD and related HAHs, the studies described here were carried out in order to assess the advantages and limitations of each assay system.

Materials and Methods:

Chemicals. A variety of chemicals, pharmacological agents, and extracts of newspaper were used in these investigations. A brief list abbreviations and chemicals used follows: TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; IDAZ, Idazoxan; PHEN, Phentolamine; EPI, Epinephrine; GUAN, Guanabenz; NP, Newspaper; TCDF, 2,3,7,8-tetrachlorodibenzofuran; PCB, 3,3',4,4',5-Pentachlorobiphenyl; 3MC, 3-Methylcholanthrene; BNF, b-Naphthoflavone; ANF, a-Naphthoflavone.

CALUX bioassay: CALUX analysis was carried out as described in detail in Garrison et al. (Fund. Appl. Toxicol. 30, 194-203 (1996)). Briefly, chemicals or extracts were applied to monolayers of stably transfected cells in DMSO and incubated at 37 C in a humidified CO2 atmosphere for 4 hours. After 4 hours cells were lysed and luciferase activity quantified with luciferase reagent (Promega) in a luminometer.\

GRAB bioassay: GRAB analysis was carried out using guinea pig hepatic cytosol as described in Bank et al. (1995). Briefly, guinea pig hepatic cytosol was incubated with chemical or extracts in DMSO and allowed to bind to Dioxin Response Elements (DREs) labeled with radioactive ^{32}P in the presence of excess nopnspecific DNA. Specific binding was detected by electrophoresis in polyacrylamide gels and quantitation of specific AhR:^{32}P-DRE complexes.

Results:

Dose dependent responses of the bioassays to TCDD.

The sensitivity of the CALUX and GRAB assays were compared by their relative response to various doses of TCDD. Shown in Figure 1 is the dose dependent response to TCDD of the two assays. The CALUX assay was approximately 10-fold more sensitive in detecting TCDD than the GRAB assay with the shapes of the dose-reponse curves being similar. The increased sensitivity of the CALUX assay may be a result of the amplification of signal due to the mechanistic basis by which the cells cells can respond to these toxins, producing more luciferase on a mole basis than measured by only binding to the receptor.

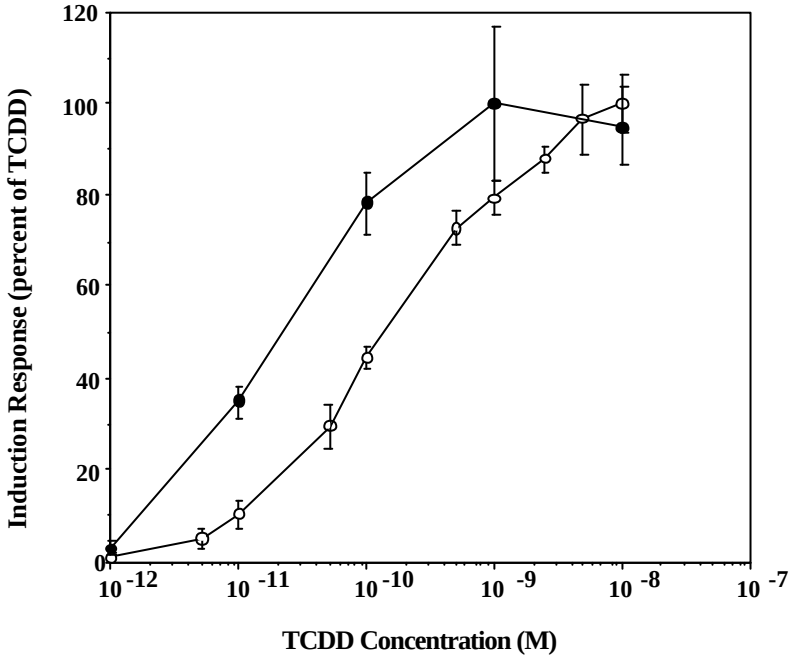

Figure 1. Dose-dependent activation of the CALUX (closed circles) and GRAB (open circles) bioassays by TCDD.

The GRAB assay estimates binding to both TCDD and specific DRE sequences without an amplification step based upon the mechanism of activation.

Response of the CALUX and GRAB bioassays to pharamcological agents and environmental extracts.

We have identified several pharmacologically-relevant chemicals including imidazoline receptor ligands (idazoxan, guanabenz and epinephrine) and benzimidazole drugs (omeprazole, albendazole and thiabendazole) which induced luciferase activity in several different CALUX cell lines. Although some of the imidazoline receptor ligands were also potent activators of AhR transformation and DNA binding in vitro, others were essentially inactive; all of the benzimidazoles were significantly less potent in the in vitro bioassay.

In contrast, the GRAB bioassay responded indiscriminatly to a number of extracts of newspapers and other common house-hold products. The CALUX assay displayed greater specificity in responding to these extracts.

Conclusions:

Our results reveal a significant discrepancy between the ability of a chemical to activate the AhR in vitro and to activate the Ah receptor system in intact cells. The cell based system (CALUX) is more sensitive and yet more discriminating in detecting AhR agonists.

The in vitro GRAB analysis may detect more activators of the AhR since chemicals are added directly to the incubation mixture and pharmacokinetics of penetrating the cell are circumvented. However, activation alone apparently generates a number of false positive results which are problematic for a screening assay for risk assessment purposes of environmental hazards.

Overall, our results demonstrate the usefulness of the CALUX assay and cell based systems for the detection and quantiation of the AhR agonists. In vitro assays may prove to be useful as environmental screening methods, however a high frequency of false positive results are of concern.

References:

1. Garrison, et al. Fund. Appl. Toxicol. 30, 194-203 (1996).
2. Bank et al. Arch. Biochem. Biophys. 317, 439-448 (1995).

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