AH RECEPTOR AGONIST MEASUREMENT WITH A CELL-BASED BIOASSAY

Chu, A.C.[1] , Chu, M.D.[2] , Matherly, C.K.[1], Gordon, J.D.[1], and Clark, G.C.[1]

[1]Xenobiotic Detection Systems Inc., 1601 E. Geer St. Suite S, Durham, NC 27704 Phone: (919) 688-4804; FAX: (919) 688-4404; [2]Alta Analytical Perspectives, 2714 Exchange Drive, Wilmington, NC 28405.

Introduction

Toxic halogenated aromatic hydrocarbons (HAHs) are a class of environmental and dietary chemical pollutants that includes polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) as well as brominated species of these chemicals. The most toxic HAH, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), serves as a prototype for this class of compounds and induces a broad range of biochemical and toxic effects that range from adaptive induction of specific cytochrome P-450s (e.g., CYP1A1, CYP1A2) to neoplastic alterations in animals such as development of liver foci, adenomas, and adenocarcinomas (1, 2). The biochemical and toxic mode of action for TCDD and related HAHs involves initial binding to and activation of the cytosolic aryl hydrocarbon receptor (AHR), which is a ligand-activated transcription factor that regulates the expression of a rapidly increasing list of genes encoding proteins that regulate numerous cellular and tissue processes (3, 4).

A physiological purpose for the AHR has yet to be confirmed although a number of naturally occurring AHR ligands, some of which are naturally occurring or even formed endogenously, have been identified and studied (5). These include certain indole carbinols and their derivatives, heterocyclic aromatic amines, vitamin A derivatives, tryptophan derivatives, catechins, resveratrol, flavonoids, and carotenoids (5, 6, 7). Many of these constituents are present in foods such as vegetables, fruits, nuts, and herbs in milligram quantities. Further, specific indole carbinole derivatives have exhibited AHR activation potencies comparable to TCDD (8). The current study was undertaken to investigate the dose-response curves and kinetics of activation of selected natural AHR ligands in a cell based luciferase reporter gene bioassay.

Methods and Materials

Compound Preparation, Dosing Procedures, and Cellular Bioassay: Compounds that have been suggested to have AHR agonist activity were acquired from Sigma-Aldrich Chemical and included indole-3-carbinol (I3C), resveratrol, daidzein, 5-cholesten-3β-ol-7-one (7-keto-cholesterol), and 3-methylcholanthrene (3MC); 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was obtained from Wellington Laboratories. Indolo[3,2-β]-carbazole (ICZ) was a kind gift of Dr. Leonard Bjeldanes, University of California at Berkeley. All of the test compounds were solubilized in Dimethyl Sulfoxide (DMSO) to prepare 5 mg/ml stock solutions except for ICZ, which was not soluble until diluted to a concentration of 1 mg/ml. TCDD and 3MC both served as reference compounds for the bioassays; TCDD was used to prepare a standard curve for each set of samples, while 3-MC was handled as a test compounds in the dose-response analysis, but also run at a single concentration of 9.3×10^{-5} M throughout the assays. Serial dilutions of the compounds were analyzed for activity in the luciferease reporter gene bioassay and dose-response curves generated at either 4 or 20 hours of incubation with the H1L1.6.1 murine hepatoma cell that stably expresses luciferase in response to AHR agonists.

Measurement of Luciferease Activity. After lysing the cells (Promega lysis buffer), the luciferase activity was measured in a Berthold Orion Microplate Luminometer, with automatic injection of 50 microliters of luciferase

enzyme reagent (Promega) into each well. The relative light units (RLUs) measured were plotted in compared to that induced by a serial dilution of TCDD standard after subtraction of the background activity (DMSO Blank).

Results and Discussion:

Figure 1. Induction of AHR-Dependent Luciferase Activity by TCDD and Selected NAHRAs at 4 hours.

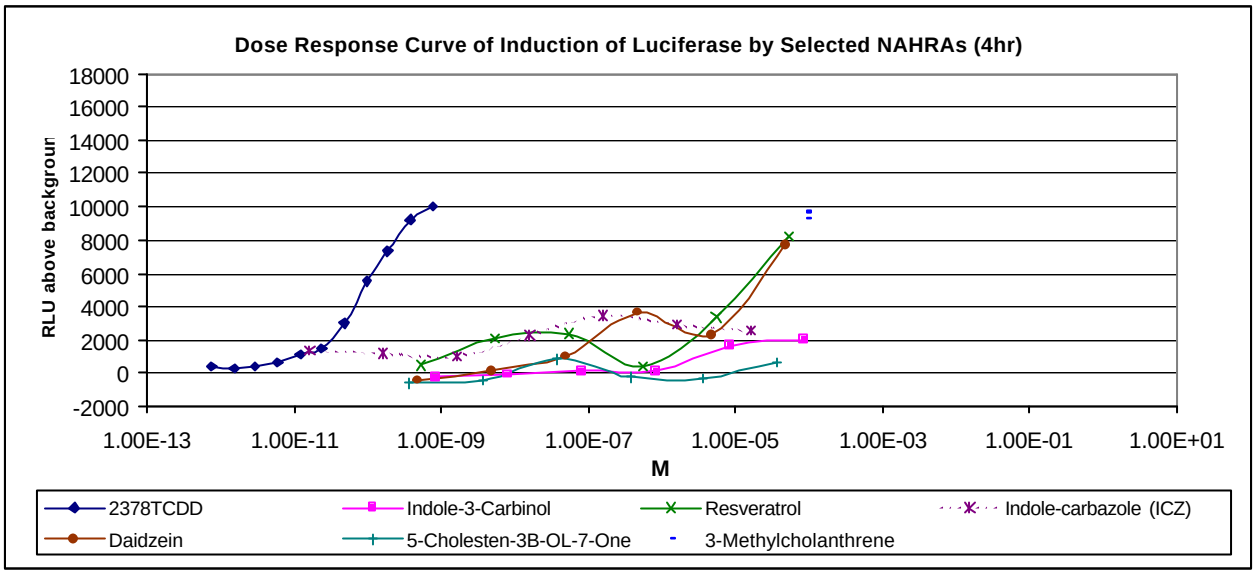
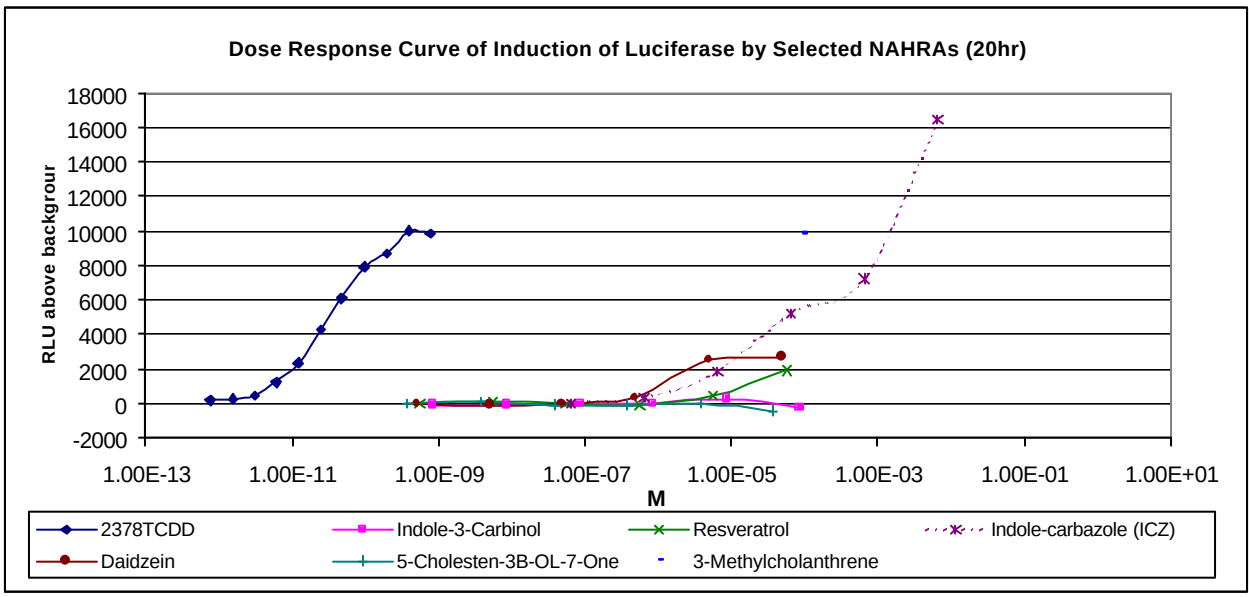


Figure 2. Induction of AHR-Dependent Luciferase Activity by Selected NAHRAs at 20 hours.



The dose response curves for TCDD and several naturally occurring AHR agonists are depicted in Figure 1 and Figure 2. The dose response curve for the TCDD is very similar at both the 4-hour incubation and the 20-hour incubation time points. There appears to be differences in the dose response curves between the 4-hour incubation and the 20-hour incubation time points for natural Ah receptor agonists. These differences might be explained by the metabolism of these natural AHR agonists and shifts in their ability to induce luciferase activity in the reporter gene bioassay; however, others investigators have tested the activity of some of these compounds in other systems finding no differences in kinetics, e.g., I3C, following 24, 48, or 72 hour incubations and have observed little declines in its ability to induce native CYP1A1 activity of AhR-regulated reporter gene activity. In addition, it is known that certain tryptophan photoproducts, which can accumulate in cell culture media, are strong AHR agonists (7). It has been observed by Stresser et al. (9) that relatively low amounts of I3C added to cell media will accumulate in the cells. More research is needed to understand the kinetics and metabolism of these natural Ah receptor agonists and how these compounds interact with the Ah receptor and affect the response to toxic halogenated aromatic hydrocarbons. These preliminary data demonstrate cell-based assays vary in their responsiveness to TCDD and the more water-soluble, naturally occurring AHR agonists. These model systems may provide a means to investigate the interactions of naturally occurring AHR agonists and the HAHs.

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