DIOXIN AND DIOXIN-LIKE CHEMICALS INHIBIT CYP1A2 ACTIVITY

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Introduction

Cytochrome P450 1A2 (CYP1A2) is an inducible hepatic protein which binds dioxins and produces a dose-dependent hepatic sequestration. Dioxins bind to the Ah receptor (AhR), which regulates a variety of effects including the expression of CYP1A2. There is a direct concordance between the ability to bind AhR and the toxic potency of each chemical. Polyhalogenated aromatic hydrocarbons (PHAHs) have been classified as dioxin-like or non-dioxin-like based on their ability to bind the aryl-hydrocarbon receptor (AhR), induce toxicity, and bioaccumulate. 90-day subchronic mouse studies have shown dose-dependent increases in liver/fat concentrations of several dioxin-like chemicals, supporting the presence of an inducible hepatic binding protein[1]. Our laboratory demonstrated the importance of CYP1A2 as the specific hepatic binding protein responsible for hepatic sequestration of TCDD and dioxin-like compounds by exposing CYP1A2 knockout mice and parental strains to TCDD, 4-PeCDF (a dioxin-like PHAH), and PCB 153 (a nondioxin-like PCB)[2]. The ability of the liver to sequester TCDD and 4-PeCDF was significantly decreased in the knockouts resulting in increased concentrations of these chemicals in extrahepatic tissues, while the distribution of PCB 153 was unaltered in the knockout mice. These studies demonstrate the influence of CYP1A2 on the distribution of dioxins in rodents.

Human data demonstrating hepatic sequestration is limited. Although the data is inconclusive, several human studies have failed to show a dose-dependent increase in CYP1A2 activity following high levels of exposure to dioxin[3]. However, there is some evidence for human hepatic sequestration based on analysis of liver and adipose tissue levels in exposed people[5]. In current risk assessments, it is assumed that the disposition of these chemicals in humans is similar to rodents. This *in vitro* study investigates this assumption by comparing the ability of dioxins to inhibit human and rat CYP1A2.

Materials and Methods

Microsomes

Rat and human CYP1A2 + P450 reductase SUPERSOMES were obtained from GenTest Corporation (Woburn, MA). Microsomes were kept at –80°C until use, then rapidly thawed at 37°C and stored on ice. A 1:10 dilution of both human or rat microsomes was prepared daily using .05M (pH 8) TRIS buffer.

Chemicals

All PHAHs were obtained from AccuStandard Inc. (New Haven, CT). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachloro-*p*-dioxin (PCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), and 2,3,7,8-tetrachlorodibenzofuran (TCDF) were 98 % or greater purity. 3,3’,4,4’,5,5’-Hexachlorobiphenyl (PCB 169), 3,3’4,4’,5-pentachlorobiphenyl (PCB 126), 2,3,3’,4,4’,5-hexachlorobiphenyl (PCB 156), 2,3’4,4’5-pentachlorobiphenyl (PCB 118), and 2,3,3’,4,4’-pentachlorobiphenyl (PCB 105) were 99 % or greater purity. All chemicals were dissolved in DMSO

and stored at 4°C. b-Nicotinamide adenenine dinucleotide phosphate (b-NADPH), methoxyresorufin, and resorufin were purchased from Sigma Chemical Co. (St. Louis, MO) and prepared daily.

Enzyme assay

Methoxyresorufin O-deethylase (MROD), a specific marker for CYP1A2 enzymatic activity, was used to detect the presence of inhibition by a series of dioxin-like chemicals. Methoxyresorufin is metabolized to resorufin by CYP1A2. The production of resorufin was recorded using a Spectromax Gemini XS plate reader. The components of each well in this kinetic assay included 50 mL microsome solution, 110 mL .05M (pH 8) TRIS buffer, 5 mL chemical, and 50 mL methoxyresorufin solution. Following a ten-minute incubation at 37°C, 25mL NADPH (prepared fresh) was added to begin the reaction. The production of resorufin was recorded for five minutes at 37°C.

Analysis

The data were initially analyzed using Lineweaver-Burke plots (Prism Software, CA). Assuming competitive inhibition, inhibitor constant (K_I) approximations were calculated using the lowest inhibitor concentration and average K_{Mobs} and K_M values according to the following equation: K_I = [Inhibitor] / [($K_{M,obs}$ – 1)/ K_M]

Results

Human and rat SUPERSOMES metabolized methoxyresorufin similarly, yielding K_M values of 1.9 and 2.5 mmol resorufin and V_{max} values of 1.8 and 2.8 mmol/min respectively. All chemicals tested produced a concentration-dependent inhibition of MROD activity in both human and rat CYP1A2 SUPERSOMES. By assuming a competitive type inhibition, approximate K_I values were calculated and are reported in Table 1. The dibenzofurans appeared to be the most potent inhibitors of rat CYP1A2 activity, where as TCDF and PCB 126 were the most potent inhibitors of human CYP1A2 activity. The dibenzodioxins and co-planar PCBs were more effective inhibitors of rat than human CYP1A2. However, these chemicals were still potent inhibitors of human CYP1A2 activity. Much higher concentrations of the mono-ortho PCBs were required to inhibit activity in both species. Lineweaver-burke plots are shown for TCDD, TCDF, and PCB 126 and are representative of the dibenzodioxins, furans, and co-planar PCBs tested .

Table 1. Approximate K_I values (mM)

Chemical	KI Human	KI Rat
TCDD	0.31	0.064
PCDD	0.48	0.055
4-PeCDF	0.54	0.027
TCDF	0.082	0.016
PCB 126	0.064	0.077
PCB 169	0.28	0.069
PCB 105	3.23	60.6
PCB 118	1.26	2.45
PCB 156	2.26	1.51

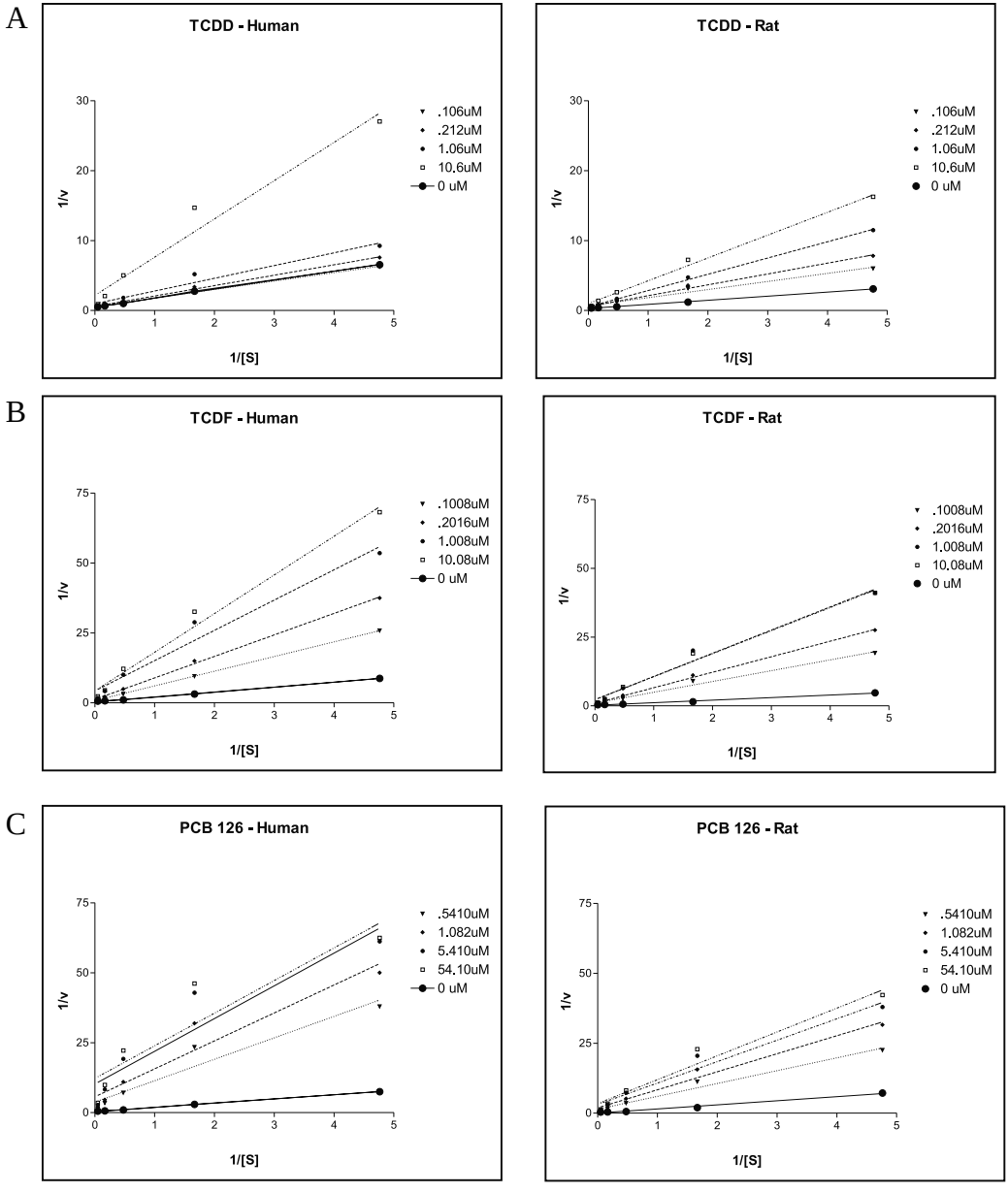

Figure 1. Lineweaver-Burke plots of human and rat MROD activity following exposure to varying concentrations of A) TCDD, B) TCDF, and C) PCB 126.

Discussion

The ability of human and rat CYP1A2 to bind dioxin and dioxin-like chemicals influences the disposition and body burden of these chemicals. This *in vitro* assay demonstrates that both human and

rat CYP1A2 enzymatic activity is inhibited by TCDD, PCDD, TCDF, 4- PeCDF, and PCBs 126, and 169 in a similar fashion. PCBs 105, 118, and 156 also inhibit enzymatic activity to a lesser extent. The inhibition of CYP1A2 activity by these chemicals could explain the lack of inducible CYP1A2 activity in several human cohorts highly exposed to TCDD as well as the plateauing of CYP1A2 activity, in contrast to CYP1A2 protein or mRNA, following treatment with high doses in rodents[4]. These results suggest that the lack of parallel increase in CYP1A2 activity observed following exposure to dioxin-like chemicals may be due to binding of the chemicals to CYP1A2, which would interfere with determination of CYP1A2 activity as a marker for CYP1A2 protein.

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