COMPARATIVE INDUCTION OF CYTOCHROME P-4501A1 (CYP1A1) AND 1A2 (CYP1A2) IN RAT AND HUMAN PRECISION-CUT LIVER SLICES EXPOSED TO TCDD IN DYNAMIC ORGAN CULTURE

Adam T. Drahushuk; Barbara P. McGarrigle; James R. Olson; Department Pharmacology and Toxicology, University at Buffalo, Buffalo, NY 14214 USA.

Abstract

The extrapolation of toxicity data from laboratory animals to humans is one of the greatest challenges of human risk assessment. Although a considerable quantity of information has been garnered on the toxicological effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds in experimental animals, the potential hazard these agents pose to humans has been less well characterized, due in part to a lack of direct experimental data in humans. In order to address this issue, the objective of the present study was to utilize precision-cut liver slices in dynamic organ culture as an *in vitro* model for comparing the ability of TCDD to induce CYP1A1 and CYP1A2 in rats and humans. The induction of these dioxin-responsive genes are sensitive biological responses which are related to the wide range of toxicological responses associated with these compounds. Therefore, these responses should prove valuable as biomarkers of exposure, effect, and susceptibility.

Introduction

Halogenated aromatic hydrocarbons (HAHs) are a concern for the environment and human health due to their ubiquitous environmental contamination and potential for toxicity. The physical and chemical properties of these agents contribute to their biological and environmental stability and lipophilicity, resulting in their exceptional ability to bioaccumulate in wildlife and humans. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent and extensively studied member of this class of compounds, which includes other polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls. TCDD and structurally related environmental pollutants elicit a broad and diverse spectrum of toxic responses in experimental animals including dermal, immunotoxic, hepatotoxic, carcinogenic, reproductive, developmental, neurobehavioral, and endocrine effects[1)2)]. Most of these effects are mediated by the aryl hydrocarbon (Ah) receptor. The group of genes, whose expression is altered by the HAHs, are collectively referred to as the 'Ah gene battery'[3)]. CYP1A1 and CYP1A2 are members of this battery and are readily inducible by exposure to TCDD and related agents[4)]. Although a considerable quantity of information has been garnered on the toxicological effects in experimental animals, the potential hazard these environmental pollutants pose to humans has been less well characterized. Uncertainty still remains in regards to the susceptibility of humans to the HAHs. In order to address this issue, we utilized precision-cut rat and human liver slices in dynamic organ culture as an *in vitro* model

for comparing the ability of TCDD to induce CYP1A1 and CYP1A2. Precision-cut liver slices in dynamic organ culture offer several advantages over other *in vitro* models, such as isolated hepatocytes. The liver slices retain the functions and morphology observed *in vivo* because hepatocytes in the slices are able to maintain the normal cell density, cell-cell contact with non-parenchymal cells, and interactions with the extracellular matrix. Moreover, the relative fat (adipocyte) content is the same as in the intact liver. Recently, precision-cut liver slices maintained in dynamic organ culture was validated as a suitable *in vitro* model for investigating the dose related hepatic uptake of TCDD and the resulting induction of CYP1A1 and CYP1A2[4]. The model was validated by measuring the dose related induction of CYP1A1 and CYP1A2 mRNAs, proteins, and activities in rat liver following a 24 hr *in vivo* (po) and *in vitro* (liver slices in dynamic organ culture) exposure to TCDD and comparing the responses with their respective tissue concentrations of TCDD. The concentration-effect relationships for induction of CYP1A1 and CYP1A2 were similar following *in vitro* and *in vivo* exposure to TCDD. Since, induction of CYP1A1 and CYP1A2 is known to be associated with exposure to these environmental contaminants and CYP1A1 induction may be the most sensitive response[5], these cytochromes should prove valuable as biomarkers of exposure, effect, and susceptibility.

Experimental Methods

Liver Slice Preparation and Incubation

Fresh liver tissue was obtained from male Sprague-Dawley rats and human organ donors when the tissue could not be transplanted (IIAM, Anatomical Gift Foundation). Precision-cut rat and human liver slices were prepared essentially as described by Smith *et al.*[6] with modifications as described previously[4]. The viability of the slices was determined by measuring intracellular K^+ content using a flame photometer. Intracellular K^+ contents of 40 μmole/g wet wt or greater were considered viable tissue.

Preparation of Microsomes

Liver slices were weighed and stored at -70°C following incubation in dynamic organ culture. Liver microsomes were prepared from the slices essentially as described by Cinti *et al.*[7].

Electrophoresis and Immunoblotting

Sodium dodecylsulfate-polyacrylamide gel electrophoresis was performed essentially according to the method of Laemmli[8]. The CYP1A1 and CYP1A2 protein contents in the microsomes were quantitated by Western immunoblotting using chemiluminescent detection as previously described[4].

Enzymatic Activities

Ethoxyresorufin-O-deethylase (EROD) activity was determined by the fluorometric assay described by Prough *et al.*[9] with the wavelengths for excitation and emission being 530 and 585 nM, respectively. The rate of fluorescence change was recorded prior to and after the addition of NADPH and the assay calibrated with the addition of a known quantity of resorufin.

The 4-hydroxylation of acetanilide (AcOH) was determined according to the method described by Liu *et al.*[10]. Liver microsomal protein (0.2 mg) was incubated for 40 minutes at 37°C in assay buffer (50 mM Tris pH 7.5, 0.3 mM $MgCl_2$, 0.6 mM NADPH, and bovine serum

albumin 1mg/ml) containing 0.4 mM acetanilide. The production of 4-hydroxyacetanilide was quantitated by reverse-phase HPLC.

Results and Discussion

In the present study, rat liver slices were exposed to medium containing TCDD (0.0001 to 10 nM) for 24 hr, as in the validation study, and maintained for an additional 72 hr (96 hr total incubation period) in culture medium without TCDD. The slices remained viable for the 96 hr incubation with intracellular K^+ values averaging greater than 45 μmole/g for all time points. The resulting detectable TCDD tissue levels in the liver ranged from approximately 9 (0.001 nM) to 57,000 (10 nM) pg TCDD/g liver wet wt and are comparable to levels previously reported[4]. Hence, precision-cut rat slices incubated in dynamic organ culture represent a reproducible *in vitro* model with low variability in regards to hepatic TCDD uptake. As a result of the TCDD exposure, a concentration-dependent increase in CYP1A1 protein and its marker activity, the O-deethylation of ethoxyresorufin (EROD), was observed for all concentrations compared to control (0 nM TCDD), with the 10 nM TCDD treatment group displaying greater than 100 fold induction at 96 hr. CYP1A1 protein level induced at 96 hr by 10 nM TCDD is ≈15 fold greater than the CYP1A1 protein observed previously at 24 hr[4]. Likewise, the EROD activity at 96 hr for the 10 nM TCDD treatment was ≈4.5 fold greater than observed previously at 24 hr[4]. Overall, prolonging the incubation time from 24 to 96 hr dramatically enhanced the induction of CYP1A1 and significantly increased the sensitivity of the tissue in regards to CYP1A1 induction. The induction of CYP1A2 was a less sensitive response than CYP1A1, with significant induction of CYP1A2 protein and its representative activity, the 4-hydroxylation of acetanilide (AcOH), occurring at a medium concentration of 0.1 nM TCDD (686 pg/g liver) and greater at 96 hr and is comparable to results reported at 24 hr. Therefore, in contrast to the beneficial effects on CYP1A1 expression, prolonging the incubation period produced little enhancement of CYP1A2 expression. Nonetheless, the 96 hr time period appears optimal for studying the expression of CYP1A1 and CYP1A2.

Human liver tissue was also utilized in the *in vitro* model following the same protocol. The human liver slices were incubated for 24 hr in medium containing TCDD and subsequently maintained up to an additional 72 hr in TCDD-free medium. The slices remained viable for the 96 hr incubation period displaying an average intracellular K^+ greater than 45 μmole/g. Incubation of human liver slices in medium containing from 0.001 to 10 nM TCDD resulted in tissue levels ranging from ≈180 to 73,000 pg TCDD/g liver wet wt, respectively. As expected there was a larger variability in the hepatic TCDD uptake between individual human livers than observed with the rat. Hepatic uptake of TCDD was correlated with the CYP1A2 protein concentration, which varied considerably between individuals. Furthermore, there was also a large interindividual variability in the enzymatic activities and as a result human data were expressed as a percentage of the respective control (0 nM). In a similar fashion as with rat liver, a concentration-related induction of CYP1A1 mediated EROD activity was observed following 24 hr of exposure to TCDD, with greater induction occurring at 96 hr. In fact, at 96 hr there was nearly a 40-fold increase in EROD activity compared to control (0 nM TCDD). Induction of human CYP1A1 protein was also demonstrated by Western immunoblotting. Therefore, analogous to the rat, prolonging the incubation period greatly enhanced CYP1A1 mediated EROD

activity. Interestingly, prolonging the incubation period resulted in enhanced CYP1A2 expression since induction of CYP1A2 protein and AcOH activity by TCDD was observed following 96 hr of incubation in dynamic organ culture, but not after 24 hr. The results clearly demonstrate that human liver slices are responsive to TCDD, exhibiting increased expression of both CYP1A1 and CYP1A2 at 96 hr. Although, it appears that the human liver displays a greater variability in response, the data suggest that rat and human liver exhibit similar relative sensitivity to TCDD (within one order of magnitude). The use of precision-cut liver slices as an *in vitro* model should also prove valuable in investigating the hepatic uptake and resulting biological/toxicological responses elicited following exposure to complex mixtures, including real world mixtures.

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