The Binding of 1,2,3,7,8-Pentachlorodibenzo-*p*-Dioxin Metabolites With Rat Urinary and Biliary Carrier Proteins

Heldur Hakk, Gerald Larsen, and Vernon J. Feil

USDA, ARS, Biosciences Research Laboratory, PO Box 5674-University Station, Fargo, ND 58105-5647 USA

INTRODUCTION

It is generally acknowledged that the major route of dioxin exposure in mammals is via ingestion. Due to the lipophilic nature of dioxins, exertion of their toxic effects in various tissues requires carrier proteins for transport. Chylomicrons have been shown to be the plasma lipoprotein carriers for dietary dioxins via the lymph system[1)].

Metabolism of dioxins by microsomal cytochromes to more polar compounds is necessary for excretion in urine or bile [2)]. Despite an increase in polarity via Phase I metabolism, these metabolites may not be sufficiently polar to be eliminated in an aqueous matrix. Therefore, carrier proteins may still be necessary for excretion. Mammalian urinary carrier proteins for polyhalogenated aromatic hydrocarbons have been described [3, 4)] and include albumin and $\alpha 2_u$ globulin ($\alpha 2_u$). Recently, a novel 79 kDa bile protein was isolated from rats that demonstrated covalent binding to numerous non-toxic tetrachlorodibenzo-*p*-dioxin congeners [5)] and the toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD)[13)].

The goal of the present study was to administer an oral dose of the toxic dioxin congener 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (12378-PeCDD), and determine the binding of the parent and/or its metabolites to carrier proteins in rat urine and bile.

EXPERIMENTAL

[UL 7,8-ring ^{14}C]-12378-PeCDD, obtained from Chemsyn Science Laboratories, Lenexa, KS, was administered orally to male Sprague-Dawley rats. Twelve rats received 2.9 mg/kg body weight (2.8μmol/rat; 17.0 μCi/rat) in the conventional study and seven rats received 3.3 mg/kg body weight (2.8 μmol/rat; 17.3 μCi/rat) in the bile-duct cannulated study. Urine and bile were collected every 24h for three days. The excreta were individually chromatographed on Sephadex G-75 (4.5 x 90cm) and Sephacryl S-200 (2.2 x 85cm), as described previously [6)]. The columns were eluted with 0.05 M phosphate buffer (pH 7.2). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 13% acrylamide) and immunoblot analysis (Western blot) were performed as described previously [7)]. TLC analysis was performed on aliquots of urine and bile after concentration by rotary evaporation. TLC plates (silica gel) were developed in 50:50 hexane:methylene chloride with a standard lane of 12378-PeCDD.

RESULTS

Daily excretion of ^{14}C from a 12378-PeCDD dose into the urine and bile of both study groups of rats did not exceed 0.31% of the dose (Table 1). This level of excretion as a percent of dose was similar to a 1.25mg/kg oral dose of 2378-TCDD administered to male rats[13)]. However, daily

biliary excretion of this high dose of 12378-PeCDD was lower when compared to smaller doses of 2378-TCDD, where the average daily biliary excretion was 1% and 2.4% following a 600ng/kg and a 200µg/kg dose, respectively [8, 9]. Saturation of the monooxygenase metabolizing enzyme systems with such a high dose was probably responsible for the decreased biliary excretion, although the severe toxicity of 12378-PeCDD may also have led to inhibition of these enzymes.

Parent 12378-PeCDD was not detected by TLC analysis in conventional or cannulated urine or bile. It was concluded that metabolism of 12378-PeCDD was necessary for elimination in the urine and bile. Greater than 67% of the urinary ^{14}C remained unbound in both study groups (Table 2), and the percentage did not change significantly with time. The protein bound fraction in conventional urine ranged from 18 to 29% of the ^{14}C, and was associated with two proteins following G-75 chromatography. The majority of ^{14}C (17.9-23.9% of total urine) was bound to a protein of MW 66.2 kDa (Figure 1; G-75 477-856 ml), which was determined to be albumin from SDS-PAGE and Western blot analysis. A smaller amount of ^{14}C (0.2-4.7% of total urine) was associated with $\alpha 2_{u,}$ and eluted in 1003-1253 ml from the G-75 (Figure 1). The identity of $\alpha 2_u$ was confirmed by SDS-PAGE (MW 18 kDa) and Western blot analysis. The amount of ^{14}C bound to $\alpha 2_u$ diminished to 0.2% by 48-72h, suggesting that the initially excreted metabolites could serve as ligands for both carrier proteins, but that metabolites excreted at the end of the experiment were preferential ligands for albumin. In contrast, all protein bound ^{14}C in urine of cannulated rats was associated with albumin; no detectable binding to $\alpha 2_u$ was observed. Indirect evidence of renal damage was observed in the 12378-PeCDD rats, in that approximately 1000 times more urinary protein was excreted per rat than in non-toxic 1278-TCDD treated rats (390mg protein/12378-PeCDD rat vs. 0.5mg protein /1278-TCDD rat).

The sex-dependent $\alpha 2_u$ is a hepatically synthesized 18 kDa protein which is passed through the glomeruli filter of the nephron, although 50% is reabsorbed by the proximal tubules[10]. $\alpha 2_u$ and albumin constitute the two principle urinary proteins in mature male rats[11]. The role of each protein in urine has not been firmly established, but albumin appears to be a carrier for small lipophilic molecules. $\alpha 2_u$ was shown to be involved in maintaining spermatogenesis [14], and there are indications that it may serve as a pheromone carrier. The native function of both proteins may be altered upon binding to 12378-PeCDD metabolites, and therefore, these binding studies may be of toxicological importance.

Unbound metabolites in bile accounted for 88-91% of the total ^{14}C applied to the G-75 columns (Table 2), and the protein bound fraction accounted for 7-10% of the ^{14}C. From linear regression MW analysis following SDS-PAGE it was found that the ^{14}C was bound to a 79 kDa protein. In previous work with 1278-, 1378-, and 1478-TCDD, and the toxic 2378-TCDD, binding of dioxins or their metabolites to the 79 kDa protein has been demonstrated in male rat bile [5, 13]. The identity of the protein has not been established, but our research has shown it is an N-terminal blocked monomeric protein with an isoelectric point of 5.7. The physiological role of this bile protein may be altered when bound to metabolites of 12378-PeCDD. Sixteen rat bile proteins have been isolated previously and characterized tentatively by 2-D PAGE and crossed immunoelectrophoresis [12]. Of these bile proteins the majority are serum proteins, but none has a molecular weight of 79 kDa. Work is currently underway in this laboratory to obtain proteolyzed amino acid sequence data in order to further characterize this novel protein.

Table 1: Recovery of ^{14}C in the urine and bile of male rats dosed orally with [^{14}C]-1,2,3,7,8-pentachlorodibenzo-*p*-dioxin.

Excreta	Percent of Dose Conventional (n=12)	Bile-duct Cannulated (n=7)
Urine		
0-24 h	0.10 ± 0.02	0.10 ± 0.07
24-48 h	0.07 ± 0.07	0.04 ± 0.02
48-72 h	0.05 ± 0.04	0.02 ± 0.01
Bile		
0-24 h	---	0.30 ± 0.09
24-48 h	---	0.12 ± 0.06
48-72 h	---	0.07 ± 0.02

Table 2: Protein binding of [^{14}C]-12378-PeCDD metabolites in rat urine and bile.

Excreta	% Unbound	% Bound			
			Albumin	$\alpha 2_u$	79 kDa
Conventional					
0-24 h Urine	74.0	24.3	21.0	3.3	---
24-48 h Urine	67.9	28.6	23.9	4.7	---
48-72 h Urine	78.1	18.1	17.9	0.2	---
Bile-duct cannulated					
0-24 h Urine	86.7	11.4	11.4	---	---
24-72 h Urine	73.4	18.2	18.2	---	---
0-24 h Bile	88.1	9.8	---	---	9.8
24-48 h Bile	88.1	9.4	---	---	9.4
48-72 h Bile	91.1	7.2	---	---	7.2

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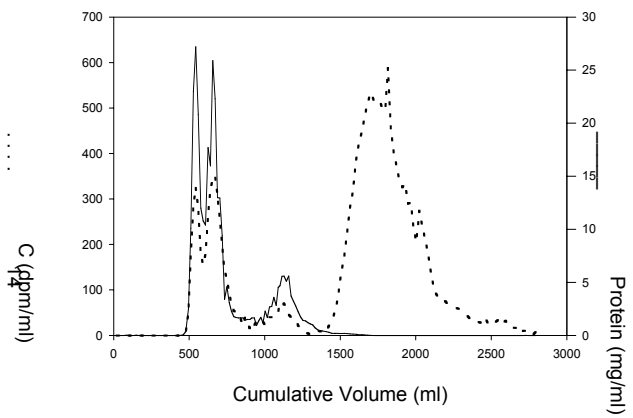

Figure 1. Sephadex G-75 elution pattern of pooled 0-24h urine from cannulated male Sprague-Dawley rats following an oral dose with [^{14}C]-12378-PeCDD.

Fractions were assayed for protein (—) and radioactivity (---).