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

Heldur Hakk, Vernon J. Feil, and Gerald Larsen

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

INTRODUCTION

Dioxins are halogenated aromatic hydrocarbons (HAH) of a highly lipophilic nature. It is generally acknowledged that the major route of mammalian exposure is via ingestion. The 2,3,7,8-chlorinated congeners are considered to be highly toxic. 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 to more polar compounds is necessary for their excretion via the urine or the bile [2]. Despite the added polarity of these metabolites, some are not sufficiently polar to adequately dissolve in the aqueous excreta and, therefore, require a carrier protein for excretion. Mammalian urinary carrier proteins for HAH's have been previously described [3,4] and include albumin and α2u-globulin (α2u). Recently a novel 79 kDa bile protein was isolated from rats that demonstrated covalent binding to numerous nontoxic tetrachloro-p-dioxin congeners [5].

The goal of this study was to characterize the protein(s) which bind(s) the toxic dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) and/or its metabolites in rat urine and bile following a single orally administered dose.

EXPERIMENTAL

[UL 7,8-ring ^{14}C] 2,3,7,8-TCDD, obtained from Chemsyn Science Laboratories, Lenexa, KS, was administered orally to twelve male Sprague-Dawley rats (1.25 mg/kg body weight; 47,038 dpm/μg) in the conventional study and six male Sprague-Dawley rats (1.67 mg/kg body weight; 47,887 dpm/μg) in the bile-duct cannulated study. Urine (conventional study), bile and urine (bile-duct cannulated study) were collected every 24 h for three days. The excreta were individually chromatographed on a Sephadex G-75 column (4.5 x 90cm; G-75) as previously described [6]. The column was 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) was performed as previously described [7]. TLC analysis was performed on aliquots of urine and bile after concentration

by rotary evaporation. TLC plates (silica gel, 5x20 cm) were developed in 50:50 hexane:CH_2Cl_2 with a standard lane containing parent 2378-TCDD.

RESULTS

Daily excretion of ^{14}C from a 2378-TCDD dose into the urine and/or bile of both study groups of rats did not exceed 0.26% of the dose (Table 1). The level of biliary excretion was lower than expected based on previous rat metabolism studies 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. The severe toxicity of 2378-TCDD may also lead to inhibition of these enzymes.

The unbound percentage of the dose remained constant in conventional urine throughout the experiment (Table 2). The protein bound fraction in urine ranged from 8.8 to 19.9 %. Two proteins responsible for the binding of ^{14}C at 0-24 and 24-48 h were evident from the G-75 column elution pattern (Figure 1A). Protein of MW 66.2 kDa eluted from the G-75 column in 518-910 ml, and was determined to be albumin from SDS-PAGE and Western immunoblot analysis. α2u-Globulin (α2u) eluted from 985-1280 ml from the G-75 column as determined by SDS-PAGE (MW 18 kDa) and Western immunoblot analysis. No detectable ^{14}C binding to α2u was noted at 48-72 h, however, binding to albumin was observed. Parent 2378-TCDD was not detected by TLC analysis in conventional urine. It was concluded that urinary ^{14}C binding to albumin and α2u was a result of metabolites of 2378-TCDD. Binding studies on bile-duct cannulated urine (0-24, 24-48, and 48-72 h) are currently underway.

Decreased binding with time was probably due to excretion of greater levels of Phase II metabolites that did not require a carrier protein. The rapid decrease in binding to α2u with time, when compared to albumin, indicated that the initially excreted metabolites could serve as ligands for both carrier proteins. The metabolites excreted at the end of the experiment were ligands only for albumin.

The sex-dependent α2u is a hepatically synthesized protein which is filtered in kidney by the glomeruli due to its size (18 kDa), although 60% is reabsorbed by the proximal tubules [10]. Albumin and α2u constitute the two principle urinary proteins in mature male rats [11]. The function of each protein in urine has not been clearly demonstrated, but they appear to be carriers for small lipophilic molecules.

Parent 2378-TCDD was not detected by TLC analysis in 0-24 h bile. It was concluded that bile contained only metabolites of 2378-TCDD. Unbound metabolites in 0-24 h bile accounted for 76.6% of the total ^{14}C applied to the G-75 column (Table 2). The bound residues in 0-24 h bile represented 9.6% of the ^{14}C and eluted from the G-75 column in 540-820 ml (Figure 1B). Linear regression MW analysis following SDS-PAGE showed that the ^{14}C was bound to a 79 kDa protein. Previous work on nontoxic dioxin congeners, i.e. 1278-, 1378-, and 1478-TCDD, demonstrated that a 79 kDa protein was the principle carrier protein in rat bile [5]. The identity of the protein was not determined, but was a monomer, was N-terminal blocked, and had a pI of 5.7.

Sixteen rat bile proteins have been previously isolated and tentatively characterized by 2-D PAGE and crossed immunoelectrophoresis [12]. Of these bile proteins the majority

are serum proteins, but none have a molecular weight of 79 kDa. Although this 79 kDa bile protein has not been identified, its physiological role may be altered when bound to metabolites of 2378-TCDD. Work is currently underway to investigate 24-48 and 48-72 h bile for the presence of the 79 kDa carrier protein, and to obtain proteolyzed amino acid sequence data in order to further characterize the protein.

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

	Percent of Dose	
Excreta	Conventional (n=12)	Bile-duct Cannulated (n=6)
Urine		
0-24 h	0.12	0.26
24-48 h	0.07	0.11
48-72 h	0.08	0.23
Bile		
0-24 h	---	0.19
24-48 h	---	0.16
48-72 h	---	0.10

Table 2: Protein binding of [^{14}C]-2378-TCDD metabolites in rat urine and bile.

Excreta	% Unbound	% Bound			
			Albumin	α2u	79 kDa
Conventional					
0-24 h Urine	62.3	19.9	17.0	2.8	---
24-48 h Urine	58.7	10.6	9.7	0.92	---
48-72 h Urine	64.2	8.8	8.8	0	---
Bile-duct cannulated					
0-24 h Bile	76.6	9.6	---	---	9.6

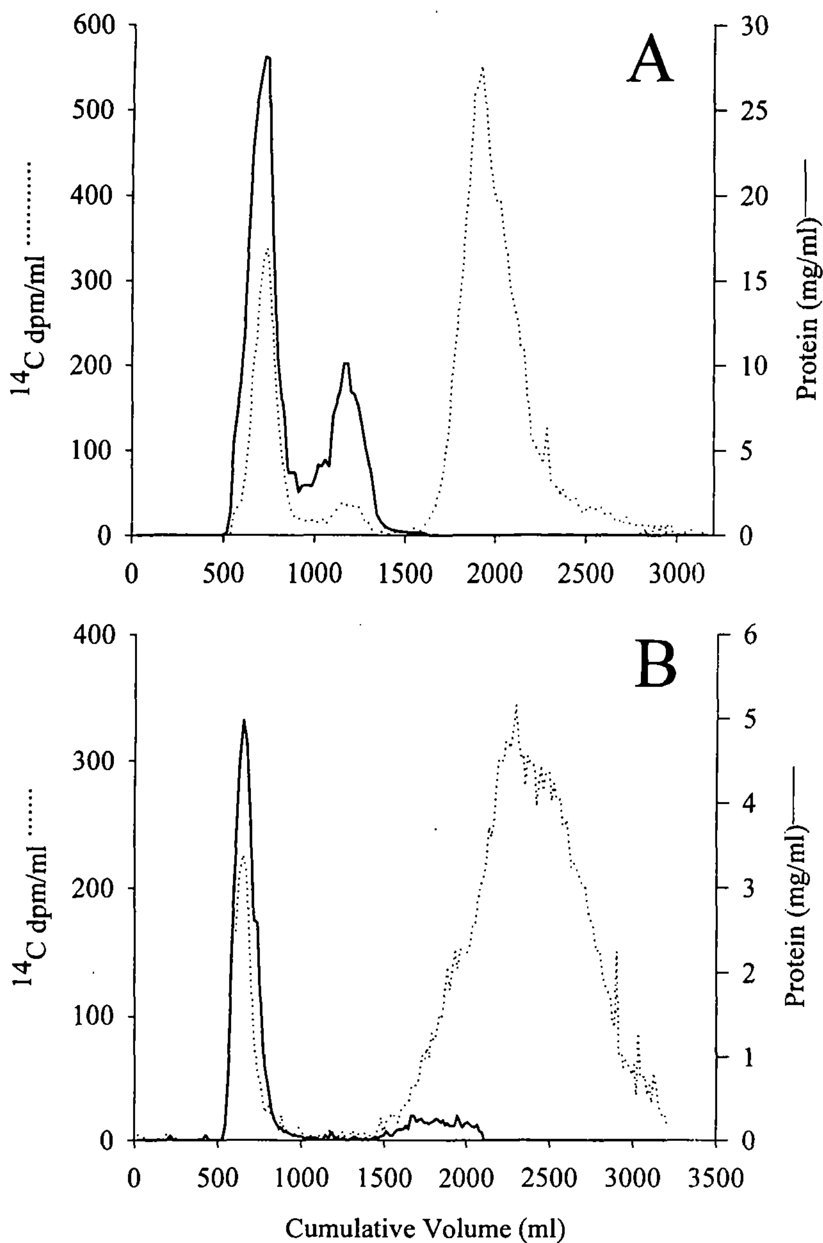

Figure 1: Elution pattern from Sephadex G-75 in the purification of 0-24h urine (A) and 0-24h rat bile protein (B) following oral dose with ^{14}C-2378-TCDD Fractions were assayed for protein (—) and radioactivity (---).

ACKNOWLEDGEMENTS

The authors would like to acknowledge the technical assistance of Barbara Magelky and Colleen Pfaff.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others than may also be suitable.

REFERENCES

(1) Lakshmanan, W.R., Campbell, B. S., Chirtel, S. J., Ekarohita, N., and Ezekiel, M. *J. Pharmacol. Experm. Therap.* **1986**, 239, 673-677.
(2) Pohjanvirta, R. and Tuomisto, J. *Pharmacol. Rev.* **1994**, 46, 483-549.
(3) Larsen, G. L., Wiener, C., Hakk, H., and Feil, V. J. *Organohalogen Cmpds.* **1995**, 25, 249-251.
(4) Larsen, G. L. and Bergman, Å. *Organohalogen Cmpds.* **1994**, 20, 451-454.
(5) Wiener, C. and Larsen, G. L. *Organohalogen Cmpds.* **1997**, 34, 195-198.
(6) Larsen, G. L., Bergman, Å., and Klasson-Wehler, E. *Xenobiotica* **1990**, 20, 1343-1352.
(7) Maizel, J. V., Jr. *Methods in Virology* **1971**, 5, 179-246.
(8) Poiger, H. and Schlatter, C. *Nature* **1979**, 281, 706-707.
(9) Poiger, H. and Buser, H-R. pp. 49-60, in *Biological Mechanisms of Dioxin Action*, Eds. A. Poland and R. Kimbrough, Banbury Report 18, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, **1984**..
(10) Borghoff, S. J., Short, B. G., and Swenberg, J. A. *Annu. Rev. Pharmacol. Toxicol.* **1990**, 30, 349-367.
(11) Neuhaus, O. W., Flory, W., Biswas, N., and Hollerman, C. E. *Nephron*, **1981**, 28, 133-140.
(12) Mullock, B. M., Dobrota, M., and Hinton, R. H. *Biochimica et Biophysica Acta* **1978**, 543, 479-507.