Interaction of 1,2,7,8-tetrachlorodibenzo-*p*-dioxin metabolites with mammalian carrier proteins

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Dioxins are compounds grouped with the halogenated aromatic hydrocarbons (HAH) which also include the furans (PCDFs) and polychlorinated biphenyls (PCBs). These highly lipophilic compounds, like PCBs, are ubiquitous environmental pollutants present at ppt levels. Dioxins are thought to be generated by natural (forest fires) and human activities (chlorine bleaching of paper products, as contaminates in the production of pesticides and incinerators). Because of their extreme lipophilic nature and resistance to metabolism, the dioxins, like PCBs, bioaccumulate up the food chain. Previously, we have shown that PCB and methylsulfonyl-containing metabolites of PCBs interact with various mammalian carrier proteins.[1)2)3)4)]

As an initial study of dioxin interaction with various mammalian carrier proteins, the nontoxic 1,2,7,8-tetrachlorodibenzo-*p*-dioxin (1278-TCDD) was studied. [UL 7,8 ring ^{14}C] 1278-TCDD was synthesized and dosed orally in peanut oil to six male Sprague-Dawley rats (wt 235 to 254 g). Rats were housed in stainless steel metabolism cages and feces and urine collected every 24 h for three days. Two rats were killed 72 h after dosing for isolation of radioactive-protein complexes from kidney and liver. Tissue samples were homogenized and centrifuged. The resulting tissue supernatants or urine samples were individually chromatographed on Sephadex G-75 column or Sephacryl S-200 (S-200) column as previously described.[1)] The method used for sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 13% acrylamide was that of Mazel[5)] and immunoblot analysis (Western Blot) was that of Towbin and Gordon.[6)]

Proteins of approximately 66.2 kDa were isolated from urine and characterized to be albumin from their MW, as determined by SDS-PAGE, and their reaction with antibodies to authentic rat albumin in the Western immunoblot analysis. The identity of the 14 kDa protein from liver and kidney has not been determined but is believed to be a fatty acid binding protein because of its MW from SDS-PAGE, which is identical to previously characterized tissue fatty acid binding proteins.[1)2)3)4)] α_{2u}-Globulin (α_{2u}) was observed in the male rat urine by its MW of 18 kDa from SDS-PAGE. Binding of 1278-TCDD (^{14}C) metabolites to α_{2u}, 14 kDa protein, or albumin from various tissues and urine was determined by isolation and co-chromatography of ^{14}C from 1278-TCDD with these proteins.

Parent 1278-TCDD was not detected in any of the urine fractions. Binding of ^{14}C from 1278-TCDD to albumin and 14 kDa was believed to be the result of metabolites of 1278-TCDD. While 14.3% of the dose was excreted in the urine in 72 h, about 3% (1%

each 24 h) was bound to albumin (Table I). It should be noted that unbound metabolites accounted for 8.0% of the dose in the first 24 h period then dropped to 3.3% in the 24-48 h period and were not detected in the 48-72 h period. ^{14}C-1278-TCDD metabolites were not extractable from the albumin with common organic solvents indicating possible covalent bonding between the 1278-TCDD metabolites and albumin. ^{14}C-1278-TCDD metabolites were not bound to α_{2u}-globulin in the urine.

Binding of ^{14}C residues to kidney and liver proteins represent most of the ^{14}C in these tissues (91% and 96%, respectively). Larger proteins bound most of this ^{14}C. Albumin, the most prevalent of these proteins, is believed to bind most of this ^{14}C, as was found in the urine. Considerable binding of ^{14}C residues to the 14 kDa protein isolated from kidney and liver tissue was also observed (12% and 4.6%, respectively)

Unbound ^{14}C in 0-24 h urine was characterized by ß-glucuronidase or aryl sulfatase enzyme hydrolysis and mass and NMR spectrometry to be the ß-glucuronide-sulfate ester diconjugate of catechol (35% of the ^{14}C in 0-24 h urine). Other 1278-TCDD metabolites are being isolated and characterized from urine at this time.

Albumin is thought to be involved in transport of free fatty acids. FABP is thought to be involved in absorption, intercellular transport and compartmentalization, and metabolism of free fatty acids and their acyl-CoA esters.[8)9)] Thus, albumin and FABP in the liver and kidney may be involved in the uptake, intercellular and extracellular transport, storage, and metabolism of 1278-TCDD. Of particular interest is the strong binding of metabolite of 1278-TCDD with urinary albumin. Ultimately, these processes may direct the accumulation of a particular dioxin metabolite in a specific tissue. In addition, these dioxin metabolites which bind to albumin or FABP may interfere with the metabolism, storage and transport of fatty acids in the cell by associating with albumin and FABP.

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Table 1. Binding of 1,2,7,8-dioxin metabolites to carrier proteins.

Urine	Total ^{14}C % of dose	Albumin 66.2 kDa (%)	α_{2u}-globulin 18 kDa (%)	14 kDa protein (%)	Unbound (%)
0-24 h	8.8	0.8 (9.2)	0	0	8.0 (91)
24-48 h	4.5	1.2 (27)	0	0	3.3 (73)
48-72 h	1.0	1.1 (100)	0	0	
Kidney	0.04	0.03 (79)	0	0.005 (12)	0.004 (8.9)
Liver	0.04	0.04 (91)	0	0.002 (4.6)	0.001 (3.0)

(% ^{14}C bound by carrier protein in urine or tissue fraction)