ASSOCIATION OF DECABROMODIPHENYL ETHER WITH URINARY AND BILIARY CARRIER PROTEINS

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Introduction

Decabromodiphenyl ether (BDE-209) is the most highly brominated congener in the brominated diphenyl ether (BDE) family. It is the most abundant commercially produced BDE, with a worldwide annual production of approximately 30,000 tons. The most important end product of BDE-209 is as an additive flame retardant in high-impact polystyrene, but it is also used for textiles in automobiles, tents, and soft furnishings. Due to its extremely low water solubility and vapor pressure, BDE-209 would be expected to be persistent and to bioaccumulate. However, BDE-209 exposures may be low because it is not extracted readily from polymers[1], and is not absorbed well from the gastrointestinal tract[2].

Halogenated aromatic hydrocarbons can associate with endogenous carrier proteins in the urine and bile of rats, either as the parent or as metabolites[3-5]. Toxic and non-toxic dioxins, PCB's, and BDE's all have this capacity. The purpose of the association has not been established, but may be used to facilitate the elimination of these lipophilic xenobiotics. However, the association may affect the normal function of these carrier proteins. The purpose of the present research was to administer a single oral dose of BDE-209 to male rats, and measure the amount eliminated in the urine and bile, as well as characterize the nature and extent of binding to any proteins in these excreta.

Materials and Methods

Male Sprague-Dawley rats (212-224 g) were divided into two groups; four conventional rats, and four bile-duct cannulated rats. A dose of ^{14}C-decabromodiphenyl ether (0.3 mg/kg; 4.0 μCi) in 0.5 ml of peanut oil was given by oral gavage. Urine and bile were collected at 24h intervals. An aliquot of each sample was mixed with Ecolite (ICN, Costa Mesa, CA), and assayed for radioactivity on a Packard 1900 CA liquid scintillation counter.

Due to the low amount of ^{14}C in urine, both 0-72h conventional and bile-duct cannulated samples were pooled separately, and chromatographed. Bile from the cannulated rats was pooled each day. Pooled excreta were chromatographed on Sephadex G-75 (4.5 x 90 cm) and Sephacryl S-200 (2.2 x 85 cm) 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% polyacrylamide) was performed as described previously[4].

Liver, kidney, intestinal mucosal cells, and lung were homogenized in 0.05M potassium phosphate buffer (pH 7.2). The samples were centrifuged at 10,000 x g for 30 min. The

supernatant was removed and assayed for ^{14}C, and the pellet was combusted on a Packard Model 307 tissue oxidizer. The 10,000 x g supernatant was centrifuged at 100,000 x g for 90 min. The supernatant was removed and assayed for ^{14}C, and the pellet was combusted. The 100,000 x g supernatant was applied to a G-75 gel filtration column (as described above), and the protein-bound regions were subjected to a fatty acid binding protein (FABP) test, as described previously[7].

Results and Discussion

Daily excretion of BDE-209 via the urine was extremely low, never exceeding 0.02% of the administered dose (Table 1). Elimination of BDE-209 via the bile was favored. Six percent of the dose was eliminated in 0-24h bile (Table 1), and cumulative 0-72h biliary elimination was greater than 9%.

Pooled 0-24h bile was chromatographed on a G-75 gel filtration column (Figure 1), and essentially all of the detectable ^{14}C was protein bound (>97%; Table 2). Further purification of the protein-bound region on an S-200 column, and chromatography on SDS-PAGE revealed the presence of a 79 kDa protein. The protein was monomeric, N-terminal blocked, and had an isoelectric point of 5.7. Neither the identity nor the function of this protein is known. Previous studies to characterize rat biliary proteins did not identify a 79 kDa monomeric protein among the 16 proteins that were observed[8]. Non-toxic[4,5] and toxic[9] dioxins and/or their metabolites, and brominated diphenyl ethers[10] have been shown to bind to a 79 kDa protein in the bile of male Sprague-Dawley rats. In those studies, the extent of xenobiotic association for the 79 kDa protein ranged from 7-47% of the biliary ^{14}C. Therefore, the present results represent the first report of nearly 100% binding of a xenobiotic by the 79 kDa bile protein. The association may be physiochemical in nature, because BDE-209 has an even higher octanol:water partition coefficient, i.e. k_{ow}= 9.97 versus 7-8 for the dioxins. The 79 kDa protein may be necessary to facilitate elimination of the highly lipophilic BDE-209. Another explanation is that BDE-209 may serve as a better ligand for the binding domain on the 79 kDa protein. There is also a possibility that the normal function of this protein may be affected by association with BDE-209. Work is currently underway in this laboratory to obtain proteolyzed amino acid sequence data to further characterize this protein.

Pooled 0-72h urine for both the conventional portion of the study and the bile-duct cannulated study were also chromatographed by gel filtration chromatography. The majority of the ^{14}C in conventional urine remained unbound in the conventional rats (Table 2), but the opposite was true of urine from cannulated rats. In both cases, however, the protein that associated with BDE-209 in the urine was identified as albumin by Western immunoblot analysis.

The tissue disposition of BDE-209 showed that a relatively high portion of the dose was retained in the liver of conventional rats (8.6%; data not shown). Lung also contained a significant portion of the dose (>2%). Intestinal cells and kidney were minor depots for BDE-209. Approximately 10% of the ^{14}C from each sampled tissue was associated with the 100,000 x g supernatant. In the liver, which was the only tissue with sufficient radioactivity, the water soluble ^{14}C was associated with liver fatty acid binding protein, presumably for solubility reasons due to the high lipophilicity of BDE-209.

Acknowledgments

The high degree of technical assistance provided by Barbara K. Magelky and Colleen Pfaff are gratefully appreciated.

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Table 1. Radioactivity present in male rat urine and bile, following a single oral dose of [^{14}C]-decabromodiphenyl ether (BDE-209) administered in 0.5 ml peanut oil (0.3 mg/kg body weight).

		Percent of Dose	
	Excreta	Conventional (n=4)	Bile-duct Cannulated (n=4)
Urine			
	0-24h	0.019	0.020
	24-48	0.008	0.020
	48-72h	0.006	0.007
Bile			
	0-24h	-----	6.0
	24-48h	-----	2.5
	48-72h	-----	0.7

Table 2. Protein binding of [^{14}C]-decabromodiphenyl ether (BDE-209) and/or its metabolites in rat urine and bile.

Excreta	% Unbound	% Bound		
			Albumin	79kDa
Conventional				
0-72h Urine	73.4	19.7	18.0	---
Cannulated				
0-72h Urine	18.2	68.3	68.3	---
0-24h Bile	---	97.5	---	94.4
24-48h Bile	---	92.0	---	89.5
48-72h Bile	---	90.4	---	87.4

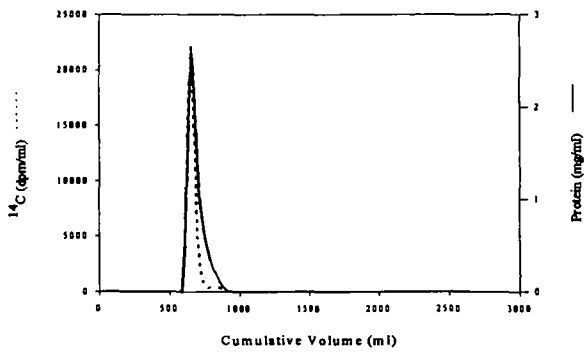

Figure 1. Chromatograph of 0-24h bile from a G-75 gel filtration column.