Interaction of 3-Methylsulfonyl-2,2',4',5,5',6-Hexachlorobiphenyl with Mammalian Carrier Proteins

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Introduction

Polychlorinated biphenyls (PCBs) have been used extensively as industrial chemicals in electrical equipment worldwide, and as a result, PCBs have become ubiquitous environmental pollutants.[1] PCBs are metabolized in mammals to sulfur-containing metabolites such as methyl sulfides and methyl sulfones.[2] The methylsulfonyl- PCBs have been shown to accumulate in mammals and some are selectively retained in certain tissues.[3,4,5,6] Introduction of methylthio-, methylsulfinyl- and methylsulfonyl-groups into PCBs is known to involve the mercapturic acid pathway.[7,8]

Previously we have shown that 4,4' bis (methylsulfonyl)-2,2',5,5'-tetrachlorobiphenyl formed a complex with the following mammalian carrier proteins: α_{2u}-globulin (α_{2u}) in kidney and urine of male rats, major urinary protein in mouse urine,[9] liver fatty acid binding protein (FABP) isolated from rat intestinal mucosa[10] and FABP from liver and intestinal mucosa of the chicken.[11] Two methylsulfonyl-containing PCBs, 3-methylsulfonyl- and 4-methylsulfonyl-2,2',4',5,5'-pentachlorobiphenyl (3-101 and 4-101) were also shown to associate with carrier proteins (i.e. α_{2u} in male rat urine, liver and kidney FABP, and 4-101 with lung 14 kDa protein).[12]

Recent work has shown that 3-methylsulfonyl-2,2',4',5,5',6-hexachlorobiphenyl (3-149) accumulated 5.5 fold in the liver of mink fed a diet containing a PCB and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethene] methylsulfone mixture.[6] Because of the extensive accumulation of 3-149, we conducted a study of 3-149 to determine its association with mammalian carrier proteins in various tissues of male rats.

Material and Methods

Sixteen male Sprague-Dawley rats (244-287 g) were each dosed orally with synthesized 3-[^{14}C]methylsulfonyl-2,2',4',5,5',6-hexachlorobiphenyl (3.3 μCi/dose, 18 μCi/mmol). The methylsulfonyl biphenyl was prepared from 3-mercapto-2,2',4',5,5',6-hexachlorobiphenyl and

[^{14}C]-methyliodide (1.0 mCi; 55.0 mCi/mmol).[13,14] Eight of the sixteen rats were bile duct cannulated as described elsewhere.[15] Bile, urine, and faeces were collected at 24 h and 48h. At 48h the animals were anesthetized with halothane, a ventral midline incision made, and killed by exsanguination from the dorsal aorta. Blood, liver, heart, lungs, thymus, kidneys, fat, testes, small and large intestine, and carcass were collected. Urine and bile samples were assayed for ^{14}C by liquid scintillation counting. Tissue samples were homogenized, centrifuged, and chromatographed on Sephadex G-75 (G-75) and Sephacryl S-200 (S-200) using a 0.05M potassium phosphate buffer (pH = 7.2) as previously described.[16] The method for sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 13% acrylamide was that of Maizel.[17] An oleic acid - FABP competitive binding assay was performed as previously described[16] on proteins suspected of being FABP by MW analysis from SDS-PAGE (14 kDa).
Binding of [^{14}C] 3-149 or its [^{14}C] metabolites to α_{2u}, FABP, albumin or the 79 kDa bile protein was determined by co-elution of ^{14}C with the respective protein on G-75 and S-200 columns.

Results and Discussion

Table 1 shows the distribution (in the tissues and excreta studied) of ^{14}C in rats dosed with [^{14}C] labelled 3-149. Table 2 shows an estimate of the percent of dose bound to carrier proteins in urine, bile, liver, kidney, and lung.

Low levels of ^{14}C were excreted in the urine (2.5 %,0 -24h, Table 1) by the conventional rat, and lower levels were observed in the bile-duct cannulated rat urine (0.45 %,0 -24h). Because albumin and α_{2u} are the major proteins found in male rat urine, we expected binding to these proteins, however, no association with urinary proteins was found except for a small amount of binding to albumin (0.01%) in 24-48 h urine from the bile-duct cannulated rats. No binding of [^{14}C] 3-149 or its metabolites to α_{2u} was observed in kidney. Higher levels of biliary excretion were observed when compared to urine (10.7%, 0-24 h, Table 1). Covalent binding to a 79 kDa biliary protein (0.7%, 0-24h, Table 2) was determined by a lack of extractability into organic solvents. Nontoxic dioxin congeners have also been shown to covalently bind to a 79 kDa biliary protein.[18]

Of the conventional rat tissues analyzed, the liver contained the highest levels of ^{14}C residues (2.6%, Table 1) followed by lung (1.1%) and kidney (0.2%). Binding to FABP and other soluble proteins was correspondingly highest in the liver (0.26%). It should be noted that of the 2.6% of the ^{14}C - dose in the liver, 62% remained in the 10k x g pellet and 27% remained in the 100k x g pellet after differential centrifugation. This radioactivity is thought to be associated with cell components in these pellets. About 1% of the radioactivity in the liver was found to be not associated with protein. This is consistent with the observation of higher levels of 3-149 in the livers of mink feed a PCB and DDE methyl sulfone mixture.[6] A lower amount of binding to FABP was observed in the kidney (0.01%). In the lung, 0.20% of the ^{14}C dose was bound to a 14 kDa protein which is believed to be the uteroglobulin-like protein reported by Lund, et al. (1988).[19] A trace of radioactivity was found to be bound to albumin in kidney and lung.

Summary

FABP is thought to be involved in absorption, intracellar transport, compartmentalization, and metabolism of free fatty acid and their acyl-CoA esters.[20,21] FABP

in liver and kidney may be involved in the uptake, intracellular transport, storage and metabolism of 3-149. The 14 kDa protein in lung may also belong to the family of FABP carrier proteins and function similarly. The covalent binding of 3-149 to the 79 kDa bile protein is believed to involve a metabolite of 3-149. Binding of 3-149 or its metabolites to these proteins may interfere with their natural functions, i.e. metabolism, storage, and transport by associating with FABP or the 79 kDa protein, and could adversely affect reproductive efficiencies. Other studies have shown that PCBs can induce reproductive insufficiencies in mink[22] and is probably the cause of poor reproductive success in seals[23] and otters[24] in the wild.

Table 1. Distribution of ^{14}C residues in rats dosed with 3-$MeSO_2$-2,2',4',5,5',6-hexachlorobiphenyl (3-149).

		Percent
Conventional Rats		
Urine		
	0-24 h	2.47 ± 0.38
	24-48 h	0.98 ± 0.32
Tissues[a]		
	Liver	2.56
	Kidney	0.2
	Lung	1.1
Bile-duct Cannulated Rats		
Urine		
	0-24 h	0.45 ± 0.50
	24-48 h	0.25 ± 0.24
Bile		
	0-24 h	10.7 ± 5.2
	24-48 h	4.6 ± 3.2
Tissues[a]		
	Liver	1.3
	Lung	0.27
	Kidney	0.13

[a] Tissues were combined.

Table 2. Binding of 3-$MeSO_2$-2,2',4',5,5',6-hexachlorobiphenyl (3-149) to carrier proteins as a percent of dose.

Conventional Rats		Percent
Albumin		
	0-24 h Urine	0
	24-48 h Urine	0
	Kidney	0.002
	Lung	0.007
α_{2u}		
	0-24 h Urine	0
	24-48 h Urine	0
14 kDa FABP		
	Liver	0.26
	Kidney	0.01
14 kDa protein		
	Lung	0.20
Bile-duct Cannulated Rats		
Albumin		
	0-24 h Urine	0
	24-48 h Urine	0.01
α_{2u}		
	0-24 h Urine	0
	24-48 h Urine	0
79 kDa bile protein		
	0-24 h Bile	0.67
	24-48 h Bile	0.33
14 kDa FABP		
	Liver	0.04
14 kDa protein		
	Lung	0.14

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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.

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