ROLE OF PROTEIN BINDING IN THE URINARY EXCRETION OF PBDES IN MICE

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Introduction

Polybrominated diphenyl ethers (PBDEs) are commercial chemical products used to prevent combustion of various consumer goods. Concern for PBDEs has risen due to their detection in the environment and in human biota, apparent persistence, and potential toxicity.[1] The congener profiles of PBDEs in human and wildlife samples are dominated by PBDE congeners 47, 99, 100, 153, and 154; all of which are components of the commercial pentaBDE mixtures commonly used in a variety of flammable consumer products.

Several of these congeners have demonstrated differences in toxicokinetic behavior between species, sex, and individual congeners. The differences in toxicokinetic behavior, specifically elimination, appear to be driven by urinary excretion.[2,3,4,5] In one study in mice, 33% of the administered dose of BDE 47 was excreted in five days and only parent compound was identified.[2] In the same study, rats excreted less than 0.5% excreted within 5 days of exposure, demonstrating a major species difference in urinary excretion.[2] Another study reported that male mice excreted up to 100-fold more BDE 47 in urine than did male rats following a single administration[5]. While not as pronounced as BDE 47, BDE 99 has also demonstrated marked species difference in urinary excretion. In a rat study, approximately 23% of a single oral dose of BDE 99 was excreted within the first 24-hours, but less than 0.5% was excreted into the urine.[6] In contrast to rats, mice in the present study excreted ~8% of a single intravenously-administered dose of BDE 99 into urine within 24 hours of exposure. Similar distinctions were found between species in other studies as well as sex-dependent differences in excretion[4].

The present study investigates urinary excretion parameters in mice for BDE congeners 47, 99, 100, and 153 in an effort to distinguish the mechanisms responsible for the observed species differences in excretion of these flame retardant congeners. Mice were administered a single intravenous dose of BDE 47, 99, 100, or 153 and excretion was monitored for five days. Resulting comparisons, metabolites, and urinary binding data are presented here.

Materials and Methods

Dosing solution

Intravenous (iv) dosing solution was prepared in an Alkamuls EL-620 (formerly Emulphor: Rhone-Pouleax, NJ), ethanol, water (1:1:8) vehicle. Solvent from [^{14}C]-labeled congeners (<97% chemical purity) was allowed to air dry in an amber vial, followed by addition of Alkamuls EL-620, ethanol and water. All solutions were made 24-hours prior to administration and sonicated two hours prior to administration.

Treatment

Female C57BL/6 mice, obtained from Charles River Laboratory (Raleigh, NC), were administered a single dose (1mg/kg) intravenously via the tail vein at a dosing volume of 4 mL/kg. For each congener, animals received the following approximate amounts of radioactivity: [^{14}C]BDE 47 (1.2 μCi), [^{14}C]BDE 99 (1.5 μCi), [^{14}C]BDE 100 (0.08 μCi), or [^{14}C]BDE 153 (1.0 μCi). Mice were housed individually in metabolism cages following BDE administration for five days. Urine was collected daily.

Urinary protein binding

0-24h urine from BDE 47 and BDE 99 dosed mice, and because of limited amounts of radioactivity, 0-120h urine from BDE 100 and BDE 153 dosed mice were chromatographed by size exclusion on Sephadex G-75 columns (4.5 x 90 cm), eluted with 0.05 M potassium phosphate buffer. Elution fractions were assayed for radioactivity. Protein-bound fractions were assayed for protein by the Bradford method (bovine serum albumin [BSA] as protein standard) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 13% acrylamide) was performed as

described previously[7] on protein bound fractions. All electrophoresis materials were purchased from Bio-Rad Laboratories (Richmond, CA). Protein samples were electrophoretically transferred to a sheet of nitrocellulose (Schleicher & Schuell, Keene, NH; Western blot) as previously described [8]. The Western blots of separated proteins were blocked by incubation for 2h in Tris-saline-Tween buffer with 1 mg/ml BSA (0.15 M Tris, 0.5 M NaCl, 0.05% Tween 20, pH 7.5), and then incubated for 2h at room temperature with anti-goat-mouse major urinary protein (Accurate Chemical, Westbury, NY; 1:500 dilution in Tris-saline-Tween buffer). The Western blots were washed three times with Tris-saline-Tween buffer (5 min each) and then incubated with the same buffer containing rabbit-anti-goat IgG horseradish peroxidase conjugate antibody (Sigma; 1:3000 dilution) for 1h at room temperature. The Western blots were washed three times with Tris-saline-Tween buffer (5 min each), once with Tris-saline buffer (0.15 M Tris and 0.5 M NaCl, pH 7.5), and then incubated in color reagent (25 ml Tris-saline buffer plus 12.5 µl 30% H_2O_2 and 5 ml of 3 mg/ml solution of 4-chloro-1-naphthol).

Urinary metabolites

The protein-bound fractions from urine were extracted with hexane and ethyl acetate (3X each). The combined organic layers were concentrated under a stream of nitrogen, and spotted onto silica gel TLC plates which were developed in 1:1 hexane:methylene chloride with a standard lane containing [^{14}C] parent compound.

Results and Discussion

BDE 47 was most rapidly excreted in the urine, followed by BDEs 99, 100, and 153 (Figure 1). Approximately 17, 8, 2, and 1%, of a single dose, respectively, were excreted within 24 hours of an iv administration. Cumulatively, 40, 16, 6, and 2% were excreted at five days demonstrating major congener-dependent differences in urinary excretion. Urine was analyzed for either protein binding by GPC analysis with a Sephadex G-75 column. Analyses of BDE 47 and BDE 99 urine excreted on day 1 revealed that the majority of BDE in the urine is protein-bound (Figure 2). Pooled urine samples (days 1-5) were analyzed for protein binding for BDE 100 and BDE 153, and the majority of radiolabel in urine was also protein-bound.

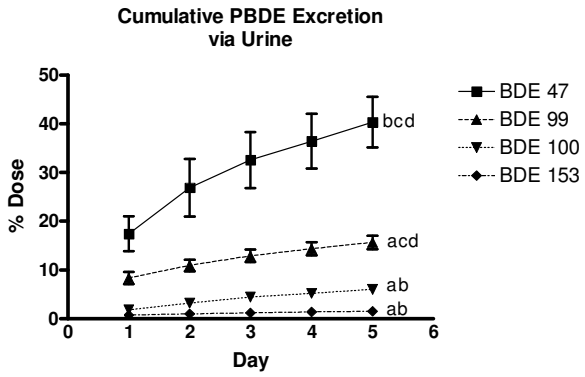

Figure 1.

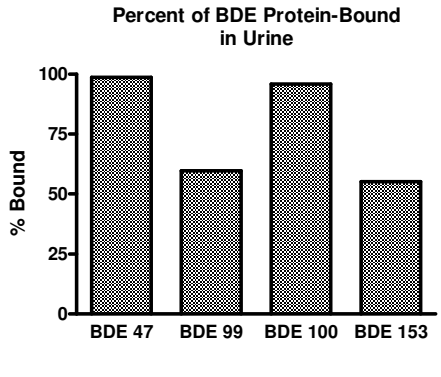

Figure 2.

Figure 1 and Figure 2.

(1) Cumulative urinary excretion of PBDE (% dose) five days following a single, iv dosing of 1 mg/kg. Statistical significance ($p<0.05$) as compared to (a) BDE 47, (b) BDE 99, (c) BDE 100, (d) BDE 153 based on cumulative % dose excreted (daily significance not shown).

(2) Percent of BDE protein-bound in urine following a single, iv dosing of 1 mg/kg: Percent

BDE 47 and BDE 99 excreted in urine for day l. Percent of BDE 100 and BDE 153 in pooled urine samples for days 1-5.

SDS-PAGE analyses of the protein-bound fraction revealed that these BDE congeners were bound to either a single 21.3 kDa protein (BDE47 and BDE 99) or to a doublet of 17.8 and 20.1 kDa proteins (BDE 100 and BDE 153; Figure 3, determined by regression analysis). The binding proteins have all been identified as mouse major urinary proteins (mMUP; Figure 4), which belong to the same superfamily of proteins which include FABP and alpha-2u-globulin, both of which are known to bind polyhalogenated aromatic hydrocarbons.[6,9,10,11]

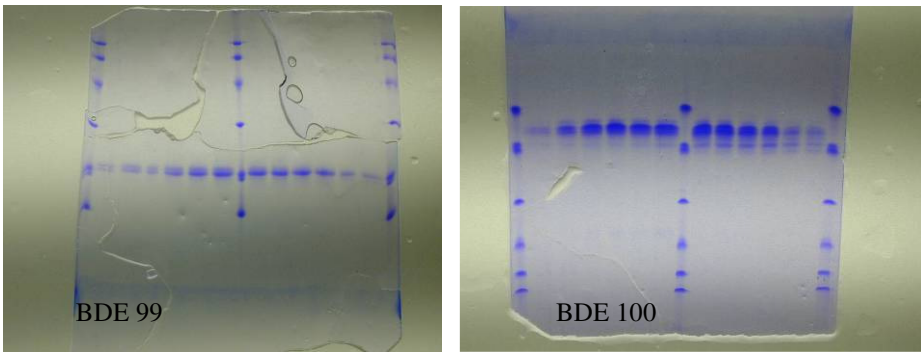

Figure 3. Preliminary representative SDS-PAGE analyses of protein binding in urine for BDE 99 and BDE 100.

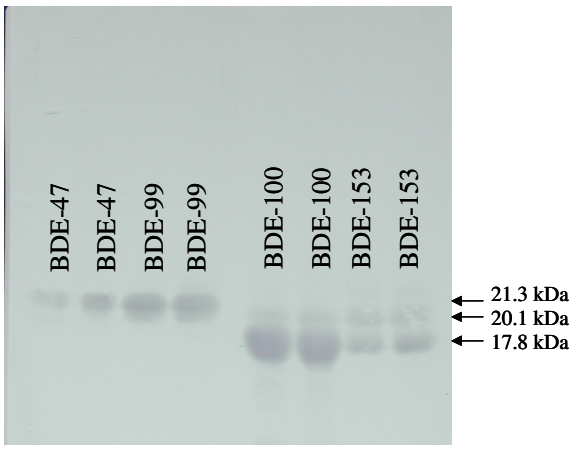

Figure 4. Western immunoblot of mouse major urinary proteins (mMUPs) from urine of mice treated with [^{14}C]BDEs 47, 99, 100, and 153, respectively.

Urinary excretion appears to have significant impact on the major elimination differences observed between PBDE congeners, animal sex, and species.[2,3,4,5] The results of the present study may offer support for these differences: all the BDEs investigated, especially BDE 47, bind to mMUPs and are readily excreted via the urine in mice. The

majority of MUP is synthesized in the liver, secreted in the serum, circulated at low levels and rapidly filtered by the kidney and excreted into the urine. Because adult male mice secrete up to 20 times more MUP from the liver than females, this could explain the gender differences in the percent of BDE 47 excreted in the urine when male and female excretion patterns are compared.[5] Studies in rats have demonstrated binding of PBDEs to the rat binding proteins alpha-2u-globulin and albumin.[12] It is important to note that the rat carrier protein alpha-2u-globulin and the mouse MUP belong to the same superfamily of proteins. Therefore, urinary binding proteins as well as bromination pattern play an important role in the pharmacokinetics of PBDEs. Because the mechanism(s) of toxicity for PBDEs is not understood, it is important to consider the differential toxicokinetic parameters associated with each congener when assessing the risk to human health from individual PBDE congeners as well as the commercial PBDE mixtures.

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