IN VIVO MDR1 TRANSPORT STUDIES WITH PCB 77

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Introduction

The brain is protected against the entry of many compounds by capillary endothelial cells which are joined together by intercellular tight junctions, thus forming the so called blood brain barrier (BBB). However, lipophilic compounds can enter the brain by passive diffusion through the lipid bilayer. In order to protect the brain, the BBB is also equipped with an efflux transporter known as the multidrug resistance (MDR) transporter. This transporter is a plasma membrane-bound, ATP-dependent, phosphorylated glycoprotein (P-gp) [1]. Studies have shown that this P-gp on the BBB is important in preventing the accumulation of a wide range of structurally- and functionally-unrelated, substances in the brain [2, 3]. Besides in the brain, this protein is also found in other vital organs, like lungs, testes, intestines, liver, and kidneys. The specific tissue location suggests a protective role of P-gp, by preventing accumulation of compounds as well as facilitating their excretion. While humans have only one MDR1 gene that encodes this P-gp, mice have two genes, mdr1a and mdr1b [4, 5]. Mdr1b is expressed predominantly in adrenal glands, placenta, and the endometrium of the uterus of pregnant animals and therefore probably not involved in the protection of the brain. Mdr1a on the other hand is predominantly expressed in the intestine and the capillary endothelial cells of the brain and testes [6].

PCBs, especially the higher chlorinated congeners are very lipophilic. Ingested PCBs would be expected to distribute from the blood and accumulate in different body compartments in proportion to their lipid content. However, the brain is an exception. Distribution studies in experimental animals, as well as in samples from wildlife and humans exposed to PCBs, have shown that PCBs accumulate in the brain to a much lesser extent than one would predict on the basis of the fat content of this tissue [7, 8]. In addition, among those individual PCB congeners identified in the brain ortho-substituted non-coplanar PCBs seem to be preferentially taken up and/or retained. In a Greenland study, analysis of brain tissues obtained from human autopsies showed increased accumulation of PCB 99, PCB 105, PCB118, PCB 138, PCB 153 and PCB 156 all of which possess one or more ortho-substitution on the biphenyl ring [9]. This suggests that while the non-coplanar PCBs are able to cross the BBB, the entry of coplanar PCBs is largely restricted. Also, the level of the non-coplanar hexachlorinated biphenyl PCB 169 was found to be 4-9 fold higher in the rat brain than the levels of PCB 153, a coplanar hexachlorinated biphenyl, after i.v. injection in the tail vein [10]. These congeners have similar lipophilicities and thus should accumulate in the brain to a similar extend. Therefore we hypothesized that coplanar PCBs are substrates for the MDR transporter on the BBB which significantly reduces their distribution into and accumulation in the brain by effluxing them into the circulation.

Materials and Methods

Compounds

^{14}C-PCB 77 (3,3',4,4'-tetrachlorobiphenyl) (specific activity 12.5 mCi/mmol) was purchased from Sigma Chemical Co (St. Louis, MO). ^{3}H-Digoxin (specific activity 19 Ci/mmol) was obtained from NEN Life Science Products Inc. (Boston, MA). Emulphor 620 was a kind gift of Rhodia Chemicals,

Cranbury, NJ. Soluene-350, Hionic-Fluor and Bio-Fluor scintillation cocktails were from Packard Instruments Co (Meriden, CT). All other chemicals were purchased from Sigma Chemical Co. if not otherwise indicated.

In vivo distribution studies

Four animals per group of 8 weeks old male FVB mice (wild type and mdr1a knock out; Taconic Farms Inc., Germantown, NY) were injected via tail vein with either ^{14}C-PCB 77 (5mg/kg, 0.214µCi/g body wt., dissolved in vehicle), ^{3}H-digoxin (0.214µCi/g body wt., dissolved in saline), or vehicle alone (emulphor 620 : ethanol (1:0.1) : saline]. After 4 hours blood was collected and organs were removed, washed with saline, and stored at –80 °C. Organs were homogenized in 9 volumes of saline. Aliquots of 333 µl tissue homogenates were digested at 50 °C for 4 hours with an equal volume of soluene-350 and decolorized with 30 % H_2O_2 (67 µl). 4 ml of the scintillation cocktail hionic-fluor was added and samples were allowed to stand in the dark for 2 hours before counting of radioactivity with a liquid scintillation counter. Blood (66.6 µl) was treated with 500 µl soluene-350: isopropanol (1:1) and decolorized with 160 µl 30 % H_2O_2 before counting. Feces (100 mg, pooled) was ground and incubated at 60 °C with 200 µl of 70 % perchloric acid and 120 µl 30 % H_2O_2 until dissolved. 4 ml of the scintillation cocktail bio-fluor was added and radioactivity was counted after allowing to stand in the dark for at least 2 hours to reduce chemiluminescence. To correct for quench by different tissues a quench curve was first established using quenched standards. Statistical calculations were performed using SAS (version 8.0) and significance was determined by ANOVA using Proc GLM (Generalized Linear Model) and post-hoc Bonferroni test.

Results and Discussion

The coplanar PCB 77 was the congener selected for this study. PCB 77 is one of the 3 highly toxic congeners following acute exposure. It is present in commercial PCBs and also detected in environmental samples. It is moderately persistent. In mice dosed orally with PCB 77, the unchanged congener constituted the major component in feces up to 2 days [11]. A high fecal and biliary excretion for this congener is reported in mice and rats [11, 12], suggesting it could be a substrate for liver canalicular and intestinal mdr transporter. Most importantly, distribution studies in experimental animals have shown that PCB 77 remains in vascular spaces and does not cross the BBB to a significant extent [8]. We used an *in vivo* mouse model to assess the contribution of P-gp in restricting the entry of PCB 77 into the brain. For this purpose, tissue distribution and excretion of ^{14}C-PCB 77 in mdr1a knock out animals and genetically matched wild type animals were compared. An early time point (4 hours after injection) was chosen for the analysis to minimize influence of metabolism on the distribution results. ^{3}H-digoxin was used as a positive control. As expected digoxin showed almost 11 times greater accumulation in the brain of knock out mice as compared to the wild type mice. The difference was statistically significant ($p = 0.004$). In contrast, no such difference was apparent in the brain of PCB 77-treated animals. A similar pattern was observed with respect to distribution of PCB 77 and digoxin in other organs, which express the P-gp efflux pump; for PCB-treated animals, the ratio of distribution between the knock out and wild type animals was at or near 1, whereas for the digoxin-treated animals differences in distribution were apparent, with ratios greater than 1.7, especially in the testes, spleen, and liver. However, these differences were not statistically significant at $p < 0.05$, probably due to the large standard deviation and the small population size ($n = 3$ or 4). The amount of digoxin that was eliminated in the feces of treated wild type animals was more than 3 times that eliminated in the knock out animals. Also, more digoxin was found associated with the colon and small intestine of wild type mice compared to knock out mice. The difference in colon was statistically significant ($p < 0.001$). Animals in the PCB 77-treated groups had equivalent amounts of the dose being eliminated in the feces. These *in vivo* results suggest that PCB 77 may not be a substrate for P-gp.

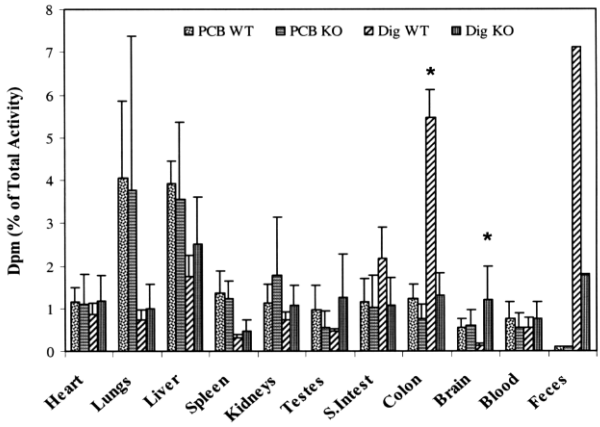

Figure 1. Level of radioactivity as percent of administered dose.

Results are expressed as means ± S.D. (n = 3 or 4), except for feces, where pooled samples were counted. * indicates statistical significance within treatment/organ at p £ 0.004.

Acknowledgements

Supported by grant DAMD 17-02-1-0241 from the DOD, grant #G0K20395 from the EPA, grant P42 ES 07380 from NIEHS, and grant 85-001-13-IRG from the American Cancer Society. The authors wish to thank Dr. C. Srinivasan, University of Kentucky, for help with the statistical analyses. N.T. was supported in part by a training grant from the Superfund Basic Research Program (ES-07380). Contents are solely the responsibility of the authors and do not necessarily represent the official views of the DOD, EPA, NIH, NIEHS, or ACS.

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