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The transepithelial transport mechanism of polybrominated diphenyl ethers in human intestine determined using a Caco-2 cell monolayer

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ABSTRACT

Oral ingestion plays an important role in human exposure to polybrominated diphenyl ethers (PBDEs). The uptake of PBDEs primarily occurs in the small intestine. The aim of the present study is to investigate the transepithelial transport characteristics and mechanisms of PBDEs in the small intestine using a Caco-2 cell monolayer model. The apparent permeability coefficients of PBDEs indicated that tri- to hepta-BDEs were poorly absorbed compounds. A linear increase in transepithelial transport was observed with various concentrations of PBDEs, which suggested that passive diffusion dominated their transport at the concentration range tested. In addition, the pseudo-first-order kinetics equation can be applied to the transepithelial transport of PBDEs. The rate-determining step in transepithelial transport of PBDEs was trans-cell transport including the trans-pore process. The significantly lower transepithelial transport rates at low temperature for bidirectional transepithelial transport suggested that an energy-dependent transport mechanism was involved. The efflux transporters (P-glycoprotein, multidrug resistance-associated protein, and breast cancer resistance protein) and influx transporters (organic cation transporters) participated in the transepithelial transport of PBDEs. In addition, the transepithelial transport of PBDEs was pH sensitive; however, more information is required to understand the influence of pH.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of typical persistent organic pollutants (POPs), which can cause adverse effects in the ecosystem and in humans. They were widely used as brominated flame retardants in various consumer products, especially in textiles, plastics, and electronic appliances [10]. As they are not chemically bound to the materials, they can be released from the materials into the environment. Due to their stability and semi-volatility, PBDEs are difficult to degrade in the environment and can be transported long distances [21]. In addition to their high lipophilicity, PBDEs are persistent with the potential for bioaccumulation in organisms and biomagnification through food chains. They have been detected in all types of biota [1,20,29,54]. In the past few years, the levels of PBDEs in biota have increased significantly and PBDEs have been detected in human tissue samples [1,20,41,43]. Toxicity studies have shown that PBDEs result in embryotoxicity, developmental neurotoxicity, and reproductive toxicity [10,3,7]. The risks due to these chemicals have attracted increasing attention in recent years. Oral ingestion is the main exposure pathway for PBDEs in the non-professional population [27].

Caco-2 cells, derived from human colon carcinoma, express tight junctions, micro villi, and many enzymes and transporters present in the normal human small intestine [40]. These cells can differentiate and polarize, and form a monolayer when cultured under specific conditions. Because of their similar morphology and function to human small intestinal epithelial cells, the Caco-2 cell model is widely used as a tool to investigate the absorption, transport, and metabolism of xenobiotics [15,16,32,44,49].

The efflux transporters, P-glycoprotein (*p*-gp), multidrug resistance-associated protein (MRP), and breast cancer resistance protein (BCRP), which belong to the ATP-binding cassette transporters (ABC transporters), have been shown to pump drugs back to the lumen, and act as a biological barrier, which can affect the bioavailability of

Abbreviations: ABC transporters, ATP-binding cassette transporters; AP, apical; BCRP, breast cancer resistance protein; BL, basolateral; DMSO, dimethyl sulphoxide; EDTA, ethylenediamine tetraacetic acid; GC/MS, gas chromatography/mass spectrometry; MEM, minimum essential medium; MRP, multidrug resistance-associated protein; OATP2B1, organic anion transporting polypeptide B; OATPs, organic anion transporting polypeptides; OCTs, Organic cation transporters; PAHs, polycyclic aromatic hydrocarbons; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; P-gp, P-glycoprotein; POPs, persistent organic pollutants; TEER, transepithelial electrical resistance.

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chemicals. Several studies have investigated the transport ratios of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and heavy metals to assess their bioavailability using the Caco-2 cell monolayer. It was also reported that PAHs can affect the gene expression of enzymes and ABC transporters in Caco-2 cells [26]. In addition, it was reported that the transport of PAHs is mediated by BCRP and the aryl hydrocarbon receptor [19,31]. Organic cation transporters (OCTs), a type of influx transporter, are expressed in Caco-2 cells. These can mediate the transport of xenobiotics with relatively low molecular weights and hydrophilic organic cations with diverse molecular structures [24]. However, research on the transepithelial transport mechanism of environmental pollutants and investigations of the roles of the influx and efflux transporters during transport are limited, although there have been many studies on the transepithelial transport mechanism of drugs using the Caco-2 monolayer model.

If there is a clear understanding of the transepithelial transport mechanism of pollutants in the human intestine, those chemicals with low bioavailability and toxicity may receive more attention for application in industrial products. It is necessary to understand the roles the transporters play in the transport process. The present study aimed to determine whether the transporters play an important role during the transport of PBDEs. Therefore, the main objective of this study was to investigate the transepithelial transport mechanism of PBDEs, and study the roles of the efflux transporters (P-gp, MRP, and BCRP) and the influx transporters (OCTs) during the transport process using a Caco-2 cell monolayer.

2. Material and methods

2.1. Caco-2 cell culture

Caco-2 cells, purchased from BioHermes Bio & Medical Technology Co., Inc. (Wuxi, China), were cultured in minimum essential medium (MEM) supplemented with 20% fetal bovine serum and 1% penicillin. The cells were grown in a 5% CO_2 atmosphere at 37 °C under humidified conditions. The culture medium was replaced every 1–2 days. The cells were passaged using 0.25% trypsin and 0.03% ethylenediamine tetraacetic acid (EDTA) until the cell monolayer reached 70–80% confluence. For the PBDE transport experiments, the cells at passages 10–30 were seeded at 2×10^5 cells/cm^2 into a 12-well Transwell insert, and then cultured for 21 days. The culture medium was changed every 2 days. The Caco-2 cell monolayer was used to carry out the PBDE transepithelial transport experiment when the transepithelial electrical resistance (TEER) values were larger than 400 Ω/cm^2 measured with an epithelial voltammeter (EVOM^2, World Precision Instruments, Sarasota, FL, USA).

2.2. MTT test

Cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl) −2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the Caco-2 cells were seeded in 96-well plastic plates at the density of 3000 per well with 100 μL medium, and cultured for 24 h. Then the cells were exposed to targets (DMSO or PBDEs) at given concentrations for 12 h. After the incubation, the medium containing targets was removed, and 100 μL of 0.25% mg·mL^{-1} MTT in medium culture was added. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 4 h. Then, the culture medium was removed, and DMSO (150 μL) was added into each well to dissolve the dark blue crystal. The absorbance was measured at 490 nm using an iMark plate reader (BIO-RAD, USA). The experiments were repeated six times.

2.3. Transepithelial transport experiment

In this experiment, the solvent nonane in the PBDE stock solution was changed to dimethyl sulphoxide (DMSO). Prior to PBDE exposure, the Caco-2 cell monolayer was washed three times with D-Hanks solution. After pre-incubation, the buffer was then gently removed using nitrogen. To investigate the influence of time on transepithelial transport, PBDEs were added into the apical (AP) side or basolateral (BL) side. The volume of medium added to the AP and BL chambers was 0.5 and 1.5 mL, respectively. Following incubation, the solutions from the AP and BL sides were collected and the cells were harvested using 0.25% trypsin and 0.03% EDTA, respectively. PBDEs in the solutions from the AP and BL sides were extracted and determined using gas chromatography/mass spectrometry (GC/MS). For PBDEs in the cells, the harvested cells were disrupted using cell breaking apparatus, and then extracted as described previously. To investigate the influence of PBDE concentrations on transport, four concentrations of PBDE (2.5, 5, 10, and 20 ng/mL) in the medium were used. Various PBDE exposure times (1, 2, 4, 8, 12, and 24 h) at the PBDE concentration of 10 ng/mL were used to study the time effects. In subsequent experiments, the PBDE concentration was set at 10 ng/mL, and the PBDE exposure time was 12 h. The influence of pH on the transport of PBDEs in both directions, i.e., AP to BL and BL to AP, was determined using the following paired pH values in the AP/BL compartments, i.e., 5.5/7.4 and 7.4/7.4. The experiment was carried out at two temperatures (37 and 4 °C) to investigate the influence of energy. To determine the roles of P-gp, MRP, BCRP, and OCTs during transport, 100 μM verapamil [6], 100 μM MK571 [52], 10 μM Ko143 [12], and 50 μM cimetidine [2] were used as inhibitors, respectively. We found that concentrations of DMSO below 0.2% had no significant effect on the viability of Caco-2 cells (data not shown). Therefore the concentrations of DMSO used in the present study were below 0.2%.

2.4. Analytical protocol

The methods used for sample extraction and cleanup were similar to those reported in our previous study [55]. After spiking with the surrogate standard ^{13}C-PCB141, solutions from the AP and BL sides were extracted three times with acetone and a mixed solution of *n*-hexane and dichloromethane. The obtained organic solutions were passed through a silica-alumina column to remove impurities. The mixed solvent of dichloromethane and *n*-hexane (v/v=1:1) was used as the mobile phase. The eluents containing PBDEs were collected and concentrated. Finally, the internal standard ^{13}C-PCB208 was added. The samples were stored in 50 μL *n*-octane at −20 °C until GC/MS analysis. For PBDEs in the cells, following ultrasonic decomposition of the cells, the mixtures including cells were extracted and cleaned up as mentioned above.

2.5. Instrumental analysis

The PBDE concentrations were determined using a Hewlett-Packard (HP) 6890N gas chromatograph coupled with a 5975 mass spectrometer. Negative chemical ionization mode was used. Splitless injection of a 1-μL sample was performed. The temperature of the injector and ion source were set at 280 and 250 °C, respectively. Quantification of tri- to hepta-BDE congeners was achieved using a DB-5MS capillary column (12 m×0.25 mm×0.1 μm, J & W Scientific, USA) with no interfering peaks. The selective ion monitoring mode was selected, and the ions *m/z*=79/81 were monitored for tri- to hepta-BDE congeners, 476/478 for ^{13}C-PCB208, and 372/374 for ^{13}C-PCB141.

2.6. Quality assurance/quality control

For each batch of seven samples, a procedural blank was processed to monitor interfering peaks. BDE209 was observed in some of the blank samples. Therefore, BDE209 is not discussed in the present study. Each experiment was carried out three to five times. Calibration