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Effects of perfluoroalkyl carboxylic acids on the uptake of sulfobromophthalein via organic anion transporting polypeptides in human intestinal Caco-2 cells

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ABSTRACT

We performed a detailed investigation of the uptake of sulfobromophthalein (BSP) from the apical membrane of Caco-2 cells, which is a substrate for organic anion transporting polypeptides (OATPs), and calculated the kinetic parameters of BSP uptake as follows: $K_m = 13.9 \pm 1.3$ μM, $V_{max} = 1.15 \pm 0.07$ nmol (mg protein)$^{-1}$ (5 min)$^{-1}$, and $k_d = 38.2 \pm 0.53$ μL (mg protein)$^{-1}$ (5 min)$^{-1}$. Coincubation with medium-chain (C7–C11) perfluoroalkyl carboxylic acids (PFCAs), such as perfluoroheptanoic acid (PFHpA, C7), perfluorooctanoic acid (PFOA, C8), perfluorononanoic acid (PFNA, C9), perfluorodecanoic acid (PFDA, C10) and perfluoroundecanoic acid (PFUnDA, C11), significantly decreased BSP uptake by 27–55%, while coincubation with short- (C3–C6) and long-chain (C12–C14) PFCAs decreased the uptake only slightly. Dixon plotting suggested that PFOA, PFNA and PFDA competitively inhibited the BSP uptake with inhibition constant (K_i) values of 62.2 ± 1.3 μM, 35.3 ± 0.1 μM and 43.2 ± 0.3 μM, respectively. PFCAs with medium-chains could be substrates for OATPs, probably OATP2B1, which is the most abundantly expressed OATP isoform in Caco-2 cells.

1. Introduction

Human organic anion transporting polypeptides (OATPs) are sodium ion-independent transporters expressed in a number of tissues, including the brain, liver, lungs, kidneys, testes and intestines [1]. OATPs are known to mediate the cellular uptake of various endogenous and exogenous amphiphilic organic compounds in those organs [1,2]. OATP2B1 is localized at the brush border membrane of the human intestinal epithelium and the apical membrane of Caco-2 cells [3,4], with the amount of OATP2B1 expressed in Caco-2 cells higher than that of other OATP isoforms such as OATP1A2, OATP3A1, OATP4A1 and OATP1B3 [4–8]. Caco-2 cells could, therefore, provide a useful in vitro model with which to investigate the intestinal absorption of drugs via OATP2B1.

Sulfobromophthalein (BSP) is a well-known dye used for liver function tests, and has been identified as a substrate for OATP family such as OATP1A2, OATP1B1, OATP1B3, and OATP2B1 [1,9]. The recent study suggests that BSP uptake in Caco-2 cells occurs via OATPs, probably via OATP2B1 [10]. However, this BSP uptake in Caco-2 cells via OATPs has not yet been investigated in detail.

Perfluoroalkyl carboxylic acids (PFCAs) are a family of fluorine-containing compounds with fully fluorinated carbon chains. PFCAs have been widely used as surface tension depressants in the manufacturing industry as they possess both a hydrophilic region (carboxylic group) and a hydrophobic carbon chain. They are very stable against metabolic and environmental degradation, and are frequently detected in human serum from the general population in various countries [11–14]. Human exposure occurs via food and drink. Among PFCAs, perfluorooctanoic acid (PFOA, C8) is a common environmental contaminant. Animal experiments indicated that PFOA was readily absorbed after oral administration, and was distributed mainly in the liver and plasma, followed by the kidneys, without metabolism [12,15]. PFOA is excreted in the urine and the feces [15], and appears to undergo enterohepatic circulation [16]. Many reports imply that PFCAs

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absorption, distribution and excretion could occur via the OATP family [17–20]. We recently suggested that PFOA uptake in Caco-2 cells is mediated via the OATP family, probably OATP2B1, as the relationship between the initial uptake of PFOA and its concentration (1–500 μM) was saturable and Lineweaver-Burk plots indicated the competitive inhibition of BSP: K_m and V_{max} were 8.3 μM and 457 pmol/mg protein/min, respectively [10]. However, the uptake mechanisms of other PFCAs with carbon chains of different lengths have not yet been investigated in Caco-2 cells.

In this study, we undertook a detailed investigation of the uptake of BSP from the apical membrane of Caco-2 cells which is a substrate for OATPs, and calculated the following kinetic parameters: Michaelis-Menten constant (K_m), maximum uptake rate for a carrier-mediated process (V_{max}) and coefficient of passive diffusion (k_d). Next, we compared the inhibitory effect of PFCAs with carbon chains of different lengths (C3 to C14) on BSP uptake in Caco-2 cells, and found that medium-chain PFCAs (C7 to C11) have a significant inhibitory effect on the BSP uptake.

2. Materials and methods

2.1. Materials

Perfluorooctanoic acid (PFOA, C8), perfluorononanoic acid (PFNA, C9), perfluorodecanoic acid (PFDA, C10), perfluoroundecanoic acid (PFUnDA, C11), and perfluorododecanoic acid (PFDoDA, C12), perfluorotetradecanoic acid (PFTeDA, C14), cyclosporin A, sodium azide (NaN_3), 2,4-dinitrophenol (DNP), Dulbecco's modified Eagle's medium (DMEM), tetrabutylammonium hydrogen sulfate and benzyl bromide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Perfluoropropionic acid (PFPrA, C3), perfluorobutanoic acid (PFBA, C4), perfluoropentanoic acid (PFPeA, C5), Perfluorohexanoic acid (PFHxA, C6), perfluoroheptanoic acid (PFHpA, C7), HPLC grade methanol and methyl *tert*-butyl ether (MTBE) were obtained from Kanto Chemicals Co., Ltd. (Tokyo, Japan). Perfluorotridecanoic acid (PFTrDA, C13), sulfobromophthalein (BSP) and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS) and nonessential amino acid (NEAA) were obtained from Life Technologies Co. (Carlsbad, CA). A mixture of $^{13}C_2$-labeled PFHxA, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled PFDoDA were purchased from Wellington Laboratories Inc. (MPFAX-MXA; Guelph, Canada). All other chemicals used were of the highest purity commercially available.

2.2. Culture of Caco-2 cells

Caco-2 cells at passage 46 were obtained from the RIKEN Cell Bank (Tsukuba, Japan). As described previously [10,21], the cells were cultured on the 35-mm six-well culture dishes coated with rat tail collagen type I (Corning Incorporated, Tewksbury, MA) at a density of 5×10^4 cells/well, and maintained in DMEM containing 10% FBS, 100 μg/mL streptomycin and 100 U/mL penicillin G, 1% NEAA at 37 °C in a humidified atmosphere of 5% CO_2-95% air. The cells used were between passage 54 and 70. The culture medium was replaced three times a week, and the cells were sub-cultured once a week. Confluent Caco-2 cell monolayers, cultured for 2 weeks, were used in this study.

2.3. Cellular uptake by Caco-2 cells

The uptake experiment was performed as described previously [10,21]. The incubation medium for the uptake study was Hanks' balanced salt solution containing 25 mM D-glucose and 10 mM MES (at pH 5.0 or 6.0) or 10 mM HEPES (at pH 7.4 or 8.5). The culture medium was removed, and the cells were preincubated at 37 °C or 4 °C for 20 min in 1.5 mL of the incubation medium (pH 7.4). After preincubation, the medium was aspirated, and the cells were incubated with 1.5 mL of fresh incubation medium containing BSP or PFCAs for the designated times at the same temperature as preincubation [10].

To examine whether the uptake of BSP was sodium-dependent, NaCl in the incubation medium was replaced with equimolar KCl or choline chloride, and Na_2HPO_4 was omitted from the medium [10,21]. BSP, PFCAs and a number of other compounds were dissolved in methanol or dimethyl sulfoxide, and added to the incubation medium at a final vehicle concentration of 0.5% or lower.

At the designated time, the uptake medium was aspirated, and the cell surface was quickly washed three times with ice-cold incubation medium at pH 7.4. The cells were then suspended in 1.0 mL of extraction solution (0.03 M phosphate buffer at pH 7.0 : methanol = 1 : 1) for 60 min at room temperature, and the cells were scraped off and collected using a cell scraper [10,21]. The suspension was centrifuged at 13,000*g* for 10 min, and the supernatant fraction was applied to the determination of BSP and PFCAs.

2.4. Determination of BSP, PFCAs and protein

Determination of BSP in the supernatant fraction was carried out by HPLC as described previously [10]. The calibration curve of the BSP used was linear over the concentration range of 0.05–1.0 nmol/mL ($r = 0.99$) and the coefficient of variance was below 4%.

Determination of PFCAs was carried out by gas chromatography-mass spectrometry (GC-MS, Agilent 6890GC/5973inertMSD) with electron-capture negative ionization based on the method used in previous studies [10,22–24]. Briefly, 10 μL of the supernatant fraction, 0.5 ng of $^{13}C_2$-labeled PFHxA, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled PFDoDA were added to a vial and dried. The dried sample was redissolved in 100 μL of a 0.1 mol L^{-1} benzyl bromide/MTBE solution and derivatized to benzyl esters. Derivatized products were analyzed by GC-MS in selected ion monitoring mode. The calibration curve (10, 1, 0.5, 0.1, and 0.01 ng/mL of standard PFCA solutions) was linear and characterized by good correlation coefficients ($r > 0.99$).

The protein concentration was determined using a Bio-rad dye reagent (Richmond, CA) with bovine serum albumin as the standard.

2.5. Kinetic analysis of BSP uptake

According to previous reports [10,21], the rate of BSP uptake by Caco-2 cells (V) could be fitted to the following equation.

$$V = V_{max}[S] / (K_m + [S]) + kd[S] \quad (1)$$

where V_{max} is the maximum uptake rate for a carrier-mediated process, [S] is the substrate concentration in the medium, K_m is the half-saturation concentration (Michaelis-Menten constant), and k_d is a coefficient of passive diffusion. The parameters V_{max}, K_m and k_d were calculated using the Kaleida Graph program (HULINKS Inc., Japan) [10].

2.6. Statistical analyses

The data were analyzed by either Student's *t*-test or Scheffe's multiple comparison test after the analysis of variance using the Statcel 3 program, and differences with values of $p < 0.05$ were considered to be significant. Data are shown as the mean ± S.E. from at least two separate experiments.

3. Results

3.1. Uptake of BSP by Caco-2 cells

Caco-2 cells were incubated with 5 μM BSP at pH 6.0 for up to 60 min (Fig. 1). The uptake of BSP increased linearly until 10 min and then

plateaued at about 15 min.

The initial uptake of BSP from incubation media containing different concentrations of BSP at pH 6.0 for 5 min was investigated (Fig. 2). The relationship between the initial uptake of BSP and the concentration of BSP (0.5–100 μM) was non-linear. These results suggested that the uptake of BSP involves a saturable process at low concentrations and a non-saturable process at high concentrations. The kinetic parameters calculated for BSP uptake were as follows: $K_m = 13.9 \pm 1.3$ μM, $V_{max} = 1.15 \pm 0.07$ nmol (mg protein)$^{-1}$ (5 min)$^{-1}$, and $k_d = 38.2 \pm 0.53$ μL (mg protein)$^{-1}$ (5 min)$^{-1}$. Uptake clearance (V_{max}/K_m) was 82.6 μL/mg protein/5 min (16.5 μL/mg protein/min). The ratio of uptake clearance to k_d was 2.2, suggesting 2.2-fold higher uptake of BSP via carrier mediated process than via simple diffusion [10,25].

3.2. Effects of pH, temperature, sodium ions and various compounds on the uptake of BSP by Caco-2 cells

The effects of pH, low temperature, sodium ions and various compounds on the uptake of BSP by Caco-2 cells were investigated (Table 1). Incubation at low temperature (4 °C) significantly reduced the uptake of BSP at pH 6.0 by 50%. Coincubation with cyclosporin A (OATP inhibitor) and glibenclamide (OATP substrate) significantly decreased the uptake of BSP by 51 and 48%, respectively. Furthermore, coincubation with PFOS (perfluorooctane sulfonate) significantly decreased the uptake of BSP by 54%. In contrast, the uptake of BSP by Caco-2 cells was not affected by extracellular pH at 5.0, 7.4 or 8.5. Sodium ion-free conditions, achieved by the replacement of NaCl in the incubation medium with KCl or choline chloride, did not affect the uptake of BSP. Further, pretreatment with metabolic inhibitors, NaN_3 and DNP, also had no effect on BSP uptake.

3.3. Effects of PFCA carbon chain length on BSP uptake

The effects of the length of the PFCA carbon chain (C3 to C14) on the uptake of BSP by Caco-2 cells were investigated (Fig. 3). Coincubation with medium-chain PFCAs, such as PFHpA (C7), PFOA (C8), PFNA (C9), PFDA (C10) and PFUnDA (C11), significantly decreased the uptake of BSP by 27–55%, while that with short- (C3–C6) and long-chain (C12–C14) PFCAs decreased the uptake only slightly. No morphologic changes due to incubation with BSP and PFCAs for 5 min were detected under microscope observation.

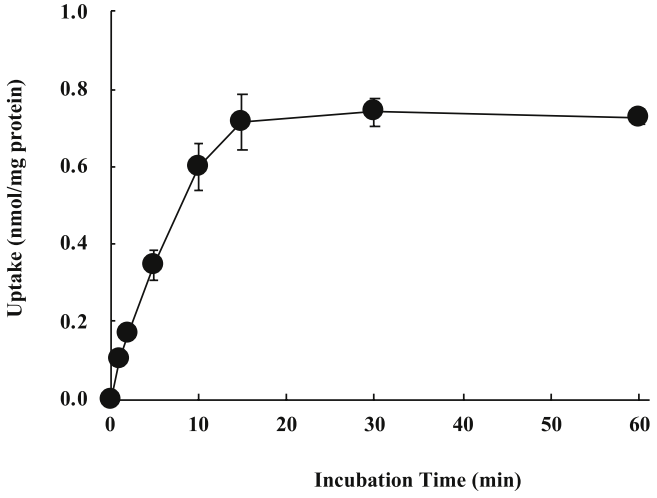

Fig. 1. Time course of the uptake of BSP by Caco-2 cells. Caco-2 cells were incubated with 5 μM BSP at 37 °C and pH 6.0. Each point represents the mean ± S.E. of 4–6 determinations.

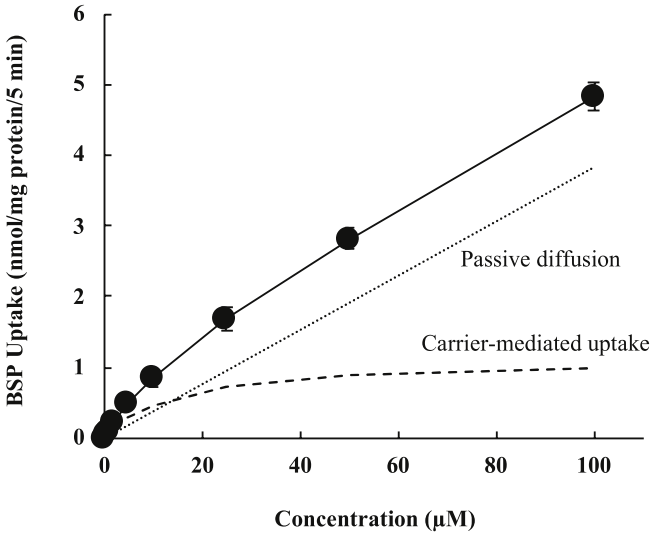

Fig. 2. Concentration-dependent uptake of BSP by Caco-2 cells. Caco-2 cells were incubated for 5 min with different concentration of BSP at 37 °C and pH 6.0. Each point represents the mean ± S.E. of 6–8 determinations.

Table 1
Effect of pH, temperature, sodium ions, metabolic inhibitors and various compounds on the uptake of BSP by Caco-2 cells.

Compound	Number of determinations	Relative uptake rate (% of control)
Control (37 °C and pH 6.0)	5	100 ± 3.0
37 °C and pH 5.0	3	101.7 ± 5.1
37 °C and pH 7.4	3	105.2 ± 5.3
37 °C and pH 8.5	3	101.6 ± 1.3
4 °C and pH 6.0	6	49.9 ± 3.1[d]
Na^+-free (KCl)[a]	5	95.0 ± 7.4
(Choline chloride)[a]	6	81.0 ± 4.0
NaN_3 (10 mM)[b]	5	106.4 ± 6.8
DNP (1 mM)[b]	5	105.9 ± 10.0
Cyclosporin A (100 μM)[c]	6	48.9 ± 4.5[d]
Glibenclamide (100 μM)[c]	5	52.2 ± 3.5[d]
PFOS (100 μM)[c]	5	46.1 ± 4.5[d]

Caco-2 cells were incubated with 5 μM BSP at 37 °C and pH 6.0 (control), or at 37 °C and pH 5.0, 7.4 or 8.5, or at 4 °C and pH 6.0 for 5 min.
Each point represents the mean ± S.E. of 3–6 determinations.
[a] Caco-2 cells were incubated with 5 μM BSP at 37 °C for 5 min in a Na^+-free medium.
[b] Caco-2 cells were treated with 10 mM NaN_3 or 1 mM DNP for 20 min, and incubated with 5 μM BSP at 37 °C for 5 min.
[c] Caco-2 cells were coincubated with 5 μM BSP and other compounds at 37 °C for 5 min.
[d] Significantly different from the control.

3.4. Competitive inhibition of PFOA, PFNA and PFDA on the uptake of BSP by Caco-2 cells

The mode of the inhibitory effects of PFOA (C8), PFNA (C9) and PFDA (C10) on the uptake of BSP by Caco-2 cells was investigated using Dixon plot analyses (Fig. 4). Caco-2 cells were incubated for 5 min at 37 °C with 2, 5 or 10 μM BSP in the absence or presence of those PFCAs (25, 50 or 100 μM). PFOA, PFNA and PFDA competitively inhibited the BSP uptake with inhibition constant (K_i) values of 62.2 ± 1.3 μM, 35.3 ± 0.1 μM and 43.2 ± 0.3 μM, respectively.

3.5. Comparison of the uptake of PFCAs by Caco-2 cells

The uptake of various PFCAs (C6–C12) by Caco-2 cells was

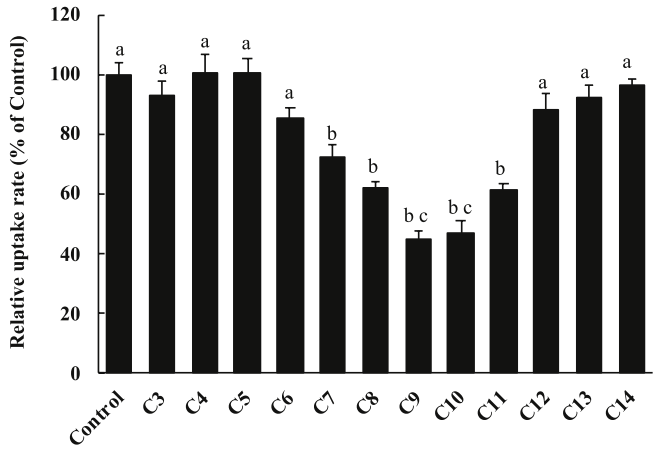

Fig. 3. Effect of various perfluoroalkyl carboxylic acids on the uptake of BSP by Caco-2 cells. Caco-2 cells were coincubated with 5 μM BSP and 100 μM perfluoroalkyl carboxylic acids (C3–C14) at 37 °C for 5 min. Each point represents the mean ± S.E. of 4–6 determinations. Significantly differences are indicated by different letters.

compared. Caco-2 cells were incubated with 1 μM PFHxA (C6), PFHpA (C7), PFOA (C8), PFNA (C9), PFDA (C10), PFUnDA (C11) and PFDoDA (C12) at pH 6.0 for 5 min (Fig. 5). The uptake of PFNA (C9) and PFDA (C10) was the highest and the second highest, respectively, followed by PFOA (C8), PFUnDA (C11), PFHpA (C7) and PFDoDA (C12), while the uptake of PFHxA (C6) was the lowest.

4. Discussion

Coincubation with cyclosporin A and glibenclamide (OATPs inhibitor and substrate), significantly decreased the uptake of BSP by 51 and 48%, respectively (Table 1), and coincubation with rifamycin SV and estrone-3-sulfate (OATPs inhibitor and/or substrate) significantly decreased the uptake of BSP in Caco-2 cells by 40 and 39%, respectively [10]. In agreement with these findings, the contributions of the carrier-mediated uptake and the uptake via simple diffusion to BSP uptake by Caco-2 cells were approximately 70 and 30%, respectively, as the ratio of uptake clearance (V_{max}/K_m) was about 2.2-fold greater than the non-saturable uptake clearance (k_d). To our knowledge, we are the first to report the kinetic data for BSP uptake in Caco-2 cells. As the amount of OATP2B1 expressed in Caco-2 cells appear to be higher than that of any other OATP isoform [4–8], it is thought that OATP2B1 is the main contributor to the carrier-mediated uptake of BSP in Caco-2 cells. Unfortunately, up to date, no specific inhibitor (substrate) for each OATP isomer has yet been reported.

The K_m value for BSP, as calculated from the Caco-2 cells (Fig. 2), was 13.9 μM, which is similar to the K_m values for estrone-3-sulfate (an OATPs substrate) in Caco-2 cells reported previously (1.8 μM, Sai et al. [4]; 6 μM, Kis et al. [26]; 23 μM, Gram et al. [27]). Similar K_m values for BSP uptake in Caco-2 cells were reported in other type of cells such as OATP2B1-transfected HEK293 cells (5.4 μM, Leonhardt et al. [28]) and ABCC2/OATP2B1 double-transfected MDCKII cells (3.4 μM, Kopplow et al. [29]). In addition, the uptake clearance of BSP (V_{max}/K_m) calculated from the present results of Caco-2 cells (16.5 μL/mg protein/min) is similar to that previously reported for estrone-3-sulfate in Caco-2 cells at a high-affinity site previously reported (Sai et al. [4]; 37 μL/mg protein/min).

BSP uptake in Caco-2 cells was sodium ion-independent and pH-independent (Table 1). In agreement, OATP2B1 activity is reported to be sodium ion-independent [1,2]. However, the pH-dependence of OATP2B1 activity is controversial: Visentin et al. reported pH-independent BSP uptake in OATP2B1-transfected cells [30], while several studies have shown that OATP2B1 activity is enhanced at a lower extracellular pH levels [3,4,31].

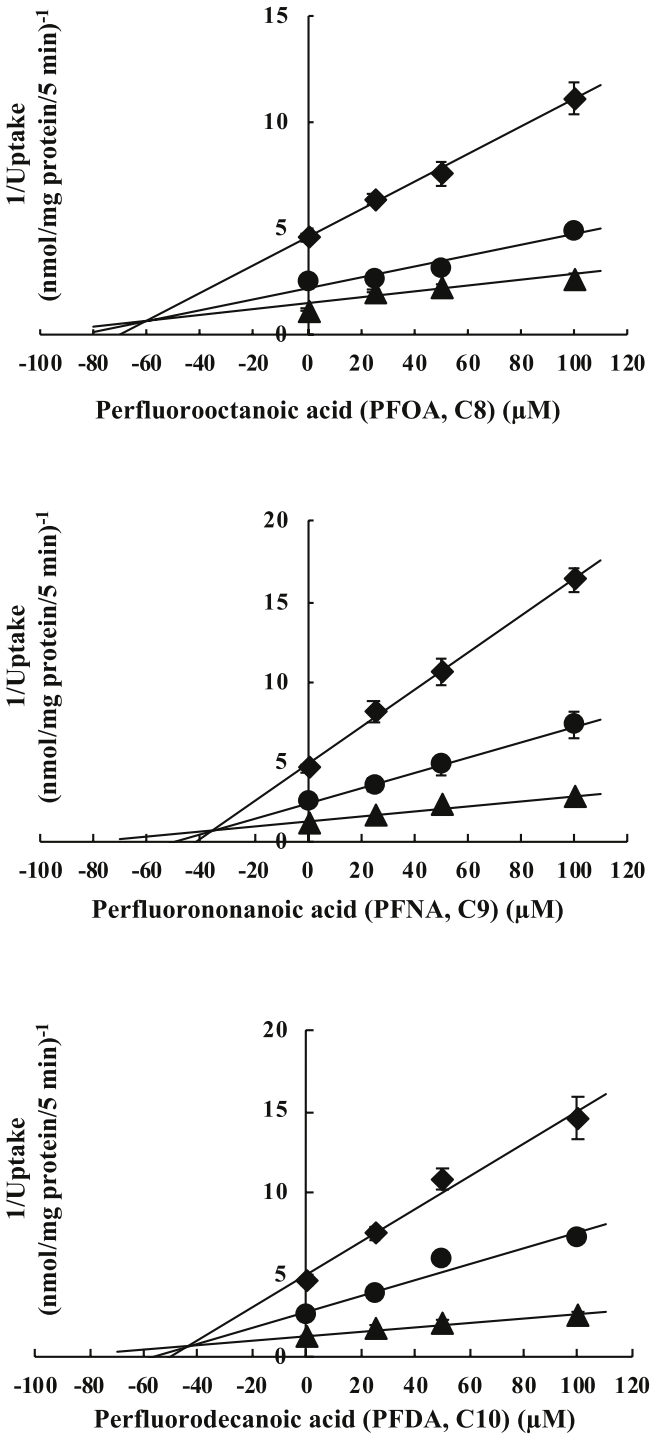

Fig. 4. Dixon plots of BSP uptake into Caco-2 cells. Uptake of BSP for 5 min at pH 6.0 from the medium containing 2, 5 or 10 μM in the absence or presence of perfluorooctanoic acid (PFOA, C8), perfluorononanoic acid (PFNA, C9) or perfluorodecanoic acid (PFDA, C10) (25, 50 or 100 μM) was measured. Each point represents the mean ± S.E. of 4–6 determinations. Diamonds: 2 μM BSP; circles: 5 μM BSP; triangles: 10 μM BSP.

Coincubation with medium-chain PFCAs (C7–C11) significantly decreased BSP uptake, while those with short- and long-chains induced only slight decreases (Fig. 3). Among the medium-chain PFCAs (C7–C11), the inhibitory effects of PFNA (C9) and PFDA (C10) on BSP uptake were higher than those of PFHpA (C7), PFOA (C8) and PFUnDA (C11). Dixon plotting (Fig. 4) revealed that PFOA (C8), PFNA (C9) and

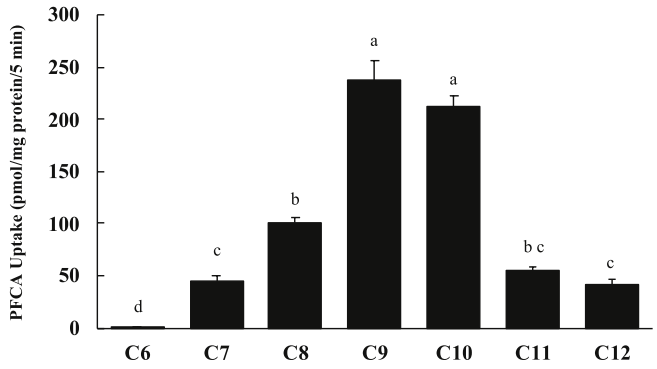

Fig. 5. Uptake of perfluoroalkyl carboxylic acids by Caco-2 cells. Caco-2 cells were incubated with 1 μM perfluoroalkyl carboxylic acids (C6–C12) at 37 °C for 5 min. Each point represents the mean ± S.E. of 6 or 7 determinations. Significantly differences are indicated by different letters.

PFDA (C10) competitively inhibit BSP uptake, and the K_i values of PFNA (C9) (35.3 μM) and PFDA (C10) (43.2 μM) were lower than that of PFOA (C8) (62.2 μM), which is in agreement with the order of the inhibitory effects of those PFCAs (Fig. 3). Similar inhibitory trends of PFCAs with carbon chains of different lengths were previously reported on the uptake of estrone-3-sulfate (OATPs substrate) by Oatp1a1-expressing CHO cells [17] and OATP1A2-expressing HEK293 cells [32] and on the uptake of estradiol-17β-glucuronide (OATPs substrate) by Oatp1a1-expressing CHO cells [18]. However, they did not confirm whether PFCAs competitively inhibit the uptake of estrone-3-sulfate and estradiol-17β-glucuronide.

The uptake levels of PFNA (C9), PFDA (C10) and PFOA (C8) by Caco-2 cells were the highest, second highest and third highest, respectively, and that of PFHxA (C6) was the lowest among the PFCAs tested (C6–C12) (Fig. 5). The uptake of PFOA (C8), PFNA (C9) and PFDA (C10) in Caco-2 cells could be mediated via OATPs, probably via OATP2B1, as these PFCAs (C8, C9 and C10) competitively inhibited the BSP uptake (Fig. 4). The order of PFCA uptake (Fig. 5) was in agreement with the order of the inhibitory effects of those compounds on BSP uptake (Fig. 3). Previous studies using Oatp1a1 and OATP1A2 transfected cells [17,32] reported the inhibitory effects of PFCAs on the uptake of estrone-3-sulfate (OATPs substrate), and indicated a similar trend of inhibitory effect shown in Fig. 3. Similar orders of PFCAs uptake and its inhibitory effects were found in the in vivo experiment of AUC after the oral administration of PFCAs (C7 to C14) to mice [33]: C9 > C8 > C10 > C11 > C7 > C12 > C13 > C14.

Zhao et al. recently quantified the uptake of PFHpA (C7), PFOA (C8), PFNA (C9) and PFDA (C10) in OATP1B1-, OATP1B3- and OATP2B1-transfected HEK293 cells and non-transfected HEK293 cells [20], and reported that (I) the uptake of PFOA (C8), PFNA (C9) and PFDA (C10) in each of the transfected cells was higher than that in non-transfected cells, but the uptake of PFHpA (C7) in each of the transfected cells was similar to that in non-transfected cells; and (II) the uptake of PFNA (C9) in each of the transfected cells was the highest among the four compounds. Their results suggest that the uptake of PFOA (C8), PFNA (C9) and PFDA (C10) are well mediated via OATP1B1, OATP1B3 and OATP2B1, with the affinity of PFNA (C9) for those OATPs likely to be the highest. OATPs, probably OATP2B1, may contribute the readily absorption after the oral administration of PFOA [12,15] and the medium-chain PFCAs (C8–C10) [33]. Further study is necessary to confirm the uptake of PFCAs and BSP using OATP2B1 transfected cells.

Some previous reports suggested that the concentration of PFCAs in environmental water (e.g., seawater) and that in organisms (e.g., marine mammals and fish) do not correspond with each other. For example, in seawater around Japan, PFCA concentrations were observed in the order of C8 (100 pg/L) > C9 (66 pg/L) > C10 (less than 35 pg/L) > C11 (less than 21 pg/L) [34]. On the other hand, PFCAs in the blood of Dall's porpoises around Japan were observed in the order of C11 (30 ng/L) > C10 (6.5 ng/L) > C9 (3.3 ng/L) > C8 (0.5 ng/L), with the order reversed [35]. In the present study, the highest level of uptake by Caco-2 cell was found in C9 among PFCAs (C6 to C12) (Fig. 5). This result is consistent with those of a previous animal study in which the level of C9 in the mouse serum was the highest among PFCAs (C6–C14), when equimolar amounts of each were orally administrated [33]. The absorption rates of PFCAs may depend on the chain length and the affinity of each PFCA for OATPs.

In conclusion, we presented the kinetic parameters for BSP uptake from the apical membrane of Caco-2 cells, and suggested that BSP uptake occurs via OATPs, probably OATP2B1. Medium-chain PFCAs competitively inhibited BSP uptake in Caco-2 cells, while those with short and long chains did not. More recent studies have revealed that OATP2B1 is localized not only in the apical membrane but also in the basolateral membrane of Caco-2 cells [36]. Further study is necessary to investigate the contribution of OATP2B1 located in the basolateral membrane in the BSP uptake.

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CRediT authorship contribution statement

Osamu Kimura: Conceptualization, Investigation, Methodology, Formal analysis, Writing - Original Draft. Yukiko Fujii: Investigation, Resources, Writing - Review & Editing. Koichi Haraguchi: Resources, Writing - Review & Editing. Yoshihisa Kato: Resources, Writing - Review & Editing. Chiho Ohta: Resources, Writing - Review & Editing. Nobuyuki Koga: Resources, Writing - Review & Editing. Tetsuya Endo: Writing - Review & Editing, Supervision.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Transparency document

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