Oral Availability of Cefadroxil Depends on ABCC3 and ABCC4

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ABSTRACT:

Some cephalosporins, such as cefadroxil, are orally available. H⁺-coupled peptide transporter 1 mediates the transport of cephalosporins across the apical membrane of enterocytes. It is not known which mechanism(s) is responsible for the subsequent transport of cephalosporins across the basolateral membrane toward the circulation. We have tested whether ATP-binding cassette (ABC) transporters ABCC3 and/or ABCC4 are involved in the latter process. Transport experiments with plasma membrane vesicles expressing these transporters were involved (Nakashima et al., 1984). Likewise, cephalosporins cannot be transported into the basolateral membrane, with a higher expression apically (Li et al., 2008). It is not exactly clear where ABCC4 resides in the intestine: in the colonic cell line HT29-CL19A the protein was localized to both the apical and basolateral membrane, but in renal proximal tubule cells, ABCC4 resides at the apical membrane (Russel et al., 2008). The subcellular localization of ABCC4 is cell type dependent. In prostate tubuloacinar cells, hepatocytes, and choroid plexus epithelium, ABCC4 is localized on the basolateral membrane, but in renal proximal tubule cells, ABCC4 resides at the apical membrane (Russel et al., 2008). It is not exactly clear where ABCC4 resides in the intestine: in the colonic cell line HT29-CL19A the protein was localized to both the apical and basolateral membrane, with a higher expression apically (Li et al., 2007). However, data from Caco2 cells show preferential basolateral expression (Ming and Thakker, 2010). In the present study, we explored whether ABCC3 and/or ABCC4 are able to transport cefadroxil in plasma membrane vesicles from cells overexpressing the corresponding genes. In addition, we used Ussing chamber experiments and intestinal uptake experiments with wild-type, Abcc3−/−, Abcc4−/−, and Abcc3−/−/Abcc4−/− mice.

ABC-3- and ABC-4-mediated transport of estradiol-17β-glucuronide was dose-dependently inhibited by cephalosporins in vesicular transport experiments. Furthermore, transport of cefadroxil by ABCC3 and ABCC4 was saturable with Km values of 2.5 ± 0.7 and 0.25 ± 0.07 mM, respectively. Transport of cefadroxil from the apical to the basolateral side of jejunal tissue explants was unchanged in Abcc3−/− but significantly reduced (approximately 2-fold) in Abcc4−/− and Abcc3−/−/Abcc4−/− mice compared with wild-type tissue. Upon instillation of cefadroxil in the jejunum, portal and peripheral blood concentrations were similar in Abcc3−/− and Abcc4−/− but approximately 2-fold reduced in Abcc3−/−/Abcc4−/− compared with wild-type mice. Our data demonstrate that intestinal absorption of cefadroxil depends partly on ABCC3 and ABCC4.

Introduction

Infectious diseases are often treated with cephalosporins (Livermore, 2009), which belong to the class of β-lactam antibiotics. Because of antibiotic resistance, there is a continuous need for the development of new cephalosporins (Livermore, 2009). Therefore, knowledge of their pharmacokinetics is of eminent interest. One way of classifying cephalosporins is the route of administration: topical, oral, or parenteral. Oral administration is the most uncomplicated form of administration but requires passage through the enterocytes. Because it is known that cephalosporins are ionized at physiological pH and have very low lipid solubility, luminal enterocytic uptake was considered to be transporter mediated (Tsui et al., 1981). The H⁺-coupled peptide transporter 1 (PepT1) was identified as the transporter involved (Nakashima et al., 1984). Likewise, cephalosporins cannot passively cross the basolateral membrane, implicating that transport over the basolateral membrane must also be transporter mediated. A transporter known to mediate transport of cephalosporins (such as ceftriaxone, cefaperazone, and cephalixin) is the efflux pump multi-drug resistance-associated protein 2 (Mrp2/Abcc2) (Oude Elferink and Jansen, 1994; Kato et al., 2008). ATP-binding cassette (ABC) transporter ABCB2 is a broad-spectrum transporter and can transport many different substrates, including unconjugated anions, glucuronate, glutathione, and sulfate conjugates and, among others, leukotriene C4 and methotrexate (Oude Elferink and de Waart, 2007). ABCB2 has overlapping substrate specificity with its close homologs ABCB3 and ABCB4 (Borst et al., 2007; Rius et al., 2008). Whereas ABCB2 resides in the apical membrane of enterocytes (Mottino et al., 2000), ABCB3 resides in the basolateral membrane of epithelial cells, including enterocytes (Scheffer et al., 2002). The subcellular localization of ABCB4 is cell type dependent. In prostate tubuloacinar cells, hepatocytes, and choroid plexus epithelium, ABCB4 is localized on the basolateral membrane, but in renal proximal tubule cells, ABCB4 resides at the apical membrane (Russel et al., 2008). It is not exactly clear where ABCB4 resides in the intestine: in the colonic cell line HT29-CL19A the protein was localized to both the apical and basolateral membrane, with a higher expression apically (Li et al., 2007). However, data from Caco2 cells show preferential basolateral expression (Ming and Thakker, 2010). In the present study, we explored whether ABCB3 and/or ABCB4 are able to transport cefadroxil in plasma membrane vesicles from cells overexpressing the corresponding genes. In addition, we used Ussing chamber experiments and intestinal uptake experiments with wild-type, 515

ABBREVIATIONS: PepT1, H⁺-coupled peptide transporter 1; E17βG, estradiol-17β-glucuronide; HPLC, high-performance liquid chromatography; Sf21, Spodoptera frugiperda 21; Mrp, multidrug resistance-associated protein.
Abcc3(−/−), Abcc4(−/−), and Abcc3(−/−)/Abcc4(−/−) mice to test whether Abcc3 and/or Abcc4 are involved in the basolateral transport of cefadroxil in vivo.

Materials and Methods

Materials. Acetonitrile [high-performance liquid chromatography (HPLC) grade] was from Mallinkrodt Baker, Inc. (Phillipsburg, NJ). [1H]Estradiol-17β-glucuronide ([1H]E217βG) was from GE Healthcare (Chalfont St. Giles, Buckinghamshire, UK). All other chemicals and reagents were from Sigma-Aldrich (St. Louis, MO).

Animals. All animal experiments were approved by the Institutional Animal Care and Use Committee of the Academic Medical Center. Wild-type and Abcc3(−/−) mice were bred as wild-type littermates. Inbred 129/SvEvTac B6C3F1 mice, generated by crossing Abcc3(−/−) mice with wild-type littermates, were used for transfection experiments. Dose–response experiments were performed with animals at 9 to 16 weeks old. All animals were on a 99% FVB/N background and kept in a temperature-controlled environment with a 12-h light/dark cycle. Standard diet (CRM; SDS, Witham, Essex, UK) and water were available ad libitum.

Methods. Preparation of membrane vesicles. The cytosol of the ABCC2 gene (Paulusma et al., 1997) was cloned into the pFastBac1 (Life Technologies, Breda, The Netherlands) donor plasmid. This was followed by transfection of the donor plasmid into DH10Bac (Invitrogen, Carlsbad, CA) cells, which allowed for transposition of the ABCC2 gene into bacmid DNA.

ABCG2, ABCC1, ABCC3, and ABCC4 recombinant baculoviruses were a kind gift from Prof. P. Borst, Netherlands Cancer Institute, Amsterdam, The Netherlands (Bakos et al., 2000; Zelcer et al., 2001; van Aubel et al., 2002; Breedveld et al., 2004). Spodoptera frugiperda 21 (516 DE WAART ET AL.) cells grown at 27°C were infected with ABCB1, ABCB2, ABCB3, ABCB4, or ABCC2 recombinant baculovirus. Isolation of membrane vesicles was as described previously (de Waart et al., 2006). In brief, cells were harvested 2 days (ABCC3), 3 days (ABCC1, ABCB2, and ABCC2), or 4 days (ABCC4), respectively, after infection, resuspended in ice-cold hypotonic buffer (250 mM mannitol/20 mM HEPES/Tris-buffered, pH 7.4/2 mM EDTA) supplemented with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin), and incubated for 30 to 60 min on ice. Cells were homogenized using a tight-fitting Dounce homogenizer. The cell homogenate was layered on top of a discontinuous sucrose gradient (i.e., 56, 38, and 19% sucrose) and centrifuged at 141,000g (SW28 rotor and a Beckman Optima L-90K centrifuge (Beckman Coulter, Inc., Fullerton, CA)). The 19 to 38% interface was collected, washed, and resuspended in 250 mM sucrose/20 mM HEPES/Tris-buffered, pH 7.4. Membrane vesicles were obtained after resuspension of the preparation by passing it 30 times through a 27-gauge needle. Vesicles were aliquoted and stored at −80°C until use. Protein content was determined using the bichinchonic acid method.

Western Blotting and Protein Analysis. Membrane vesicles were fractionated by 6% SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), which were blocked in phosphate-buffered saline/5% milk powder/0.05% Tween 20. The following antibodies were used: anti-ABCG2, BXP-21 (Maliepaard et al., 2004); anti-ABCC1, M31116 (Scheffer et al., 2000); anti-ABCC3, M31211 (Scheffer et al., 2000); and anti-ABCC4, M31110 (Leggas et al., 2004) [all antibodies were kind gifts from Dr. B.G. Scheffer (Free University Hospital, Amsterdam, The Netherlands)].

Immune complexes were visualized with horseradish peroxidase-conjugated IgG (Bio-Rad Laboratories, Veenendaal, The Netherlands) and detected using the ECL Western blot detection kit (GE Healthcare).

Vesicular Transport Assays. Vesicular transport studies were performed using the rapid filtration technique as described previously (Heijn et al., 1992). In brief, 5- to 50-µg membrane vesicles were added to a prewarmed (37°C) apical buffer at the apical side. Cefadroxil was quantified by reversed-phase HPLC with UV detection (Dionex, Amsterdam, The Netherlands). In brief, a 100-µl sample was applied to a BDS Hypersil C18 (3 µm, 15 cm) HPLC column (Thermo Fisher Scientific, Waltham, MA). The starting eluent consisted of 10% acetonitrile/90% ammonium formate (20 mM), pH 3.5, followed by a linear gradient to 30% acetonitrile in the same buffer in 20 min. Cefadroxil had a retention time of 14 min. Detection of cefadroxil was done at 260 nm. Quantification of cefadroxil was done by using a calibration curve of cefadroxil.

In Vivo Experiment: Jejunal Administration of Cefadroxil and Portal Blood Collection. Mice were anesthetized with a combination of Hypnorm (11.8 mg/kg fluanisone and 0.37 mg/kg fentanyl citrate; Vetapharma, Leeds, UK) and Valium (5.9 mg/kg diazepam; Centrafarm, Eten-Leur, The Netherlands). Body temperature was maintained between 35 and 37°C by the mice on thermostatically controlled heating pads. After induction of anesthesia, the vena porta was cannulated, followed by ligation of 10 cm of the middle part of the jejunal. Five hundred microliters of cefadroxil (5 µM; 2 µCi) was subsequently injected into the cannulated jejunum, and portal blood samples were collected after the indicated time points. At the end of the experiment, a peripheral blood sample was taken by cardiac puncture. Mice were subsequently euthanized, and liver, the ligated jejunum, kidneys, and gallbladder were collected. After the addition of H2O2 (30%) to blood, liver, jejunal, kidney, liver, and jejunal radioactivity was measured by liquid scintillation counting.

Statistical Analyses. Statistical differences were determined by an unpaired Student’s t test. All data were expressed as means ± S.D.

Results

To study the contribution of ABCC3 and ABCC4 in the transport of cephalosporins, we expressed the human proteins in S21 insect cells. ABCC2 protein was expressed as a positive control for cephalosporin transport. Transport activity was determined by measuring uptake of substrates into isolated membrane vesicles. First, ABCC2-, ABCC3-, and ABCC4-dependent uptake of the model substrate E217βG was studied in the absence and presence of cefadroxil. A concentration-dependent inhibition of E217βG uptake by cefadroxil was observed in membranes expressing ABCB2 (Fig. 1A), ABCB3 (Fig. 1B), and ABCC4 (Fig. 1C), albeit with very different IC50 values. The same inhibition studies were performed for nine other cephalosporins (Table 1). For all cephalosporins, the concentration at which E217βG uptake was inhibited by 50% (IC50) was determined and plotted as a function of the molecular weight of the cephalosporins (Fig. 1D; Supplemental Fig. 1). For all three ABC transporters, a near-linear, inverse correlation between IC50 values and molecular weight of the cephalosporins was observed. The orally available cephalosporins (Table 1) have the lowest molecular weight, indicating that molecular weight/bulkiness of the substrate is an important determinant for oral availability. Moreover, the orally available cephalosporins show the largest difference with regard to IC50 values of cephalosporins toward upper a vacuum. The filters were washed three times and counted in a liquid scintillation counter.

Calculation of Half-Maximal Inhibition. Transport of [1H]estradiol-17β-glucuronide was plotted against cephalosporin concentration. The program GraphPad (GraphPad Software Inc., San Diego, CA), using nonlinear regression, was used to calculate the concentration of cephalosporin, at which half-maximal inhibition was achieved.

Using Chamber Experiments and Quantification of Cefadroxil. Jejunal tissues from wild-type, Abcc3(−/−), Abcc4(−/−), and Abcc3(−/−)/Abcc4(−/−) animals were mounted in an Ussing chamber. The basolateral compartment contained 1.5 ml of basolateral buffer, which consisted of Krebs-Ringer bicarbonate, glucose (10 mM), and HEPES (10 mM, pH 7.4). Furthermore, the apical compartment contained 1.5 ml of apical buffer, which consisted of Krebs-Ringer bicarbonate, mannitol (10 mM), and MES (10 mM, pH 6.0). Both buffers were continuously gassed with carbogen. The temperature was set at 37 ± 1°C. After a 5- to 10-min equilibration, the experiment was started by the addition of 100 µl of cefadroxil containing 8 or 80 nmol (in apical buffer) at the apical side. Cefadroxil was quantified by reversed-phase HPLC with UV detection (Dionex, Amsterdam, The Netherlands). In brief, a 100-µl sample was applied to a BDS Hypersil C18 (3 µm, 15 cm) HPLC column (Thermo Fisher Scientific, Waltham, MA). The starting eluent consisted of 10% acetonitrile/90% ammonium formate (20 mM), pH 3.5, followed by a linear gradient to 30% acetonitrile in the same buffer in 20 min. Cefadroxil had a retention time of 14 min. Detection of cefadroxil was done at 260 nm. Quantification of cefadroxil was done by using a calibration curve of cefadroxil.
transport of E$_{217}$G mediated by ABCC4 versus ABCC2 and ABCC3, respectively. Competitive inhibition is an indication that the inhibitor may be a transported substrate. Cefadroxil is an orally prescribed cephalosporin that is commercially available in a radiolabeled form allowing its use in direct transport experiments. Therefore, we used cefadroxil as a model substrate for orally prescribed cephalosporins, in vesicular transport experiments. ABCG2 did not transport cefadroxil. However, membrane vesicles containing ABCC1, ABCC2, ABCC3, and ABCC4 all showed ATP-dependent uptake of cefadroxil (Fig. 2). In line with the inhibition experiments, transport of cefadroxil mediated by ABCC4 was 3- to 6-fold higher compared with transport by ABCC2 and ABCC3 (Fig. 2). Furthermore, cefadroxil transport was time dependent (Fig. 3, A–C) and saturable (Fig. 3, D and E) for ABCC1, ABCC3, and ABCC4 with $K_m$ values of 3.9 $\pm$ 0.6, 2.5 $\pm$ 0.7, and 0.25 $\pm$ 0.07 mM, respectively (Supplemental Table 1; Supplemental Fig. 2).

We subsequently tested whether there could be a role for Abcc3 and/or Abcc4 in vivo in the transport of cefadroxil across the basolateral membrane of the enterocyte in Ussing chamber experiments. No difference was found in transport of cefadroxil from the apical to the basolateral compartment using jejunal tissue from wild-type and Abcc3(−/−) mice. Transport of cefadroxil, (5 $\mu$M) was reduced to approximately 50% of normal levels, when jejunal tissue of Abcc4(−/−) and Abcc3(−/−)/Abcc4(−/−) mice were used (Fig. 4). Similar results were found with 50 $\mu$M cefadroxil (data not shown).

Finally, in intestinal loop experiments, we found that injection of

**TABLE 1**

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<th>Cephalosporins used in the inhibition studies</th>
<th>Molecular Weight</th>
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<tr>
<td>Cephalexin</td>
<td>347.4</td>
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<tr>
<td>Cephradine</td>
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<td>Cefuroxime</td>
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<tr>
<td>Cefoxitin</td>
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</tr>
<tr>
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<tr>
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<td>Ceftriaxol</td>
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</tr>
<tr>
<td>Cefoperazone</td>
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**FIG. 1.** Inhibition of estradiol-17β-glucuronide transport by cephalosporins. Cephalosporin inhibition of E$_{217}$G (0.05 $\mu$Ci; 1.0 $\mu$M) transport into ABCC2-, ABCC3-, and ABCC4-containing S21 membrane vesicles is shown. A–C, measurements of the IC$_{50}$ values for E$_{217}$G (0.05 $\mu$Ci; 1.0 $\mu$M) transport mediated by ABCC2 (A), ABCC3 (B), and ABCC4 (C), inhibited by different concentrations of cefadroxil. D, IC$_{50}$ values for inhibition of E$_{17}$βG transport by cephalosporins. Transport mediated by ABCC2 (dashed line), ABCC3 (solid line), and ABCC4 (dotted line) versus molecular weight of these cephalosporins is shown.

**FIG. 2.** Vesicular transport assay. Transport of cefadroxil (0.2 $\mu$Ci; 10 $\mu$M) with (filled bars) or without (open bars) ATP into mock-transduced ABCC1-, ABCC2-, ABCC3-, ABCG2-, and ABCC4-expressing membrane vesicles is shown. The incubation time is 6 min, and the amount of total protein used is 10 $\mu$g. Data represent the average $\pm$ S.D. Shown is the result of one of two independent experiments. Significance was tested using the two-sided Student’s $t$ test: *, $P < 0.05$ for transport of ABCC1-, ABCC2-, ABCC3-, and ABCC4-expressing membrane vesicles versus membrane vesicles from mock-transduced cells.
Cefadroxil in the ligated jejunum resulted in the time-dependent appearance of cefadroxil in portal blood of wild-type mice (Fig. 5). However, the appearance of cefadroxil in portal blood of Abcc3 \((/H11002)/H11002) and Abcc4 \((/H11002)/H11002) mice was not different from wild-type mice, with the latter result being in contrast to the Ussing chamber data. It is noteworthy that the appearance of cefadroxil in portal blood of Abcc3\((-/-))\) and Abcc4\((-/-)) mice was reduced. Similar results were found for peripheral blood: a significantly lower concentration of

**Fig. 3.** Time- and concentration-dependent transport. A–C, time-dependent transport of cefadroxil \((0.2 \mu Ci; 10 \mu M)\) into plasma membrane vesicles from \(/S/21\) cells expressing ABCC1 (A), ABCC3 (B), and ABCC4 (C). Open squares (with dotted line) and open diamonds represent incubations with or without ATP, respectively. Filled triangles (with solid line) represent ATP-dependent transport. D–F, Concentration-dependent transport of cefadroxil \((0.2 \mu Ci; 10 \mu M)\) into plasma membrane vesicles (open diamonds with solid line) from \(/S/21\) cells expressing ABCC1 (D), ABCC3 (E), and ABCC4 (F). Shown are the average \pm S.D. of ATP-dependent transport of a representative experiment with triplicate incubations performed three to four times.
cefadroxil was found in Abcc3(-/-)/Abcc4(-/-) mice compared with wild-type mice. There was no difference in the cefadroxil tissue content of intestine, kidney, and liver or in its urinary excretion between the mice strains tested. However, an increased amount of cefadroxil was noticed in the very low (less than 0.4% of dose) biliary content in Abcc3(-/-)/Abcc4(-/-) versus wild-type mice (Fig. 5D).

Discussion

Orally prescribed cephalosporins are efficiently taken up in the gut. At the luminal side of the small intestine, the dipeptide transporter PepT1 is involved in the import of cephalosporin. At present, the transporter(s) involved in the translocation of cephalosporins from the enterocytes into blood is still unknown. ABCC2 transports several cephalosporins, such as ceftriaxone, cefoperazone, and cephalaxin (Oude Elferink and Jansen, 1994; Kato et al., 2008). Because of its apical expression, ABCC2 cannot be responsible for the transport of oral cephalosporins into blood. Because ABCC3 and ABCC4 are related transporters, we tested their ability to mediate transport of one of the orally prescribed cephalosporins, namely cefadroxil. In this study, we show that both ABCC3 and ABCC4 mediate transport of cefadroxil, albeit with different kinetics: of the ABC transporters under study, ABCC4 mediates transport with the highest affinity (Figs. 2 and 3). This is in line with a previous report showing that the nonoral available cephalosporins ceftriaxone, cefazolin, cefotaxime, and cefmetazole are substrates of ABCC4 (Ci et al., 2007). Because cefadroxil is a smaller compound than both of these cephalosporins, the $K_m$ value of 246 μM is higher, as expected. This is based on our findings that an inverse correlation exists between molecular weights of cephalosporins and $IC_{50}$ values (Fig. 1D) and a relationship between $K_m$ and $IC_{50y}$ values. Our in vivo experiments with wild-type, Abcc3(-/-) and Abcc4(-/-) single knockout mice showed no difference in transport of cefadroxil from the luminal to serosal side (Fig. 5). We have two explanations for this result. One explanation is that murine Abcc3 and Abcc4, unlike their human orthologs used in the vesicular transport experiments, are unable to mediate transport of cefadroxil (Figs. 2 and 3). This explanation is unlikely because we observed in Ussing chamber experiments that transport was reduced in intestinal explants from Abcc4(-/-) mice compared with wild-type mice. Another explanation is that these transporters can compensate for the loss of each other. Therefore, we extended the experiments with Abcc3(-/-)/Abcc4(-/-) mice. The Abcc3(-/-)/Abcc4(-/-) mice had reduced levels of cefadroxil in their portal blood (Fig. 5). Because similar results were found in Ussing chamber experiments using jejunal explants from Abcc3(-/-)/Abcc4(-/-) and wild-type mice, we conclude that both Abcc3 and Abcc4 transport cefadroxil and when one transporter is lacking, the other can fully compensate. Human ABCC4 transports cefadroxil at higher rates than ABCC3 in vesicular transport experiments. Expressions of Abcc3 and Abcc4 were assessed in vivo using the two-sided Student's t test. * $P < 0.05$ for Abcc3(-/-)/Abcc4(-/-) versus wild-type mice, ko, knockout.

![Fig. 4. Transport of cefadroxil across jejunal explants in Ussing chambers. Appearance of cefadroxil on the basolateral side of jejunal explants from Abcc3(-/-), Abcc4(-/-), Abcc3(-/-)/Abcc4(-/-), and wild-type (wt) mice. Cefadroxil was applied to the apical compartment of Ussing chambers at a final concentration of 5 μM. At the indicated time points, samples were taken from the basolateral side and analyzed by HPLC. Shown is the average ± S.D. of three to four experiments with triplicate incubations. ‡, $P < 0.05$ comparing appearance of cefadroxil using tissue from Abcc3(-/-) versus wild-type mice for the indicated time points; #, $P < 0.05$ comparing appearance of cefadroxil using tissue from Abcc3(-/-)/Abcc4(-/-) versus wild-type mice for the indicated time points.](image)

![Fig. 5. In vivo uptake of cefadroxil by the jejunum. A–C, appearance of cefadroxil in portal (filled symbols) and peripheral (open symbols) blood of Abcc3(-/-) versus wild-type (wt) mice (A), Abcc4(-/-) versus wild-type mice (B), and Abcc3(-/-)/Abcc4(-/-) versus wild-type mice (C) after injection of 500 μl of cefadroxil (5 μM; 2 μCi) into ligated jejunum. Portal blood was collected after the indicated time points, and peripheral blood was collected after the last portal blood sample was drawn. D, appearance of cefadroxil in bile of Abcc3(-/-)/Abcc4(-/-) versus wild-type mice after injection of 500 μl of cefadroxil (5 μM; 2 μCi) into ligated jejunum. Data represent average ± S.D. of at least five mice. Significance was tested using the two-sided Student’s t test. * $P < 0.05$ for Abcc3(-/-)/Abcc4(-/-) versus wild-type mice.](image)
sion of murine Abcc3 is more than murine Abcc4 in the gut. This might be the reason that although Abcc3 transports cefadroxil, only at moderate rates can it still compensate for the loss of Abcc4. Because the difference in expression level between Abcc3/Abcc3 and Abcc4/Abcc4 in jejunum is similar in mouse and man, we speculate that also in the human situation, Abcc3 and Abcc4 are involved in transport of cefadroxil from enterocyte to blood (Taipalensuu et al., 2001; Maher et al., 2005).

The potential involvement of Abcc4 in the basolateral transport of cefadroxil is important because literature data are not consistent about the cellular localization of Abcc4. In the human colonic cell line HT29-CL.19A, Li et al. (2007) found Abcc4 in both the apical and basolateral membrane, with a higher expression apically. In Caco2 cells, Ming and Thakker (2010) detected Abcc4 mainly in basolateral membrane. Our data that intestinal Abcc4 influences cefadroxil uptake supports the notion that Abcc4 is present at the basolateral membrane of the enterocytes. Our functional data suggest that Abcc4 is present at the basolateral membrane of enterocytes and confirm the data obtained in Caco2 cells by Ming and Thakker (2010).

Abcc4(−/−)/Abcc4(+/−) mice still show a considerable amount of transport of cefadroxil over the basolateral membrane of enterocytes. In theory, Abcc1 could be responsible for the residual transport of cefadroxil, because we found that human ABCC1 is able to mediate transport of cefadroxil (Figs. 2 and 3). However, Abcc1 is expressed in the small intestine, mainly in the crypts (Peng et al., 1999; Kato et al., 2009), which does not colocalize with PepT1, which is abundantly present in the villus tip with decreasing levels toward the villus base. Therefore, the localization of Abcc1 argues against a role of Abcc1 in the basolateral efflux of cefadroxil in enterocytes. However, we cannot fully exclude that there is a role of Abcc1 in the efflux of cefadroxil because this transporter protects against the intestinal toxicity evoked by methotrexate (Kato et al., 2009). This means that Abcc1 is pumping methotrexate out of the cell at a physiological relevant speed. The same might be the case for Abcc1-mediated transport of cefadroxil from blood to the enterocyte, especially in the absence of Abcc3 and Abcc4 in the used murine model: Abcc3(+/−)/Abcc4(+/−) mice.

Abcc4(−/−)/Abcc4(+/−) mice. In conclusion, the data presented in this study demonstrate that murine intestinal uptake depends partly on Abcc3 and Abcc4. Therefore, we speculate that in the human situation, oral availability involves, at least partly, uptake via Abcc3 and Abcc4.

### Authorship Contributions

**Participants in research design:** de Waart and Oude Elferink.

**Conducted experiments:** de Waart, Kunne, and Duijst.

**Contributed new reagents or analytic tools:** van de Wetering and Paulusma.

**Performed data analysis:** de Waart.

Wrote or contributed to the writing of the manuscript: de Waart, van de Wetering, Paulusma, and Oude Elferink.

### References


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