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Species differences in the pharmacokinetics of cefadroxil as determined in wildtype and humanized *PepT1* mice

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ABSTRACT

PepT1 (SLC15A1) is a high-capacity low-affinity transporter that is important in the absorption of digested di/tripeptides from dietary protein in the small intestine. PepT1 is also crucial for the intestinal uptake and absorption of therapeutic agents such as the β-lactam aminocephalosporins and antiviral prodrugs. Species differences, however, have been observed in PepT1-mediated intestinal absorption and pharmacokinetics, thereby, making it more difficult to predict systemic drug exposure. In the present study, we evaluated the *in situ* intestinal permeability of the PepT1 substrate cefadroxil in wildtype and humanized *PepT1* (*huPepT1*) mice, and the *in vivo* absorption and disposition of drug after escalating oral doses. The *in situ* perfusions indicated that cefadroxil had a twofold higher affinity (i.e., twofold lower K_m) for jejunal PepT1 in *huPepT1* mice, lower but substantial permeability in all regions of the small intestine, and low but measureable permeability in the colon as compared to wildtype animals. The *in vivo* experiments indicated almost superimposable pharmacokinetic profiles between the two genotypes after intravenous bolus dosing of cefadroxil. In contrast, after oral dose escalation, the systemic exposure of cefadroxil was reduced in *huPepT1* mice as compared to wildtype animals. Moreover, the AUC and C_{max} versus dose relationships were nonlinear for *huPepT1* but not wildtype mice, and similar to that observed from human subjects. In conclusion, our findings indicate that *huPepT1* mice may provide a valuable tool in the drug discovery process by better predicting the oral pharmacokinetic profiles of PepT1 substrates in humans.

1. Introduction

The peptide transporter PepT1 (SLC15A1) is expressed predominately at the apical side of enterocytes in the small intestine with 50% abundance as compared to the total protein content of clinically relevant transporters [1]. As a consequence, PepT1 is mainly responsible for the uptake of di/tripeptides and peptide-like drugs from the intestinal lumen [2–5]. However, differences in specific protein activities have been observed between mammalian species which, in turn, may affect the absorption, disposition, metabolism and excretion of drugs [6]. Using a yeast system expressing mouse, rat and human *PepT1* cDNA, a species difference in PepT1 activity was demonstrated for glycylsarcosine (GlySar) where the uptake was saturable in all three species and 3- to 5-fold differences were observed in their K_m values [7]. Recognizing the need to improve prediction of human pharmacokinetics, drug–drug interactions and safety concerns because of species differences, genetically humanized and chimeric liver humanized mouse models were proposed by Scheer and Wilson [8]. At present, most humanized mouse models have focused on addressing the species differences in drug metabolizing enzymes [9], xenobiotic receptors [10,11] and, to a lesser extent, drug transporters [12].

Cefadroxil, (6*R*,7*R*)-7-{[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, is a first generation aminocephalosporin with good patient compliance [13], a long-acting therapeutic effect, high solubility and relatively broad spectrum of anti-bacterial activity [14,15]. It is used to treat urinary tract infections [16], skin and soft tissue infections [17,18], pharyngitis [19,20] and tonsillitis [21]. Cefadroxil has low plasma protein binding (~20%) and good oral bioavailability of at least 90% [22,23]. Renal excretion is the primary route of elimination, with more than 90% of the orally administered drug being excreted unchanged in urine over 24 h [22,24]. Cefadroxil is also a substrate of the intestinal peptide transporter PepT1, which is primarily responsible for the drug's uptake across the apical membrane of small intestine [25–29].

Species differences in PepT1-mediated permeability were first observed for the synthetic dipeptide GlySar during *in situ* jejunal

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perfusions in which the K_m was reduced two- to fourfold in humanized *PepT1* (*huPepT1*) mice as compared to wildtype animals [12]. In addition, during oral dose escalation studies with cefadroxil, there was no evidence of nonlinear intestinal absorption of drug in both wildtype and *PepT1* knockout mice as demonstrated by dose-proportional increases in area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{max}) [28]. The latter finding in mice, however, was contrary to other studies in humans where non-proportional increases in AUC were reported after increasing oral doses [23,24,30]. These studies clearly demonstrated that a species difference existed in the intestinal absorption and/or systemic exposure of peptides/mimetics as attributable to mouse and human PepT1.

In the present study, we hypothesized that cefadroxil would have a greater affinity (i.e., lower K_m) for intestinal PEPT1 when present in *huPepT1* mice as compared to wildtype mice. We further hypothesized that, given these differences in PEPT1 affinity, a nonlinear intestinal absorption should be more evident in the humanized mice. With this in mind, *in situ* permeability studies were performed with cefadroxil during small and large intestinal perfusions, along with *in vivo* absorption and disposition studies of drug after intravenous bolus injection at low and high doses, and after oral dose escalation. Our findings indicated that the humanized *PepT1* mouse model could provide a valuable tool in the drug discovery process, as well as better predict the pharmacokinetic profiles of PepT1 substrates in humans.

2. Materials and methods

2.1. Chemicals

[^{3}H]Cefadroxil (0.7 Ci/mmol) and [^{14}C]inulin 5000 (1.1 mCi/g) were purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). Unlabeled cefadroxil, glycyl-proline (GlyPro), glycyl-glycyl-histidine (GlyGlyHis), glycine, L-histidine, probenecid, p-aminohippuric acid (PAH), tetraethylammonium (TEA), quinidine, N^1-methylnicotinamide (NMN), carnosine, cephalexin, cephalothin, dimethylamiloride (DMA) and inulin 5000 were purchased from Sigma–Aldrich (St. Louis, MO). CytoScint™ scintillation solution and hyamine hydroxide were purchased from MP Biomedicals (Solon, OH). All other chemicals were acquired from standard sources.

2.2. Animals

In–house breeding of gender- and weight-matched, 8–10 week, *mPepT1*$^{+/+}$ (wildtype), *mPepT1*$^{-/-}$/*hPepT1*$^{-/-}$ (*PepT1* knockout) and *mPepT1*$^{-/-}$/*hPepT1*$^{+/-}$ (humanized *PepT1*, *huPepT1*] mice, on a C57BL/6 background, were used for these experiments as reported previously [12]. Wildtype, *PepT1* knockout and humanized *PepT1* mice were identified by genotyping and culled from the same litter. The mice were housed in a temperature-controlled environment with 12-h light and 12-h dark cycles, receiving a standard diet and water ad libitum (Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI). All mouse studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

2.3. In situ single-pass intestinal perfusion studies

Wildtype, *PepT1* knockout and *huPepT1* mice were fasted overnight (~12 h) with free access to water and then anesthetized with sodium pentobarbital (40–60 mg/kg ip). Perfusion studies of the jejunum, as well as all regional segments, were carried out according to methods described previously [5,31]. In brief, after sterilizing the abdominal area with 70% ethanol and keeping the mice on top of a heating pad to maintain body temperature, the intestines were exposed by a mid-line incision of the abdomen. When studying regional segments, 2 cm of the duodenum, 8 cm of the proximal jejunum (i.e., ~2 cm distal to the ligament of Treitz), 6 cm of the ileum (i.e., ~1 cm proximal to the cecum) and 4 cm of the colon (i.e., ~0.5 cm distal to the cecum) were isolated, and incisions then made at both the proximal and distal ends. For jejunal studies, only the 8-cm segment of proximal jejunum was isolated. The segments were rinsed with 0.9% isotonic saline solution, and a glass cannula (2.0 mm outer diameter) was inserted at each end of the intestinal segment and secured in place with silk suture. The isolated intestinal segment(s) were covered with saline-wetted gauze and parafilm to prevent dehydration. After cannulation, the animals were transferred to a temperature-controlled chamber, at 31 °C, to maintain body temperature during the entire perfusion procedure. The cannulas were then connected to inlet tubing, which was attached to a 10-mL syringe (BD, Franklin Lakes, NJ USA) placed on a perfusion pump (Model 22: Harvard Apparatus, South Natick, MA), and to outlet tubing, which was placed in a collection vial.

The perfusate buffer contained 135 mM NaCl, 5 mM KCl and 10 mM MES/Tris (pH 6.5) plus 10 μM of [^{3}H]cefadroxil (0.5 μCi) and 0.01% (w/v) [^{14}C]inulin 5000 (0.25 μCi) (which served as a non-absorbable marker to correct for water flux). The buffer was perfused through the intestinal segments at a flow rate of 0.1 mL/min, and the exiting perfusate was collected every 10 min for 90 min. A 100-μL aliquot of each perfusate collection was added to a vial containing 6.0 mL of scintillation solution, and the samples measured for radioactivity using a dual-channel liquid scintillation counter (Beckman LS 6000 SC, Beckman Coulter Inc., Fullerton, CA). At the end of experimentation, the actual length of intestinal segments was measured.

For the inhibition studies in jejunum, 10 mM of potential inhibitors was added to the perfusate except for DMA (0.1 mM). For the concentration-dependent studies in jejunum, cefadroxil varied from 0.01 to 25 mM in perfusate buffer containing [^{3}H]cefadroxil (0.5 μCi) and 0.01% (w/v) [^{14}C]inulin 5000 (0.25 μCi).

2.4. In vivo intravenous pharmacokinetic studies

Wildtype and *huPepT1* mice were anesthetized with sodium pentobarbital (40–60 mg/kg ip) prior to an intravenous bolus injection of [^{3}H]cefadroxil (11 and 528 nmol/g body weight, 5.0 μCi per dose) in 100 μL of saline. Serial blood samples were collected at 1, 2.5, 5, 10, 20, 30, 45, 60, 90 and 120 min after dosing via tail transections. Blood samples (15–20 μL) were placed into tubes containing 1.0 μL of EDTA-K3 and centrifuged for 3 min × 3000*g* to obtain the plasma (10 μL). A 30-μL aliquot of 30% H_2O_2 was then added, followed by 6.0 mL of scintillation solution and 20 μL of 0.5 M acetic acid. Radioactivity in the plasma samples was measured using a dual-channel liquid scintillation counter.

For the biodistribution studies, 0.2 μCi of [^{14}C]inulin in 100 μL of saline was given by bolus intravenous injection, 2.0 min prior to the time at which the tissue samples were harvested (i.e., 120 min). Following decapitation, the tissues (including a blood sample) were weighed and 300 μL of hyamine hydroxide was added to the samples and then incubated at 37 °C until the entire tissue was dissolved. After the samples cooled down to room temperature, 30 μL of 30% H_2O_2 was added, followed by 6.0 mL of scintillation solution and 20 μL of 0.5 M acetic acid. Radioactivity in these samples was measured using a dual-channel liquid scintillation counter. The cefadroxil tissue-to-plasma concentration ratios were also determined at 120 min.