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Evaluating the Intestinal and Oral Absorption of the Prodrug Valacyclovir in Wildtype and huPepT1 Transgenic Mice

Daniel Epling[a], **Yongjun Hu**[a], and **David E. Smith**[a,*]

[a]Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor MI, USA

Abstract

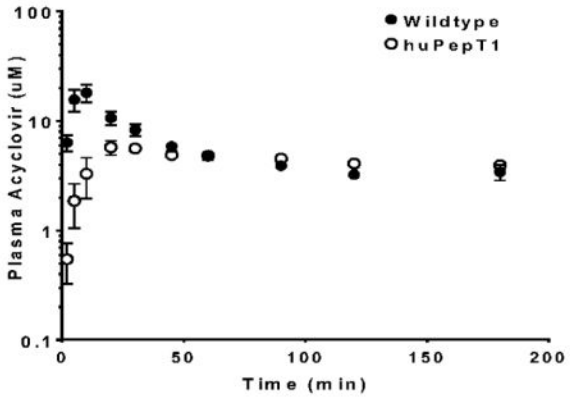
The purpose of this work was to evaluate the intestinal permeability, oral absorption and disposition of the ester prodrug valacyclovir in wildtype mice and a huPepT1 transgenic mouse model. PepT1 (SLC15A1) is a transporter apically expressed along the lumen of the gastrointestinal tract and is responsible for the absorption of di-/tripeptides, ACE inhibitors, β-lactam antibiotics and numerous prodrugs. Unfortunately, PepT1-mediated substrates that have been extensively studied were shown to exhibit species-dependent absorption and pharmacokinetics. Accordingly, in situ intestinal perfusion studies were conducted and valacyclovir uptake was shown to have a 30-fold lower K_m and 100-fold lower V_{max} in huPepT1 compared to wildtype mice. Moreover, inhibition studies demonstrated that the huPepT1 transporter alone was responsible for valacyclovir uptake, and segment-dependent studies reported significant reductions in permeability along the length of small intestine in huPepT1 mice. Subsequent oral administration studies revealed that the in vivo rate and extent of valacyclovir absorption were lower in huPepT1 mice, as indicated by 3-fold lower C_{max} and 3-fold higher T_{max} values, and an $AUC_{0–180}$ that was 80% of that observed in wildtype mice. However, no significant changes in drug disposition were observed between genotypes after intravenous bolus administration of acyclovir. Lastly, mass balance studies established that the bioavailability of acyclovir, after oral dosing of valacyclovir, was 77.5% in wildtype mice and 52.8% in huPepT1 mice, which corroborated values of 51.3% in clinical studies. Thus, it appears the huPepT1 transgenic mice may be a better model to study prodrug absorption and disposition in humans than wildtype mice.

Graphical Abstract

*Corresponding author at: University of Michigan, College of Pharmacy, 428 Church Street, Ann Arbor, MI 48109-1065, USA., Telephone: 734-647-1431, Facsimile: 734-615-6162, smithb@umich.edu.

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.



Keywords

Valacyclovir; Pharmacokinetics; huPepT1 Mice; Permeability; Absorption; Bioavailability

1. Introduction

The proton-coupled oligopeptide transporter PepT1 (SLC15A1), which is expressed along the apical side of intestinal enterocytes, has become a prime target for prodrug binding and uptake. A prodrug is similar in structure to a pharmacologically active parent compound but contains an added chemical promoiety that, in this case, confers PepT1-mediated transport upon the entire molecule, thus enhancing intestinal absorption [1,2]. One reason PepT1 has become a preferential target is because of this transporter's abundant expression along the gastrointestinal tract. This fact is substantiated in a study by Drozdzik et. al., showing that relative PepT1 protein expression in the human small intestine constitutes half of all tested transporter proteins [3]. Secondly, PepT1 is targeted due to its high capacity transport and broad substrate specificity. Known substrates to be transported by PepT1 include di/tripeptides, peptidomimetic compounds such as β-lactam antibiotics and ACE inhibitors, and a growing number of prodrugs (e.g. valacyclovir, valganciclovir, L-dopa-L-Phe, and zanamivir [1,2,4,5].

Valacyclovir is the L-valine prodrug of acyclovir, a nucleoside antiviral that is used for the treatment of genital herpes and herpes zoster [6]. Importantly, this compound has been studied extensively as a substrate for PepT1-mediated transport. In a novel study by Yang and Smith [7], the authors found that PepT1 contributed >80% of the *in situ* intestinal permeability of valacyclovir in wildtype mice and, in a subsequent study [8], they observed a substantial increase in the oral absorption of valacyclovir as compared to PepT1 knockout mice. Many *in vitro* studies have also demonstrated that cells transfected with human PepT1 exhibit functional activity for valacyclovir transport [9–13].

Although studies investigating PepT1-mediated uptake are numerous, a definitive species difference in PepT1 transporter kinetics was first reported in Pichia pastoris expressing the mouse, rat, and human orthologs [14]. In light of this finding, Hu et al [15] developed a novel humanized transgenic mouse line (huPepT1) that was proven to be viable, express

human PepT1 protein along the intestinal tract, and demonstrate functional activity with the model dipeptide glycylsarcosine. Recently, these same researchers [16] validated huPepT1 with the β-lactam antibiotic cefadroxil. The huPepT1 mice clearly showed a higher affinity (i.e., lower K_m) and lower capacity for transport (i.e. lower V_{max}) as compared to wildtype mice. Equally important, oral dose escalation studies revealed that AUC and C_{max} profiles in huPepT1 mice over a large dose range exhibited non-linear pharmacokinetics [16], as seen in human clinical trials, but unlike the linear pharmacokinetics observed in wildtype mice [17]. The huPepT1 mouse line, therefore, demonstrated its use as a potential model for predicting human pharmacokinetics involving compounds that are mainly absorbed by the action of PepT1. Nonetheless, huPepT1 mice have not been tested with respect to their *in situ* intestinal permeability, and *in vivo* absorption and disposition kinetics of prodrugs.

With this in mind, the purpose of the current study was to evaluate the intestinal permeability, oral absorption and disposition of the ester prodrug valacyclovir in a huPepT1 transgenic mouse model. We hypothesized that valacyclovir would exhibit a higher affinity for intestinal transport in huPepT1 mice, and that the rate of absorption and extent of systemic exposure would be lower than that observed in wildtype mice. In particular, initial studies evaluated the *in situ* intestinal permeability and kinetics of the transport of valacyclovir, the specificity of prodrug transport, and the intestinal segment-dependent permeability of valacyclovir in both wildtype and huPepT1 mice. Subsequent studies evaluated the *in vivo* oral and intravenous pharmacokinetics of valacyclovir (and acyclovir) as related to oral absorption, systemic exposure, and bioavailability.

2. Materials and Methods

2.1. Chemicals

Valacyclovir, acyclovir, glycylsarcosine (Gly-Sar), cephalexin, tetraethylammonium (TEA), L-histidine, L-lactate and L-valine were purchased from Sigma-Aldrich (St. Louis, MO). CytoScint™ scintillation solution were purchased from MP Biomedicals (Solon, OH). [^{3}H]Valacyclovir (1.1 Ci/mmol) and [^{3}H]acyclovir (10.6 Ci/mmol) were purchased from Moravek Biochemicals and Radiochemicals (Brea, CA). [^{14}C]Inulin 5000 (1.1 mCi/g) was purchased from American Radiolabeled Chemicals (St. Louis, MO).

2.2. Animals

Gender-matched and age-matched (8–12 weeks) mice utilized in these studies have a C57BL/6 background and consisted of the mPepT1$^{+/+}$ (wildtype) and mPepT1$^{-/-}$/huPepT1$^{+/-}$ (humanized PepT1) genotypes. The viability and functional activity of the humanized PepT1 mouse line were previously established in our laboratory [15]. Inheritance of the huPepT1 gene was confirmed by genotyping. All mice were bred and housed in a temperature-controlled environment with 12 hour light and dark cycles, and a standard diet and water a*d libitum* (Unit for Laboratory Animal Medicine, University of Michigan, Ann Arbor, MI). Animal studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3. *In Situ* Single-Pass Intestinal Perfusion Studies

As reported previously [7,18], wildtype and huPepT1 mice were fasted overnight (14–18 hours) before experimentation. Mice were then given 40–60 mg/kg of pentobarbital as an intraperitoneal injection for anesthesia. The abdominal area was sanitized with alcohol before a 1.5 cm incision was made along the midline section. An 8-cm segment of jejunum (~2 cm distal to the Ligament of Trietz) was then isolated, cannulated (2.0 mm outer diameter), and tied off with silk sutures. The mouse was then placed in a temperature-controlled chamber (31°C) and saline-wetted gauze, along with Parafilm, was laid out across the abdominal section to prevent dehydration. A syringe containing pH 6.5 perfusion buffer (10 mM MES/Tris, 135 mM NaCl, 5 mM KCl), and 50 μM valacyclovir, was housed in a syringe pump (Harvard Apparatus PHD Ultra, South Natick, MA). Inlet tubing connected the syringe to the perfused segment and outlet tubing connected the perfused segment to the collection vials. Buffer solution was perfused through the cannulated segment at a programmed flow rate of 0.1 mL/min for 90 minutes. Perfusate samples were collected every 10 minutes for the last 60 minutes of the perfusion. No samples were collected during the first 30 minutes in order to pass over the pre-equilibration period. Upon completion, the perfused intestinal segments were removed, and the length was measured and recorded.

Concentration-dependent studies were conducted by perfusing valacyclovir through the jejunum at concentrations ranging from 0.05–30 mM in wildtype mice and from 0.025–1 mM in huPEPT1 mice. Additionally, inhibition studies conducted in both genotypes evaluated the specificity of valacyclovir transport from the jejunum. Valacyclovir was perfused at 50 μM either alone (control) or with inhibitors at 25 mM. Potential inhibitors included glycylsarcosine (Gly-Sar), cephalexin, tetraethylammonium (TEA), L-histidine, L-lactate, and L-valine. All perfusion buffers containing inhibitors had their pH readjusted to 6.5, as needed.

For segment-dependent studies, 50 μM valacyclovir was simultaneously perfused through a 2 cm segment of duodenum (~0.25 cm distal to the pyloric sphincter), the jejunum (as described before), a 6 cm segment of the ileum (~1 cm proximal to the cecum), and a 3 cm segment of the colon (~0.5 cm distal to the cecum).

2.4. UPLC Analytical Method

All perfusate samples were analyzed by a UPLC Waters Acquity H-class system. Peaks corresponding to valacyclovir and acyclovir were separated by a gradient method through a 100-cm Acquity HSS T3 column. The method starts with 100% water in 0.1% TFA, and changes linearly over the next five minutes to 90% water/10% acetonitrile. This ratio holds for 2 minutes before reverting back to 100% aqueous content almost instantly. Each sample run lasts 12 minutes to ensure column pressure equilibration. Column temperature was maintained at 40°C and the flow rate remained constant at 0.5 mL/min. Compounds were detected by UV detection at 254 nm and retention times were approximately 2.4 and 4.9 minutes for acyclovir and valacyclovir, respectively. All perfusate samples were centrifuged at 15000 rpm for 10 minutes and 25 μL aliquots of supernatant were injected into the column by automation.

Method validation over three consecutive days showed accuracy within 6% of specified values and precision within 2% (relative standard deviation). Linearity was established from 0.5–50 μM for acyclovir and from 2–200 μM for valacyclovir. The limits of quantitation for acyclovir and valacyclovir in perfusion buffer were 50 and 10 nM respectively. Additionally, the examination of blank perfusate samples showed that endogenous compound noise was essentially absent within the analyte retention time windows.

2.5. Intravenous and Oral Administration Studies

IV bolus and oral administration studies were conducted in a similar manner to that reported previously [5]. For IV bolus studies mice were administered, by tail vein, 100 μL of saline solution containing 24 nmol/g of acyclovir and [^{3}H] acyclovir (5 μCi/mouse). Blood samples (~20 μL) were collected by tail nick at 1, 5, 10, 20, 30, 45, 60, 90, 120 and 180 minutes after administration. Prior to the oral studies, mice were fasted overnight (16–18 hours) before being administered 200 μL of saline solution containing 24 nmol/g of valacyclovir and [^{3}H] valacyclovir (10 μCi/mouse) by oral gavage. Blood samples were collected similarly as described for IV bolus, with the exception that the first collection time was at 2 minutes. Blood samples from both studies were placed in microcentrifuge tubes with K3-EDTA and centrifuged for 3 minutes at 3000 rpm. Aliquots (5 μL) of plasma were placed in scintillation vials along with 6 mL of CytoScint scintillation fluid (MP Biomedicals, Solon, OH). The plasma samples were placed in a dual-channel liquid scintillation counter (Beckman LS 6000 SC, Beckman Coulter Inc., Brea, CA) and radioactivity was measured.

2.6. Mass Balance Studies

Mice were fasted overnight (16–18 hours) and then 100 μL [^{14}C]inulin (5 μCi/mouse) was injected by IV bolus in the tail vein. Immediately after, these mice were administered 24 nmol/g valacyclovir with [^{3}H]valacyclovir (10 μCi/mouse) by oral gavage. Mice were then placed in a metabolic cage for 24 hours for the purpose of separately collecting the urine and feces. Food and water were provided to the mice while in the cage. After several cage washes, 5–10 μL aliquots of the diluted urine and feces were placed in separate vials with 6 mL of scintillation fluid, and radioactivity was measured by the scintillation counter.

2.7. Data Analysis

For in situ perfusion studies, the calculation of effective permeability (P_{eff}) assumed a complete radial mixing (parallel tube) model [19,20]:

$$Peff = \frac{-Qin \times \ln\left(\frac{C'out}{Cin}\right)}{2\pi RL} \quad (1)$$

where Q_{in} is the inlet perfusion flow rate (0.1 mL/min), C'_{out} is the sum of valacyclovir and acyclovir concentrations in the outlet perfusate corrected for water flux, Cin is the concentration of valacyclovir in the inlet perfusate, R is the intestinal radius (0.1 cm for small intestine and 0.2 cm for colon), and L is the intestinal segment length. The equation used to determine C'_{out} was calculated as:

$$C'out = (Cout, VACV + Cout, AVC - Cin, ACV) \times (\frac{Qout}{Qin}) \quad (2)$$

where Q_{out} is determined gravimetrically [21]. Concentration-dependent studies in both wildtype and huPepT1 mice were modeled according to Michaelis-Menten kinetics:

$$V = Peff \times Cin = \frac{Vmax \times Cin}{Km + Cin} \quad (3)$$

where V_{max} is the maximal velocity of transport and K_m is the transporter affinity in terms of the inlet concentration of valacyclovir. The kinetic data was modeled by nonlinear regression and assessed for goodness of fit using Prism version 7 software (GraphPad Software, Inc., La Jolla, CA).

Data were reported as mean ± SD, unless noted differently. Comparisons between two groups were assessed by the unpaired t–test. Comparisons between multiple groups were analyzed by one-way ANOVA followed by either Dunnett's or Tukey's test using Prism software. Statistical significance was attributed when $p \leq 0.05$. For the oral and IV bolus studies, pharmacokinetic parameters were determined by noncompartmental analysis using Phoenix WinNonlin 8.0 software (Certara, St Louis, MO).

Results

2.1. Valacyclovir Transport Kinetics During In Situ Jejunal Perfusions

Both wildtype and huPepT1 mice exhibited distinctive Michaelis-Menten kinetics for valacyclovir uptake with characteristic transport saturability (Figure 1). The kinetic parameters for wildtype mice (Figure 1A) were V_{max} = 782 ± 53 pmol/cm^2/sec and K_m = 7.7 ± 1.0 mM (r^2=0.992), and for huPepT1 mice (Figure 1B) were V_{max} = 7.83 ± 1.37 pmol/cm^2/sec and K_m = 0.25 ± 0.10 mM (r^2=0.898). The V_{max} value was approximately 100-fold greater in wildtype mice than in huPepT1 mice, while the K_m value was approximately 30-fold less (i.e. affinity was 30-fold greater). The inclusion of a nonsaturable term did not improve the goodness of fit, so only a saturable term was considered in the final model.

2.2. mPepT1 and huPepT1 Specificity For Valacyclovir Transport During In Situ Jejunal Perfusions

The co-perfusion of various potential inhibitors with valacyclovir in wildtype mice (Figure 2A) demonstrated that glycylsarcosine and cephalexin significantly reduced the permeability of valacyclovir by approximately 2-fold from the control value. In contrast, the substrates tetraethylammonium, L-histidine, L-lactate, and L-valine did not significantly change valacyclovir permeability. These same inhibitor studies in huPept1 mice (Figure 2B) showed a significant 16-fold reduction in the permeability of valacyclovir by glycylsarcosine, whereas cephalexin did not induce a significant reduction. Moreover, co-perfusion of

valacyclovir with other potential inhibitors did not significantly change the permeability from control values, as similarly observed in the wildtype results.

2.3. Regional Valacyclovir Permeability During In Situ Intestinal Perfusions

The permeability of valacyclovir, obtained from segments of the small intestine, were shown to be significantly different between, but not within, the two genotypes (Figure 3). The huPepT1 mice had permeability values that were substantially smaller than for wildtype mice and were approximately 12-fold lower in the duodenum, 10-fold lower in the jejunum, and 7-fold lower in the ileum. On the other hand, permeability values from the colon were not different between wildtype and huPepT1 mice and, in huPepT1 animals, were not different from other segments of the small intestine.

2.4. Pharmacokinetic Parameters In Vivo from IV Bolus and Oral Administrations

The IV bolus administration of acyclovir in wildtype and huPepT1 mice yielded pharmacokinetic profiles that were nearly superimposable between the two genotypes (Figure 4A). This observation is supported by the pharmacokinetic analysis in which all key parameters showed no significant differences between the wildtype and huPepT1 mice (Table 1). In contrast, the oral administration of valacyclovir produced plasma concentration-time curves of acyclovir that were visually different between the two genotypes (Figure 5). This difference was noted by the 3-fold higher C_{max} in wildtype mice, the 3-fold larger T_{max} in huPepT1 mice, and the $AUC_{0–180}$ of huPepT1 mice that was approximately 80% of the value seen in wildtype animals (Table 2). Despite these trends, only the C_{max} values were statistically different from each other.

2.5. Acyclovir Bioavailability In Vivo from Mass Balance Studies

As shown in Table 3, the bioavailability of acyclovir, after oral administration of valacyclovir, was 77.5% and 52.8% for wildtype and huPepT1 mice, respectively. This significant difference corresponds to a relative bioavailability in huPept1 mice that was 68% of the value seen in wildtype animals. Although the urine from huPepT1 mice contained 20% less of the administered dose, their feces contained approximately 20% more of the dose, as compared to wildtype mice. Inulin, a marker for urine collection efficiency, revealed adequate recoveries of drug-related radiolabel in these studies (~90%). Additionally, trace amounts of inulin in the feces (~1%) confirmed that there was a minimal level of urine-to-feces cross-contamination.

Discussion

Applicability of the huPepT1 transgenic mouse line for predicting clinical pharmacokinetic parameters has been validated recently through studies performed with cefadroxil [16]. However, these findings were limited in scope because the substrates that PepT1 can transport encompass more than just β-lactam antibiotics [1,2,4]. The current study has sought to broaden the utility of these transgenic mice by further validating the model with the ester prodrug valacyclovir. Specifically, several major findings were revealed: 1) huPepT1 mice had a greater affinity (30-fold lower K_m) and reduced capacity (100-fold lower V_{max}) for valacyclovir during jejunal perfusions than wildtype mice; 2) the jejunal

permeability of valacyclovir was specific for PepT1; 3) the permeability of valacyclovir was significantly lower in all segments of huPepT1 mouse small intestine as compared to wildtype; 4) the disposition kinetics of intravenously administered valacyclovir did not differ between the two genotypes; and 5) the relative bioavailability of orally dosed valacyclovir was about 30% lower in huPepT1 mice than wildtype animals, and its absolute bioavailability in huPepT1 mice was better correlated with clinical studies.

In a previous perfusion study, Yang and Smith [7] reported the jejunal transport kinetics of valacyclovir as V_{max} = 1.4 nmol/cm^2/s and K_m = 10.2 mM in wildtype mice. These values were comparable to those obtained in the current study (i.e., V_{max} = 0.78 nmol/cm^2/s and K_m = 7.71 mM). In regard to huPepT1-mediated transport, previous cell culture studies revealed K_m values ranging from 0.29 – 5.94 mM [9–13]. In the current study, huPepT1 mice had a K_m of 0.25 mM, a value approaching the lower bounds of the literature range. The reasons for variation between our in situ perfusion studies and other in vitro studies may be attributed to the use of different cell expression systems, a lack of blood flow during in vitro studies, and differences in buffer pH that may alter valacyclovir affinity for PepT1 [12].

An understanding of PepT1 specificity for valacyclovir in the huPepT1 mouse model is paramount for model validation. Inhibition studies, conducted in both wildtype and huPepT1 mice, indicated that L-valine, L-histidine, L-lactate and tetraethylammonium had little to no effect on valacyclovir-mediated uptake, thereby, ruling out an influence of $ATB^{0,+}$, PhT1/2, MCT1 and OCT transporters. Conversely, the reduction in valacyclovir uptake during co-perfusion of glycylsarcosine and cephalexin indicated that valacyclovir was transported substantially by PepT1 in both mouse models. This finding is in agreement with a similar study in wildtype mice by Yang and Smith [7], with the novelty of also testing MCT1 given its known expression in mouse small intestine [22]. It is noteworthy to mention that cephalexin did not significantly reduce valacyclovir uptake in huPepT1 mice, although a trend was observed. This may have to do with differences in inhibitor affinity of cephalexin for this transporter. Two previous studies in Caco-2 cells (pH 6.0 uptake buffer) revealed that the K_m values for glycylsarcosine and cephalexin were approximately 4-fold different (i.e., 2 and 8 mM respectively) [23,24].

With respect to segment-dependency, wildtype mice showed uptake trends that were roughly similar to the relative levels of protein expressed in each segment [18]. However, segment-dependent uptake in huPepT1 mice did not correspond well with protein levels. While uptake was low and statistically similar across all segments, our previous study reported higher protein levels in the small intestine and only trace amounts in the colon [16]. This “apparent” discrepancy is hard to reconcile. However, a similar study with cefadroxil revealed non-significant differences in uptake between the small and large intestines of huPepT1 mice, although greater differences were observed [16].

In a clinical study, where 10 μM of valacyclovir was perfused through the subject’s jejunum using the Loc-I-Gut® method (pH 6.5 sodium phosphate buffer), the effective permeability was reported as 1.66×10^{-4} cm/s [25]. In the current study, the jejunal permeability of 50 μM valacyclovir was 0.92×10^{-4} in wildtype mice and 0.12×10^{-4} cm/s in huPepT1 mice.

Using these permeability values, it was possible to estimate the absorption rate constant (Ka) of valacyclovir by the equation:

$$Ka = (2 \times Peff)/R \quad (4)$$

where R is the intestinal radius of the jejunum (1.75 cm for humans and 0.1 cm for mice) [26]. Accordingly, the K_a values were 0.68 hr^{-1} in humans, 6.62 hr^{-1} in wildtype and 0.86 hr^{-1} in huPepT1 mice, indicating a better correlation in the absorption rate constant between humans and huPepT1 mice. Thus, it appears that huPepT1 transgenic mice may predict the absorption of valacyclovir in humans with improved accuracy.

The 24 nmol/g valacyclovir dose chosen for all *in vivo* experiments in the current study was selected from the 10–100 nmol/g dosing range established previously [8]. These authors justified the dosing range by relating the C_{max} values of acyclovir obtained from their wildtype mice with the C_{max} values determined from clinical studies in which 250, 500, 1000 and 2000 mg oral doses of valacyclovir were administered [27]. Apart from establishing dosing rationale, pharmacokinetic trends can be elucidated from these studies as well. For example, escalating oral doses of valacyclovir in wildtype mice revealed linear increases in acyclovir AUC and C_{max}, but clinical studies showed less than proportional increases in these parameters. Investigators [8,27] have attributed these clinical trends to saturation of transporter-mediated intestinal absorption, which we believe is substantiated by comparing the oral absorption results in the current study between wildtype and huPepT1 mice.

The *in vivo* oral absorption and disposition of 25 nmol/g valacyclovir has been reported previously in wildtype and PepT1 knockout mice [8]. In these studies, PepT1 knockout mice had a lower C_{max} (5- to 6-fold), a higher T_{max} (5-fold), and an $AUC_{0–180}$ for acyclovir that was 35% of that observed in wildtype mice. While PepT1 ablation clearly demonstrated a deep reduction in the rate and extent of oral valacyclovir absorption, more modest differences were observed between wildtype and huPepT1 mice (Table 2). Nonetheless, as clearly shown by our mass balance studies (Table 3), the bioavailability of orally administered valacyclovir was 52.8% in huPepT21 mice, as compared to 77.5% for wildtype animals. Notably, a similar bioavailability was observed in the clinical setting where a value of 51.3% was observed after the oral administration of 1000 mg valacyclovir [28].

As shown previously by our group, jejunal permeabilities that differed by 10-fold between wildtype and PepT1 knockout mice resulted in systemic availabilities that differed by only 2-fold for glycylsarcosine [18,29] and by 2- to 3-fold for valacyclovir [7,8] after oral dosing. In these reports, we advanced the concept that PepT1 knockout mice may take advantage of the residual length and prolonged residence times along the entire length of small and large intestines, resulting in greater than expected passive absorption and, thereby, minimizing the reduced systemic availability of substrate expected during PepT1 ablation. This concept was subsequently validated during an in silico analysis by our group [30], demonstrating that whereas wildtype mice primarily absorb valacyclovir in the duodenal and jejunal segments of the small intestine (i.e., 66% of a total 70% absorbed), the absorption of valacyclovir in

PepT1 knockout mice was slow but sustained throughout the entire intestinal tract (i.e, 4% duodenum, 14% jejunum, 10% ileum and 12% caecum/colon for a total 40% absorbed). Although a similar analysis was not performed for valacyclovir in huPepT1 mice, this transgenic group appears to fall in the middle of the results observed between wildtype and PepT1 knockout mice, namely, having an absorption of 53%.

An underlying assumption throughout these studies was that valacyclovir was rapidly degraded to its parent compound *in vivo* and that the radiolabel being measured was essentially all acyclovir. We believe, for several reasons, that this was a reasonable assumption. First, Yang and Smith [7] found that after a 90-min intestinal perfusion of valacyclovir, only acyclovir was observed in the portal venous blood of mice. Second, these investigators reported very similar plasma concentration-time profiles of acyclovir in mice following oral administration of either radiolabeled or unlabeled valacyclovir, as measured by liquid scintillation counting or HPLC, respectively. Finally, studies in humans, monkeys and rats also confirmed the rapid and efficient metabolic conversion of prodrug [27,31,32].

In conclusion, the *in situ* mechanistic studies provided here demonstrate that valacyclovir has a greater affinity (and lower capacity) for PepT1-medidated transport in huPepT1 than wildtype mice. The *in vivo* pharmacokinetic and mass balance studies reflect the saturability of intestinal uptake in huPepT1 mice, as judged by decreases in valacyclovir's rate and extent of absorption or bioavailability. Taken as a whole, the discrete differences in wildtype and huPepT1 mice, when held against clinical data, further rationalize the usefulness of this transgenic model for studying other prodrugs. The huPepT1 mice, along with other transgenic mouse models, are currently being used to evaluate the feasibility of developing oral prodrugs for parenteral anti-cancer therapeutics.

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Abbreviations

ACV	Acyclovir
$AUC_{0–180}$	Area under the plasma concentration-time curve from 0 to 180 minutes
CL	Clearance
C_{max}	Maximum plasma concentration
F	Bioavailability
Gly-Sar	glycylsarcosine
huPepT1	Humanized peptide transporter 1
Inu	Inulin

K_m	Michaelis-Menten constant
mPepT1	mouse peptide transporter 1
P_{eff}	Effective permeability
PepT1	Peptide transporter 1
$T_{0.5}$	Half-life
TEA	tetraethylammonium
T_{max}	Time to reach the maximum plasma concentration
VACV	Valacyclovir
Vd_{ss}	Volume of distribution steady-state
V_{max}	Maximum velocity

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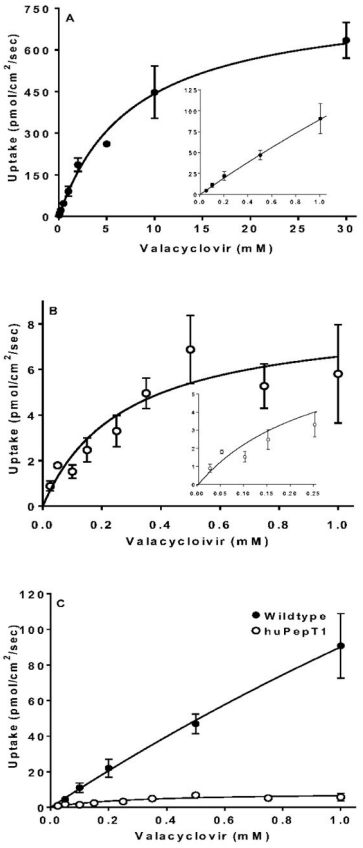

Figure 1.

Uptake of valacyclovir during in situ perfusions of (A) wildtype mice (n=3–4) and (B) huPepT1 mice (n=3–7) at given perfusate concentrations. Data are expressed as mean ± SE and mean data was fit to the model. Insets show the data points at low valacyclovir concentrations. (C) Comparison of valacyclovir uptake in wildtype and huPepT1 mice at lower concentrations.

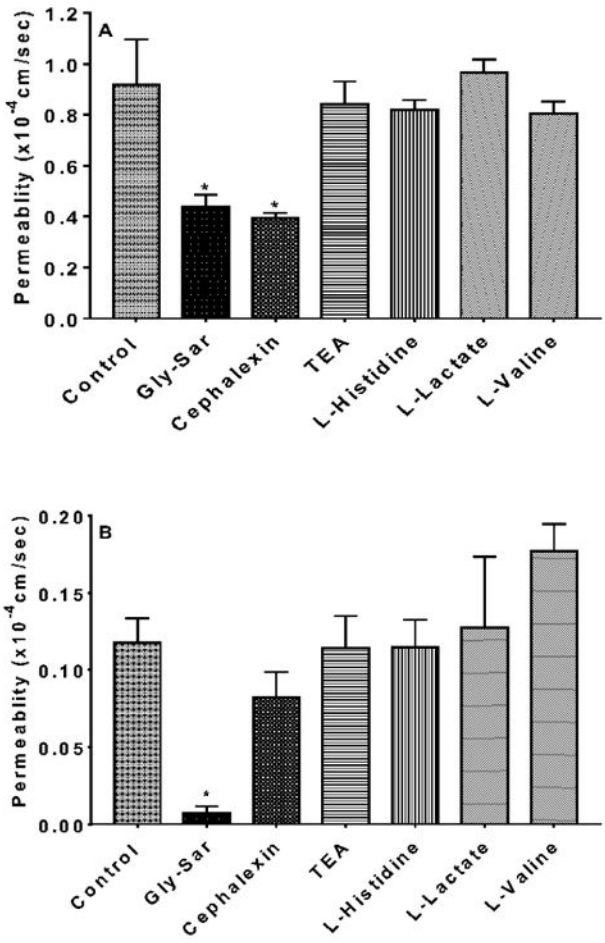

Figure 2.

Valacyclovir at 50 μM was co-perfused (pH 6.5) with various potential inhibitors at 25 mM in (A) wildtype mice (n=3–7) and (B) huPepT1 mice (n=5–8). The control column represents the perfusion of valacyclovir alone. *$p \leq 0.05$ compared to control, as determined by ANOVA and Dunnett's' test.

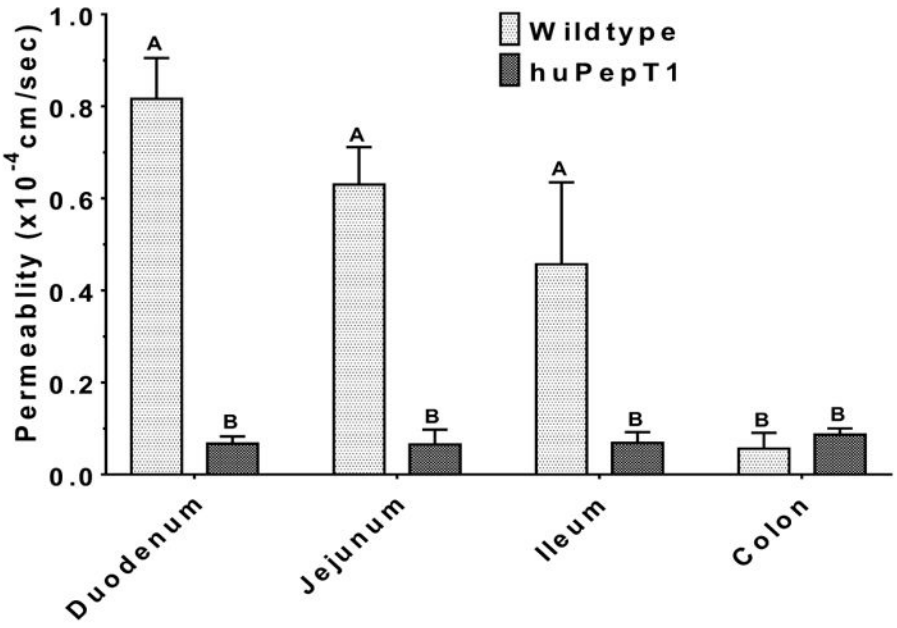

Figure 3.

All four intestinal segments were perfused simultaneously with valacyclovir in both wildtype and huPepT1 mice. Data are expressed as mean ± SE (n=4). Segments with the same letter were not significantly different, as determined by ANOVA and Tukey's test.

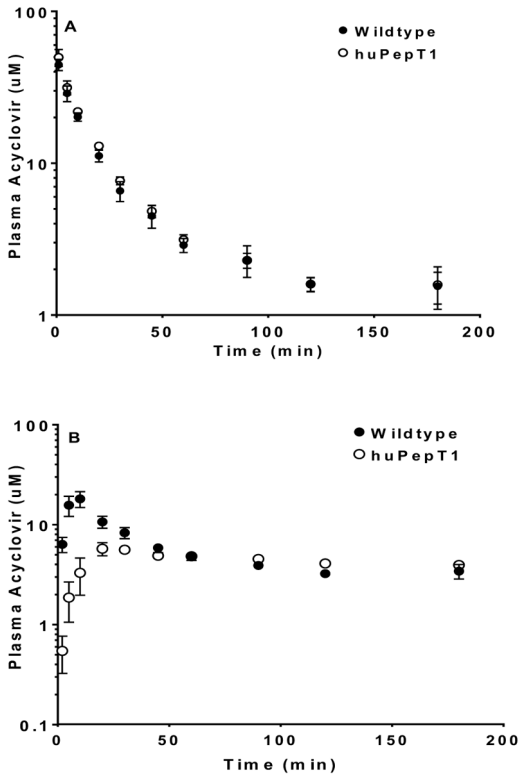

Figure 4.

(A) Acyclovir plasma concentration-time profile after IV bolus administration of 24 nmol/g of acyclovir in wildtype and huPept1 mice. (B) Acyclovir plasma concentration-time profiles after oral administration of 24 nmol/g of valacyclovir in wildtype and huPept1 mice. Data are expressed as mean ± SE (n=4).

Table 1

Pharmacokinetic Parameters of Acyclovir after IV Bolus Administration of Acyclovir (24 nmol/g) in Wildtype and huPeptT1 Mice

Parameter (unit)	Wildtype	huPepT1	Ratio (huPepT1/WT)
Vd_{ss} (L/kg)	2.1 ± 0.8	1.4 ± 0.2	0.67
CL (L/hr/kg)	1.3 ± 0.2	1.3 ± 0.2	1.0
$T_{0.5}$ (min)	106 ± 46	63.4 ± 4.0	0.60
$AUC_{0–180\ min}$ (μM*min)	929 ± 126	1015 ± 92	1.1

Data are shown as mean ± SD (n=4). No significant difference was observed for any of the key parameters, as determined by unpaired t-test.

Table 2

Pharmacokinetic Parameters of Acyclovir after Oral Administration of Valacyclovir (24 nmol/g) in Wildtype and huPept1 Mice

Parameter (unit)	Wildtype	huPepT1	Ratio (huPepT1/WT)
T_{max} (min)	10 ± 0	33 ± 19	3.3
C_{max} (μM)	18.0 ± 6.5	6.1 ± 1.4*	0.34
$AUC_{0–180\ min}$ (μM*min)	982 ± 230	775 ± 86	0.79

Data are shown as mean ± SD (n=4).

* $p \leq 0.05$ compared to wildtype mice, as determined by unpaired t-test.

Table 3

Bioavailability of Acyclovir after the Oral Administration of Valacyclovir (24 nmol/g) in Wildtype and huPepT1 as Determined from Mass Balance

	Wildtype			huPepT1		
	Urine	**Feces**	**F**	**Urine**	**Feces**	**F**
% Acv	68.1	11.6	77.5	47.4	31.6	52.8*
% Inu	87.9	1.4	89.3	89.1	0.5	89.6

Data are expressed as mean values and represent three mice per group. Each animal was injected with [^{14}C]inulin (Inu) by IV bolus, and then dosed with [^{3}H]valacyclovir [VACV] by oral gavage. Animals were then placed in a metabolic cage, and the urine and feces collected over 24 hours. Bioavailability (F) of acyclovir (ACV) was calculated as: %ACV recovered in the urine divided by %Inu recovered in the urine. Bioavailability (F) of Inu was calculated as: %Inu recovered in the urine plus %Inu recovered in the feces.

* $p \leq 0.05$ compared to wildtype mice, as determined by unpaired t-test.