Transport of Valganciclovir, a Ganciclovir Prodrug, via Peptide Transporters PEPT1 and PEPT2

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ABSTRACT: In clinical trials, valganciclovir, the valyl ester of ganciclovir, has been shown to enhance the bioavailability of ganciclovir when taken orally by patients with cytomegalovirus infection. We investigated the role of the intestinal peptide transporter PEPT1 in this process by comparing the interaction of ganciclovir and valganciclovir with the transporter in different experimental systems. We also studied the interaction of these two compounds with the renal peptide transporter PEPT2. In cell culture model systems using Caco-2 cells for PEPT1 and SKPT cells for PEPT2, valganciclovir inhibited glycylsarcosine transport mediated by PEPT1 and PEPT2 with K_i values (inhibition constant) of 1.68 ± 0.30 and 0.043 ± 0.005 mM, respectively. The inhibition by valganciclovir was competitive in both cases. Ganciclovir did not interact with either transporter. Similar studies done with cloned PEPT1 and PEPT2 in heterologous expression systems yielded comparable results. The transport of valganciclovir via PEPT1 was investigated directly in PEPT1-expressing *Xenopus laevis* oocytes with an electrophysiological approach. Valganciclovir, but not ganciclovir, induced inward currents in PEPT1-expressing oocytes. These results demonstrate that the increased bioavailability of valganciclovir is related to its recognition as a substrate by the intestinal peptide transporter PEPT1. This prodrug is also recognized by the renal peptide transporter PEPT2 with high affinity. © 2000 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 89: 781–789, 2000

Keywords: prodrug; bioavailability; valyl ester; ganciclovir; peptide transporter; intestine; kidney

Introduction

Cytomegalovirus (CMV) is a herpes virus that causes disease in severely immunocompromized patients, such as those who have received organ transplantation and those with acquired immunodeficiency syndrome (AIDS). CMV infection can occur in any part of the body but commonly involves the retina of the eye, nervous system, and gastrointestinal system. CMV retinitis in AIDS patients may lead to blindness if not treated. CMV infection can be treated with the nucleoside analog ganciclovir, which is available in three formulations. Intravenous administration gives therapeutically effective blood levels but complications due to intravenous access occur because the patients should be on the drug indefinitely to suppress the infection. Intraocular implantation needs a surgical procedure and delivers the drug only locally in the eye. Together with the intraocular implantation, the drug should be given orally to maintain blood levels to suppress infection. The currently available oral formulation of the drug shows very low bioavailability requiring intake of two capsules (3 g) every day. Patients with AIDS

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who take a variety of other drugs for HIV and opportunistic infections fail to adhere to the suppressive doses of oral ganciclovir.

The ganciclovir prodrug (RS-79070-194), a valyl ester of ganciclovir (valganciclovir), is rapidly converted to ganciclovir during the first pass process, primarily by hydrolysis in the gut wall. The bioavailability of valganciclovir is high and that of ganciclovir itself is very low. Pharmacokinetic studies have shown that the bioavailability is 60.9% with 360 mg of prodrug and only 5.57% with 1000 mg of the now available oral ganciclovir. Furthermore, the t_{max} with the prodrug was earlier than with the oral ganciclovir. There were no new or unexplained toxicities from the prodrug.[1]

Drug delivery through known transport systems has been under investigation for many years. The peptide transporters of the intestine (PEPT1) and kidney (PEPT2) have been shown to transport a variety of therapeutically active agents, such as β-lactam antibiotics.[2,3] Because PEPT1 transports small peptides and the intestinal mucosal cells possess high levels of intracellular peptidases that hydrolyze small peptides very efficiently, drug delivery using peptide-based prodrugs through PEPT1 has been suggested as an effective approach to enhance the oral bioavailability of drugs that are otherwise not readily absorbable in the intestine.[4] The feasibility of this approach has been supported with studies on the intestinal absorption of dihydroxyphenylalanine (DOPA) in the form of peptide-based DOPA derivatives.[5, 6] The intestinal peptide transporter is a low-affinity transporter that mediates the absorption of the peptides arising from the digestion of dietary proteins.[7, 8] The high-affinity kidney peptide transporter plays a role in the reabsorption of the peptides present in the glomerular filterate.[7–10] The transport process is energy dependent, involving the transmembrane electrochemical H^+ gradient and the underlying mechanism is the cotransport of the peptide substrates with H^+. β-Lactam antibiotics and other compounds that share the structural features of the endogenous peptides are recognized by the peptide transporters PEPT1 and PEPT2.[11–16] Recently, we and others have shown that valaciclovir, the valyl ester of aciclovir, is a substrate for the peptide transporters.[17–19] Valaciclovir is a prodrug with the amino acid valine in an ester linkage. There is no peptide bond in this prodrug and yet this compound is recognized as a substrate by the intestinal and renal peptide transporters. The ganciclovir prodrug valganciclovir is also a valyl ester of ganciclovir. Therefore, it is very likely that this prodrug is also a substrate for the peptide transporters. We investigated in the present study the interaction of ganciclovir and valganciclovir with intestinal and renal peptide transporters using different experimental approaches. The results of this investigation show that ganciclovir by itself is not recognized as a substrate by PEPT1 and PEPT2, whereas addition of a valyl residue in an ester linkage makes it a transportable substrate for these transporters.

MATERIALS AND METHODS

Materials

The human colon carcinoma cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD). The rat renal proximal tubule cell line SKPT was provided by Dr. Ulrich Hopfer, Case Western University, Cleveland, OH. [2-^{14}C]-Glycyl-[1-^{14}C]-sarcosine (specific radioactivity, 109 mCi/mmol) was custom synthesized by Cambridge Research Biochemicals (Cleveland, United Kingdom). The ganciclovir prodrug valganciclovir and ganciclovir were generous gifts from Roche Pharmaceuticals (Boulder, CO) and Roche Bioscience (Palo Alto, CA), respectively. Minimal Essential Medium (MEM) with Earle's salts and L-glutamine were purchased from Mediatech Inc. (Herndon, VA). Dulbecco's Modified Eagle Medium:Nutrient Mixture F12 (Ham) 1:1 (DMEM/F12) with Hepes (15 mM) and L-glutamine, MEM nonessential amino acids, penicillin (10,000 units/mL)-streptomycin (1,000 μg/mL), and trypsin were obtained from Life Technologies, Inc. (Gaithersburg, MD). Fetal bovine serum was purchased from Atlanta Biologicals (Atlanta, GA). Dexamethasone and apotransferrin were obtained from Sigma. Recombinant insulin was from Novo Nordisk (Denmark) and epidermal growth factor was from Upstate Biotechnology, Inc. (Lake Placid, NY). All chemicals were of analytical grade.

Cell Culture and Uptake Measurements

Caco-2 cells were cultured in MEM supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% MEM nonessential amino acids.[20] The rat renal proximal tubule cells (SKPT) were cultured in DMEM/F12 (1:1) medium supplemented with fetal bovine serum (10%), penicillin-streptomycin (1%), insulin (5 μg/

mL), dexamethasone (4 μg/mL), and epidermal growth factor (10 ng/mL).[21] Uptake of [^{14}C]glycylsarcosine in cells was measured with the uptake buffer whose composition was 15 mM Mes/Tris (pH 6.0), 140 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, and 5 mM glucose. Caco-2 and SKPT cells were trypsinized when confluence was noted in the 75-cm^2 flasks, and 200,000 cells were seeded per well in 24-well culture plates. The Caco-2 cells were confluent on the fourth day of seeding, and uptake measurements were done the next day. The SKPT cells were confluent on the third day, and the uptake was measured on the fourth day after seeding. The culture medium was aspirated and the cell monolayers were washed with 250 μL of the uptake buffer. Then, 250 μL of uptake buffer containing the radiolabeled substrate was added. Incubation was done for 10 min at room temperature, at the end of which the uptake was terminated by aspiration of the buffer. The cells were then washed three times with the ice-cold uptake buffer. The cells were then solubilized with 0.5 mL of 0.2 M NaOH and 1% SDS,and the contents were transferred to a counting vial for measurement of radioactivity.

Vaccinia Virus Expression of PEPT1 and PEPT2 in HeLa Cells

The cloned human PEPT1[22] and rat PEPT2[23] were functionally expressed in HeLa cells heterologously using the vaccinia virus expression technique as described previously.[11, 12] Subconfluent HeLa cells in 24-well culture plates were first infected with a recombinant vaccinia virus $VTF_{7\text{-}3}$, which carries the gene for T7 RNA polymerase as a part of its genome. This procedure enables the HeLa cells to express T7 RNA polymerase. Following the infection, the cells were transfected with pSPORT-hPEPT1 cDNA construct or with pSPORT-rPEPT2 cDNA construct. In these constructs, the cDNAs were under the control of T7 promoter in the plasmid. Cells transfected with plasmid alone served as controls. Transfection was mediated by Lipofectin. The virus-encoded T7 RNA polymerase catalyzes the transcription of the cDNA, allowing transient expression of hPEPT1 and rPEPT2 proteins in the HeLa cell plasma membrane. After 12 h postinfection, transport measurements were made at room temperature. The uptake buffer was the same as that used in the cell culture experiments. The uptake was initiated by adding 250 μL of the uptake buffer containing the radiolabeled substrate. The time of incubation was 5 min. The uptake was terminated by removing the buffer from the wells and washing the cells three times with ice-cold uptake buffer that did not contain radiolabeled substrate. The cells were then solubilized with 0.5 mL of 0.2 M NaOH/1% SDS and transferred to counting vials, and then the radioactivity was measured.

Oocyte Expression and Electrophysiological Measurements

The procedures for oocyte preparation from *Xenopus laevis* and microinjection of cRNA into the oocytes have been described previously.[24] A two-microelectrode voltage clamp system was used to measure steady-state currents associated with hPEPT1-mediated transport. The hPEPT1-expressing oocytes were perifused with either ganciclovir or valganciclovir (10 mM) at pH 5.5, and the currents were recorded.

Data Analysis

Experiments were repeated 2–3 times, each done in duplicate or triplicate, and the results are given as mean ± SE of these replicates. Only the specific, carrier-mediated uptake values were used in data analysis. Specific uptake was calculated by subtracting the nonsaturable uptake from the total uptake. The nonsaturable uptake was determined from the uptake of radiolabeled glycylsarcosine measured in the presence of an excess amount (20 mM for PEPT1 and 5 mM for PEPT2) of the competitor alanylvaline. The kinetic parameters, Michaelis- menten constant (K_t) and the maximal velocity (V_{max}), were calculated by nonlinear regression and confirmed by linear regression. Inhibition constants (K_i) were calculated from IC_{50} values (concentration of the unlabeled test compound necessary to inhibit 50% of the uptake of radiolabeled substrate) according to the method of Cheng and Prusoff.[25]

Results and Discussion

Interaction of Ganciclovir and Valganciclovir with Peptide Transporters Expressed in Caco-2 Cells and SKPT Cells

The human intestinal cell line Caco-2 constitutively expresses the intestine-specific peptide transporter PEPT1. This cell line has been used as a model system to investigate the functional and regulatory aspects of PEPT1.[20, 26–28] Similarly, the rat kidney proximal tubular cell line SKPT expresses the kidney-specific peptide

transporter PEPT2.[21] PEPT2 is not expressed in Caco-2 cells and PEPT1 is not expressed in SKPT cells.[11] The differential expression of PEPT1 and PEPT2 in Caco-2 and SKPT cell lines makes it possible to examine the functional aspects of these two transporters independently. We first investigated the interaction of ganciclovir and valganciclovir with PEPT1 and PEPT2 expressed in these cells. The transport function of these transporters was monitored by the uptake of the dipeptide glycylsarcosine into the cells in the presence of an inwardly directed H^+ gradient (i.e., extracellular pH 6.0). The interaction was assessed by the ability of ganciclovir and valganciclovir to inhibit the uptake of glycylsarcosine. In Caco-2 cells as well as in SKPT cells, valganciclovir inhibited the uptake of glycylsarcosine in a dose-dependent manner (Figure 1). The IC_{50} value (i.e., concentration at which the inhibition was 50%) was 1.69 ± 0.30 mM in Caco-2 cells and 0.046 ± 0.005 mM in SKPT cells. In contrast, ganciclovir had very little effect on the dipeptide uptake in both cell lines, even at a concentration as high as 10 mM. We then examined the kinetic nature of the inhibition caused by valganciclovir (Figure 2). In both cell lines, the inhibition was competitive. In Caco-2 cells, glycylsarcosine was taken up into the cells with a K_t of 0.66 ± 0.04 mM and V_{max} (maximal velocity) of 2.4 ± 0.1 nmol/mg of protein/10 min in the absence of valganciclovir. The corresponding kinetic parameters were 1.39 ± 0.11 mM and 2.5 ± 0.1 nmol/mg of protein/10 min in the presence of 3 mM valganciclovir. In SKPT cells, the K_t and V_{max} values for glycylsarcosine uptake in the absence of valganciclovir were 0.068 ± 0.003 mM and 0.62 ± 0.01 nmol/mg of protein/10 min. The corresponding values in the presence of 0.1 mM valganciclovir were 0.160 ± 0.022 mM and 0.58 ± 0.05 nmol/mg of protein/10 min. Thus, in both cell lines, the inhibition of dipeptide uptake by valganciclovir was associated with an increase in K_t without any noticeable change in V_{max}.

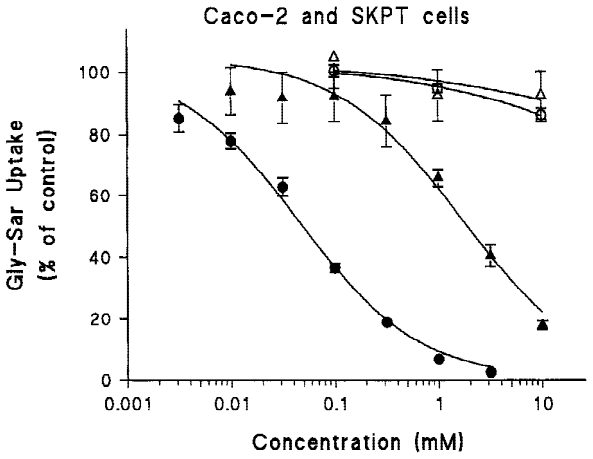

Figure 1. Differential interaction of ganciclovir and valganciclovir with PEPT1 in Caco-2 cells and PEPT2 in SKPT cells. Uptake of [^{14}C]glycylsarcosine (5 μM) was measured in Caco-2 cells (▲, △) and SKPT cells (●, ○) in the presence of increasing concentrations of ganciclovir (△, ○) or valganciclovir (▲, ●). Uptake measured in the absence of inhibitors was taken as 100%.

Interaction of Ganciclovir and Valganciclovir with Cloned Peptide Transporters PEPT1 and PEPT2

We then investigated the interaction of ganciclovir and valganciclovir with PEPT1 cloned from human intestine and PEPT2 cloned from rat brain.[22, 23] The rat brain PEPT2 is identical to the rat kidney PEPT2.[29] These two cloned transporters were expressed heterologously in HeLa cells using the vaccinia virus expression technique, and their transport function was monitored by measuring the uptake of glycylsarcosine into these cells in the presence of an inwardly directed H^+ gradient (i.e., extracellular pH 6.0). HeLa cells do not possess any detectable endogenous peptide transport activity and thus are ideal for heterologous expression of the cloned peptide transporters. Figure 3 describes the ability of ganciclovir and valganciclovir to inhibit glycylsarcosine uptake in HeLa cells expressing PEPT1 and PEPT2. Valganciclovir inhibited the uptake of the dipeptide in a dose-dependent manner with IC_{50} values of 2.84 ± 0.42 mM in PEPT1-expressing cells and 0.176 ± 0.012 mM in PEPT2-expressing cells. Ganciclovir caused very little effect on the uptake even at a concentration as high as 10 mM. The inhibition of glycylsarcosine uptake by valganciclovir was competitive in the case of PEPT1 as well as PEPT2 (Figure 4). The cloned PEPT1 exhibited a K_t value of 0.43 ± 0.05 mM and a V_{max} value of 8.6 ± 0.6 nmol/10^6 cells/5 min in the absence of valganciclovir. When studied in the presence of 3 mM valganciclovir, the K_t value increased to 0.91 ± 0.16 mM, with no detectable change in V_{max} (8.4 ± 1.0 nmol/10^6 cells/5 min). Qualitatively, similar results were obtained with cloned PEPT2. The uptake of glycylsarcosine in PEPT2-expressing cells exhibited a K_t value of 0.054 ± 0.005 mM and a V_{max} value of 2.05 ± 0.08 nmol/10^6 cells/5 min in the absence of valganciclovir. When studied in the presence of 0.3 mM

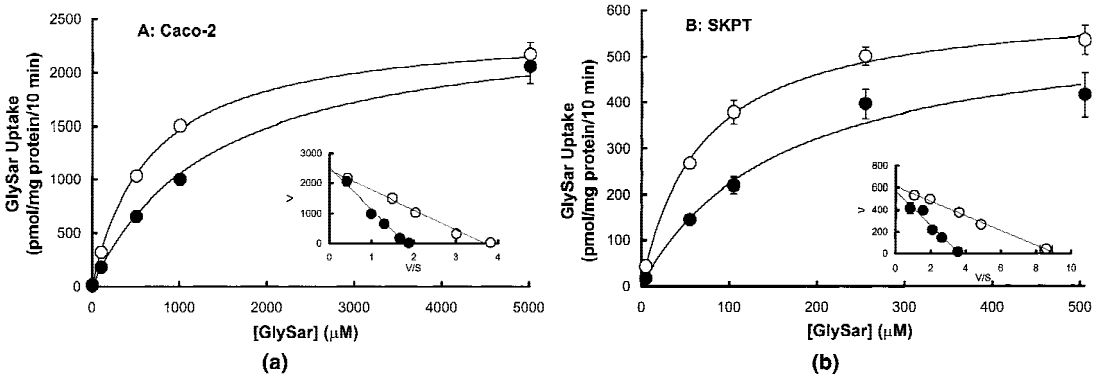

Figure 2. Competitive inhibition of glycylsarcosine uptake by valganciclovir in (A) Caco-2 cells (PEPT1) and (B) SKPT cells (PEPT2). Uptake of glycylsarcosine was measured in cells in the absence (○) or presence (●) of valganciclovir. The ranges of glycylsarcosine concentration were 5–5000 μM in Caco-2 cells and 5–500 μM in SKPT cells. The concentration of valganciclovir was 3 mM in Caco-2 cells and 0.1 mM in SKPT cells. Inset: Eadie–Hofstee plots.

valganciclovir, the K_t value increased to 0.122 ± 0.026 mM with no detectable change in the V_{max} value (2.13 ± 0.17 nmol/10^6 cells/5 min).

Using the values of IC_{50}, K_t and the concentration of glycylsarcosine used in the uptake studies, we calculated the inhibition constants (K_i) for valganciclovir. The K_i values in Caco-2 and SKPT cells were 1.68 ± 0.30 mM and 0.043 ±0.005 mM, respectively. The K_i values in the case of the cloned transporters PEPT1 and PEPT2 were 2.71 ± 0.40 mM and 0.113 ± 0.008 mM, respectively. Thus, for PEPT1 as well as for PEPT2, the affinity towards valganciclovir is comparable between the constitutively expressed transporter and the cloned transporter. Furthermore, the affinity of PEPT1 for valganciclovir is 20–40 fold lower than that of PEPT2. A similar difference (~10-fold) is observed between these two transporters in their affinity towards glycylsarcosine.

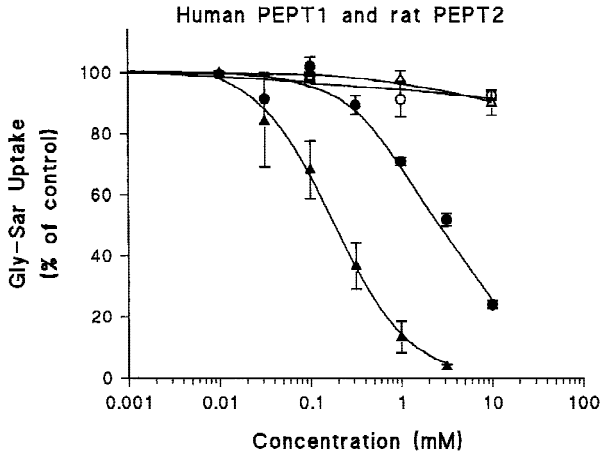

Figure 3. Differential interaction of ganciclovir and valganciclovir with cloned PEPT1 and PEPT2 expressed heterologously in HeLa cells. Uptake of [^{14}C]glycylsarcosine was measured in PEPT1-HeLa cells (●, ○) and in PEPT2-HeLa cells (▲, △) in the presence of increasing concentrations of ganciclovir (○, △) or valganciclovir (●, ▲). The concentration of radiolabeled dipeptide was 20 μM in the case of PEPT1-HeLa cells and 30 μM in the case of PEPT2-HeLa cells. Uptake measured in the absence of inhibitors was taken as 100%.

Direct Evidence for the Transport of Valganciclovir by Human PEPT1

The inhibition of PEPT1-mediated glycylsarcosine uptake by valganciclovir as observed in Caco-2 cells and in PEPT1-expressing HeLa cells does not necessarily mean that valganciclovir is a transportable substrate for PEPT1. A compound can block the transport of a substrate by competing for the substrate-binding site without itself being translocated across the membrane. The studies described thus far do not establish whether valganciclovir is merely a blocker of the peptide transporter function or actually a transportable substrate. These two modes of interaction can be easily differentiated by electrophysiological monitoring of the interaction of valganciclovir with the peptide transporters. H^+-coupled translocation of a substrate across the membrane in cells expressing the peptide transporters is expected to cause depolarization of the membrane potential due to

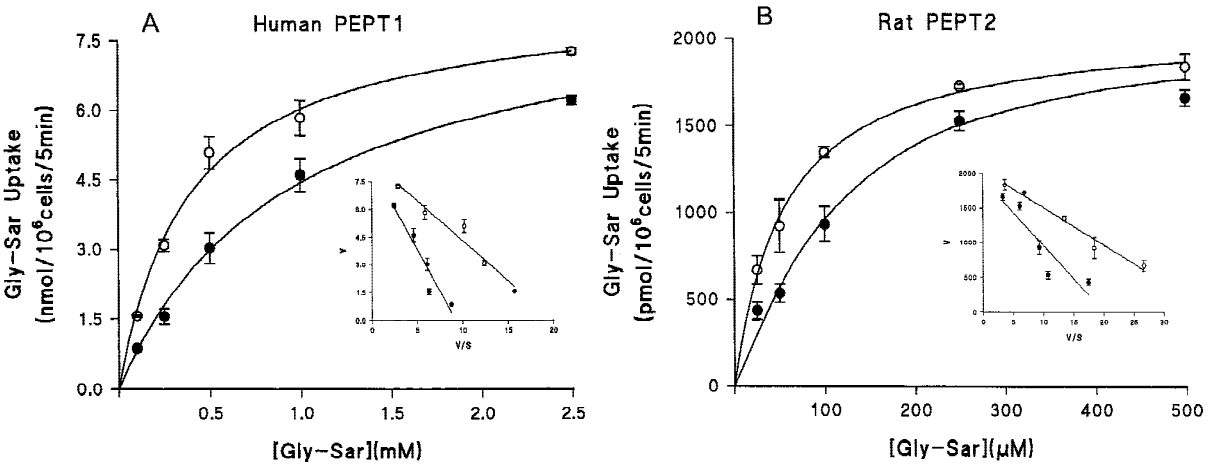

Figure 4. Competitive inhibition of glycylsarcosine uptake by valganciclovir in (A) PEPT1-HeLa cells and (B) PEPT2-HeLa cells. Uptake of glycylsarcosine was measured in cells in the absence (○) and presence (●) of valganciclovir. The ranges of glycylsarcosine concentration were 0.1–2.5 mM in the case of PEPT1-HeLa cells and 25–500 μM in the case of PEPT2-HeLa cells. The concentration of valganciclovir was 3 mM in PEPT1-HeLa cells and 0.3 mM in PEPT2-HeLa cells. Inset: Eadie–Hofstee plots.

the electrogenic nature of the translocation process. If a compound merely binds to the transporter without being actually translocated, such a process is not expected to be associated with changes in membrane potential. The *X. laevis* oocyte expression system offers an easy and convenient way to monitor peptide transporter-associated currents by the two-microelectrode voltage clamp technique. We expressed the cloned human PEPT1 in *X. laevis* oocytes and monitored currents when the oocyte was exposed to ganciclovir or valganciclovir at pH 5.5 (Figure 5). Exposure of the oocyte to ganciclovir was not associated with detectable currents. In contrast, exposure of the same oocyte to valganciclovir induced significant inward currents. At 10 mM concentration, valganciclovir induced an inward current of 20–25 nA with the oocyte membrane potential clamped at −50 mV. Induction of an inward current in this experimental protocol means that valganciclovir is actually transported across the membrane via PEPT1 and that the transport process is associated with the transfer of positive charge across the membrane. The valganciclovir-induced currents decreased markedly (<5 nA) when the pH of the perifusion buffer was 7.5 instead of 5.5 (data not shown). This result suggests that the H^+/valganciclovir cotransport is the mechanism of the transport process. Exposure of water-injected oocytes to valganciclovir did not induce any detectable currents (data not shown), indicating that the valganciclovir-induced current is obligatorily dependent on PEPT1 expression. We were unable to perform similar experiments with PEPT2 because the expression levels of this transporter in *X. laevis* oocytes are much lower than that of PEPT1.

There are other possible explanations for the observed depolarization in human PEPT1-expressing oocytes when exposed to valganciclovir. Perifusion of the oocytes with valganciclovir

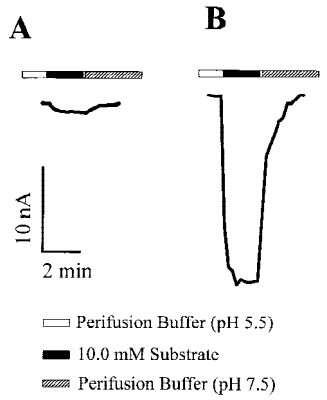

Figure 5. Induction of inward currents by valganciclovir but not by ganciclovir in *Xenopus laevis* oocytes expressing human PEPT1 heterologously. The oocyte was first perifused with a pH 5.5 buffer, then with either (A) 10 mM ganciclovir or (B) 10 mM valganciclovir (B) in the same buffer, and finally with a pH 7.5 buffer. The currents were recorded using the two-microelectrode voltage clamp technique. The membrane potential was clamped at −50 mV.

may result in the opening of cation channels leading to the influx of cations other than H^+. However, this result is unlikely because the valganciclovir-induced depolarization is seen in PEPT1-expressing oocytes only in the presence of an inwardly directed H^+ gradient. If the influx of cations other than PEPT1-mediated H^+ transfer were to be involved, one would expect no changes in the magnitude of the valganciclovir-induced depolarization whether the experiments were done in the presence or absence of a H^+ gradient. Furthermore, substrate-induced charge movements in PEPT1-expressing oocytes have been completely accounted for by the transfer of H^+ into the oocytes by intracellular pH measurements as well as by H^+:substrate stoichiometry analysis.[30–32] Another possible explanation for the valganciclovir-induced depolarization is that exposure of the oocytes to valganciclovir may activate some hitherto unidentified membrane receptors leading to influx of cations. Again, because the valganciclovir-induced depolarization was not detected in water-injected oocytes, this alternative explanation also seems very unlikely. Therefore, we suggest that the depolarization observed in PEPT1-expressing oocytes on exposure to valganciclovir in the presence of an inwardly directed H^+ gradient is most likely due to H^+ influx associated with the H^+/valganciclovir cotransport mediated by PEPT1.

Valganciclovir is a valyl ester of ganciclovir (Figure 6). It is interesting to note that the presence of a peptide bond is not a prerequisite for recognition as a substrate by the peptide transporters. Because ganciclovir itself is not recognized as a substrate by these transporters, it is the addition of the valyl residue that is responsible for the recognition of valganciclovir as a substrate. Ester linkages are easily hydrolyzable by intracellular esterases, which explains why valganciclovir is readily converted to ganciclovir in the intestine. Once transported into the mucosal cells across the brush border membrane via PEPT1, valganciclovir is hydrolyzed to generate ganciclovir by intracellular esterases.

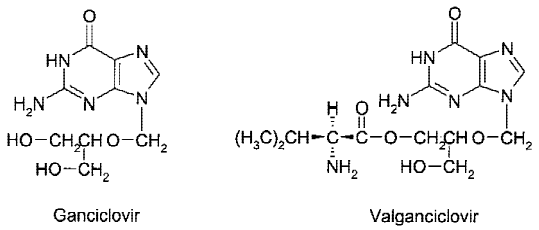

Figure 6. Structures of ganciclovir and valganciclovir.

A similar phenomenon has been described in earlier studies with valaciclovir.[17–19] Although aciclovir is not recognized as a substrate by the intestinal peptide transporter PEPT1 and consequently has poor oral bioavailability, its valyl ester valaciclovir is a good substrate for PEPT1 and hence is absorbed very efficiently in the small intestine. Valaciclovir and valganciclovir are similar in structure except for the presence of a hydroxymethyl ($-CH_2OH$) group in valganciclovir in place of a hydrogen atom in valaciclovir. However, a comparison of the affinities of these two prodrugs for the peptide transporters PEPT1 and PEPT2 reveals a significant difference (Table 1). In the case of PEPT1, whether it is the constitutively expressed transporter in Caco-2 cells or the cloned transporter expressed heterologously in HeLa cells, valaciclovir exhibits a 3- to 4-fold higher affinity than valganciclovir. In contrast, the relative affinities are reversed in the case of PEPT2. Whether it is the constitutively expressed transporter in SKPT cells or the cloned transporter expressed in HeLa cells heterologously, valganciclovir shows a 3- to 4-fold higher affinity towards PEPT2 than valaciclovir. It appears that the presence of the hydroxymethyl group reduces the affinity for PEPT1 and increases the affinity for PEPT2.

Recent studies have shown that the intestinal and renal peptide transporters have a very broad substrate specificity.[7, 8] These transporters recognize as substrates not only the physiologically occurring di- and tripeptides, but also several pharmacologically active agents such as β-lactam antibiotics and bestatin that possess peptide-like structural features. Interestingly, the presence of a peptide bond does not appear to be a prerequisite for recognition as substrates by the peptide transporters. Compounds such as ω-amino fatty acids, δ-amino levulenic acid, p-amino phenylacetic acid, and amino acid amides and esters are effectively transported by PEPT1 as well as PEPT2.[33–37] Recent studies with aciclovir and valaciclovir have shown that even though aciclovir is not a substrate for PEPT1 and PEPT2, addition of a valyl residue in an ester linkage to this compound transforms it to a good substrate for these transporters.[17–19] The present studies with ganciclovir and valganciclovir lead to a similar conclusion. These findings are significant with regard to the design of newer drugs as candidates for oral absorption via the intestinal peptide

Table 1. Comparison of the Affinities of Valaciclovir and Valganciclovir for PEPT1 and PEPT2

	Affinity (mM)			
Prodrug	Caco-2 (PEPT1)	hPEPT1-HeLa	SKPT (PEPT2)	rPEPT2-HeLa
Valaciclovir[a]	0.49 ± 0.04	0.74 ± 0.14	0.17 ± 0.01	0.39 ± 0.03
Valganciclovir	1.68 ± 0.30	2.71 ± 0.40	0.043 ± 0.005	0.113 ± 0.008

[a] Data from ref 17.

transporter PEPT1. There are several nonspecific esterases in the intestinal mucosal cells and in the blood. In contrast, peptidases exhibit a much narrower specificity. Therefore, prodrugs with an amino acid in an ester linkage rather than in a peptide linkage are likely to be more readily hydrolyzed in the intestine and blood. If these prodrugs are transportable substrates for PEPT1, the active drugs will be readily generated by the action of esterases in the mucosal tissue following the PEPT1-mediated entry of the prodrugs into the cells. This use of prodrugs provides an effective means of enhancing the oral bioavailability of drugs that are otherwise not absorbable in the intestinal tract. The present studies also show that valganciclovir is a high-affinity substrate for the renal peptide transporter PEPT2; that is, if the prodrugs that are absorbed in the intestine via PEPT1 do remain intact in the blood, these prodrugs can be absorbed effectively from the glomerular filtrate and be prevented from urinary loss. The renal tubular cells also possess a battery of esterases that might participate in the hydrolysis of amino acid ester-based prodrugs and generate the active drug. Renal tubular absorption of these prodrugs may also lead to their increased half-life in the systemic circulation, thus subjecting them to extended hydrolytic action of esterases in the blood.

Acknowledgments

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