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# Carrier-Mediated Intestinal Absorption of Valacyclovir, the L-Valyl Ester Prodrug of Acyclovir: 1. Interactions with Peptides, Organic Anions and Organic Cations in Rats

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**ABSTRACT:** The mechanism of intestinal transport of valacyclovir (VACV), the L-valyl ester prodrug of acyclovir, was investigated in rats using an *in situ* intestinal perfusion technique. VACV demonstrates an oral bioavailability that is three to five time greater than acyclovir, concentration dependent, and saturable in humans. Homogenate and perfused buffer stability results demonstrated that VACV was increasingly unstable with increasing pH. VACV was converted to ACV in a concentration dependent manner during a single pass through the intestinal segment. Perfusions were performed at 37°C, pH 6.5, and under iso-osmotic conditions ($290 \pm 10$ mOsm $L^{-1}$). Intestinal outlet concentrations were corrected for VACV that was converted to ACV during the perfusion. The effective dimensionless intestinal permeability ($P_e^*$) of VACV was concentration dependent, saturable (intrinsic $K_m = 1.2 \pm 0.7$ mM), and significantly reduced ($p < 0.05$) in the presence of peptide analogues (amoxicillin, ampicillin, cefadroxil, and cephradine), by the organic anion, *p*-amino hippuric acid and by the organic cation quinine. VACV transport was not inhibited by classical nucleoside competitive substrates or inhibitors or by valine. These results suggest that $H^+$–oligopeptide, $H^+$–organic cation, and organic anion transporters are involved in the small-intestinal uptake of VACV. The permeability of VACV in the colon was very low, indicating that VACV is predominantly absorbed from the small intestine. VACV $P_e^*$ was not altered in the presence of glucose-induced convective fluid flow, suggesting that carrier-mediated, transcellular uptake is the predominant absorption pathway of VACV in rat small intestine. Based on these results, the oral bioavailability of VACV appears to be significantly influenced by the preabsorptive conversion of VACV to the poorly absorbed ACV, by the involvement of multiple transporters in VACV small-intestinal uptake, and by the low permeability of VACV in the colon. © 1998 John Wiley & Sons, Ltd.

**Key words:** carrier-mediated transport; valacyclovir; nucleoside; rats; intestine; peptide carrier

## Introduction

Up to 30% of patients with acquired immunodeficiency syndrome (AIDS) will develop sight or life threatening opportunistic cytomegalovirus (CMV) infections [1–3]. Moreover, patients with AIDS are at high risk for developing progressive or recurrent mucocutaneous herpes simplex virus (HSV) or varicella zoster virus (VZV) disease [1]. In order to effectively treat VZV infections and for the suppression of CMV infections, intravenous or high-dose oral acyclovir (ACV) regimens are required [4]. It has also been reported that suppressive therapy in immunocompromised patients with suboptimal oral doses can lead to resistant strains of HSB and VZV [4]. Therefore, in order to develop an oral prodrug of ACV that would achieve plasma levels comparable to intravenous ACV, Beauchamp *et al*. [4] synthesized 18 amino acid esters of ACV. They found that ten of the amino acid prodrugs produced a significantly greater amount of ACV in the urine of rats. They also reported that the L-isomer produced significantly higher urinary levels than the D or DL isomers, suggesting the involvement of a stereospecific transporter or transporters in the intestinal absorption of these compounds.

Valacyclovir (VACV) is the L-valyl ester of the acyclic nucleoside analog of deoxyguanosine, ACV [4]. The mean absolute oral bioavailability of VACV (54%) is three to five times that of ACV in humans [1–5]; however, the rate and extent of VACV absorption has been shown to be dose dependent [1] in human patients. VACV is extensively and almost completely converted to ACV during first-pass intestinal and hepatic metabolism in rats, primates, and humans [4–7]. VACV hydrolysis is mediated chemically and enzymatically; however, its conversion to ACV has been demonstrated in rats to be

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more rapid in blood and tissue homogenates, suggesting that enzymatic hydrolysis is the primary hydrolytic pathway *in vivo* [6]. Similar results have been reported in primates [7] and humans [5]. In humans, VACV has not been detected in stool samples. Only ACV and, in one sample, a small amount of the hydroxy metabolite of ACV were detected, indicating that VACV is converted in the gut lumen to ACV [5]. In a preliminary report, Smith *et al*. [8] demonstrated that the uptake of VACV in primate intestinal brush border membrane vesicles (BBMVs) was concentration dependent, saturable, and inhibited by several valyldipeptides, suggesting the involvement of the intestinal $H^+$–oligopeptide carrier; however, proton dependence was not observed. In this report, the mechanism of intestinal transport of VACV is investigated using an intestinal perfusion technique in rats.

## Materials and Methods

### *Materials*

Ketaset (ketamine) and Rompun (xylazine) were obtained from A.J. Buck (Owings Mills, MD). Valacyclovir was supplied by Glaxo Wellcome (Research Triangle Park, NC). Stavudine (d4T) was supplied by Bristol–Myers Squibb (Wallingford, CT). Formycin B, valine, cefadroxil, amoxicillin, ampicillin, cephradine, quinine, MES (2-[N-morpholino]-ethanesulphonic acid), HEPES (4-(2-hydroxyethyl) -1-piperazine ethanesulphonic acid), D-(+)-glucose, *p*-nitrobenzyl-6-thioinosine (NBTI), *p*-amino hippuric acid sodium salt (PAH), PEG-3350, thymidine, and dimethyl sulphoxide were obtained from Sigma (St. Louis, MO). $^{14}$C-PEG-3350 was obtained from Du Pont NEN (Boston, MA). All other chemicals were obtained from Fisher Scientific (Fair Lawn, NJ) and were used as received.

### *Methods*

*Single-Pass Perfusion (SPIP) Procedure*. SPIP is an *in situ* uptake method [9] performed in anesthetized rats in which the intestinal segment of interest is cannulated and perfused with the drug solution. This method relies on the measurement of the rate of drug disappearance by analysing the inlet and outlet samples for drug concentration.

Viral free male Sprague–Dawley rats weighting 250–350 g were used in accordance with a protocol approved by the Institutional Review Board—Use and Care of Animals Committee (Rutgers University, 92-004). Following an overnight fast (12–18 h), with water given *ad libitum*, anesthesia was induced by an intramuscular injection of ketamine–xylazine (60 mg $kg^{-1}$ ketamine–8 mg $kg^{-1}$ xylazine). The rats were then placed on a warming pad (Vetko thermal barrier, Harvard Apparatus, South Natick, MA) under a surgical lamp to maintain body temperature. Upon verification of loss of pain reflex, a midline abdominal incision of 3–4 cm was made, the intestinal segment of interest was located and a cannula was inserted. The first cannula was inserted and the second was placed 5–10 cm aboral of the first incision. The exposed intestinal segment was moistened with warm saline and covered with moistened gauze and parafilm to prevent moisture loss. Each segment was perfused by connecting the inlet Tygon tubing (Norton, Akron, OH) to a 50 mL syringe (Popper, New Hyde Park, NY) placed in a Harvard Infusion Pump (model 55, Harvard Apparatus Company, South Natick, MA). The tubing was submerged in a water bath (model 183, Precision Scientific Instruments, Chicago, IL) maintained at 38°C. The inlet and the outlet tubing were positioned as far as possible at the same height to prevent gravitational flow. The intestinal segment of choice was perfused with VACV in an iso-osmotic (290 mOsm $kg^{-1}$) MES buffer (pH 6.5) containing 15 mM MES, 130 mM NaCl, 5 mM KCl, 0.01% (w/v) PEG-3350, and a tracer amount (1.9 nCi $mL^{-1}$) of its $^{14}$C isotope. When 0.01mM VACV was coperfused with 3 mM quinine, the perfusion medium was HEPES buffer containing 15 mM HEPES, 130 mM NaCl, 5 mM KCl, 0.01% (w/v) PEG-3350, and a tracer amount (1.9 nCi $mL^{-1}$) of its $^{14}$C isotope adjusted to pH 8.0 using 1 N NaOH. PEG-3350 was used as a non-absorbable marker for the purpose of assessing net intestinal water transport. The perfusion rate was 0.191 mL $min^{-1}$ and after 40 min, when the steady state was reached, seven or eight outlet samples were collected at 10 min intervals. The samples were then analysed by the HPLC method. The outlet VACV concentrations were corrected for net water flux based on the changes in $^{14}$C-PEG concentrations between the inlet and outlet samples. At the conclusion of the experiment, the intestinal segment was measured for its length. The rats were euthanized with an overdose of sodium pentobarbital administered intravenously in accordance with AAALAC recommendations.

The SPIP method assumes that the disappearance of drug from the lumen of the intestine during the perfusion is due to the permeation of the intestine; therefore, the determination of nonabsorptive disappearance of VACV is required. To determine the nonabsorptive disappearance of VACV, three studies were performed (i) the stability of 0.1 mM VACV was investigated in perfused MES buffer at room temperature, (ii) the stability in intestinal homogenate enzyme solution was assessed, and (iii) the *in situ* conversion of VACV to ACV was studied. In order to establish the intrinsic baseline permeability and to determine the mechanism(s) of intestinal transport, the permeability of VACV was

 

measured using various inlet concentrations: 0.01, 1, 5, 10, and 25 mM. The intestinal transport mechanism of VACV was further elucidated by coperfusion of 0.01 mM VACV with various concentrations of putative VACV transport inhibitors. The concentrations of inhibitors used were 0.5 mM formycin B, 0.5 mM thymidine, 0.1 mM NBTI, 0.5 mM d4T, 5 mM amoxicillin, 5 mM cefadroxil, 5 mM cephradine, 5 mM ampicillin, 5 mM *p*-amino hippuric acid, 5 mM valine, and 3 mM quinine. In order to investigate the effect of convective fluid flux on VACV permeability, VACV (0.01 mM) was coperfused with an iso-osmotic MES buffer containing 25 or 56 mM glucose at pH 6.5.

*Stability of Valacyclovir in Perfused MES Buffer at Room Temperature*. Stability studies of VACV in perfused MES buffer were carried out at three different pH values. Two rats were perfused with iso-osmotic MES buffer (pH 6.5) containing 15 mM MES, 130 mM NaCl, 5 mM KCl, and 0.01% (w/v) PEG-3350 with a flow rate of 0.191 mL $min^{-1}$. After 40 min, when equilibration was achieved, the perfused buffer was collected for a period of approximately 80 min and immediately put in ice to preserve the activity of the intestinal enzymes. About 15 mL of 0.1 mM solution of VACV was prepared and divided into three sets. One set was used as is, the second set was adjusted to pH 5 by adding 1 N HCl, and the third set was adjusted to pH 7.5 by adding 1 N NaOH. Samples of these solutions were then injected into the HPLC every 50 min for 5 h. The amount of VACV remaining at each time period was calculated using the initial peak area (area at time 0) as the control.

*Stability of VACV in Rat Small-Intestinal Homogenate*. Stability studies of VACV in rat small-intestinal homogenate were carried out at two different concentrations (0.1 and 1 mM) and at two different temperature conditions (25 and 37°C). Following an overnight fast (12–18 h), with water given *ad libitum*, the rats were anesthetized by an intramuscular injection of ketamine–xylazine (60 mg $kg^{-1}$ ketamine–8 mg $kg^{-1}$ xylazine). Upon verification of loss of pain reflex, a mid-line incision was made and the intestinal region of interest was located (upper small intestine), cut, and placed in ice cold Ringers buffer (pH 7.4 and iso-osmotic). The intestine was rinsed with 25 mL ice cold Ringers buffer using a 60 $cm^3$ syringe to clean the intestine of any undigested food. The brush border was scraped out of the intestine by placing the intestine on a glass plate kept on ice and scraping it with a metal spatula. A 20% (w/v) solution of the intestinal tissue was made in Ringers buffer in a chilled blender by blending it at high speed for 2 min. The homogenate was then centrifuged in a Beckman JA-17 rotor at 12 000 × *g*, 4°C, for 15 min to remove intestinal material other than the brush border present in the intestinal homogenate. The supernatant (homogenate enzyme solution) was removed and used immediately for the stability studies of VACV in the intestinal homogenate. For the stability studies performed at 37°C, 450 μL of this enzyme solution was incubated in a water bath at 37°C for 15 min. Immediately after the incubation period, the vial was spiked with 50 μL VACV stock solution. The sample was mixed immediately and 50 μL taken out and added to 450μL of the stopping solution (ice cold mixture of 200 μL methanol and 200 μL MES buffer). This comprised the initial (0 min) sample and subsequently 50 μL was taken out every 5 min and added to the stopping solution. These samples were collected for a period of 30 min and then centrifuged at 12 000 × *g* for 5 min at 4°C. The supernatant was collected and analysed using the HPLC to obtain the amount of VACV remaining non-degraded at different time intervals. For the stability studied at 25°C, about 2 mL of the homogenate enzyme solution was incubated in a water bath at 25°C for 15 min. At the end of the incubation period, the enzyme solution was spiked with the VACV stock solution followed by vigorous stirring and then injected into the HPLC every 30 min for 3 h. The amount of VACV remaining at each time period was calculated using the initial peak area (area at time 0) as the control.

*In Situ Formation of ACV and Correction of Outlet Perfusate Concentrations*. Since VACV was unstable in the presence of intestinal homogenate enzyme solution but stable in the perfusion buffer, the *in situ* conversion of VACV to ACV was investigated. The HPLC chromotograms of the outlet samples demonstrated a distinct peak for ACV. Since this peak was due to the degradation of VACV, a correction of the outlet to inlet ratio of VACV concentrations was determined. The outlet concentrations of VACV were multiplied by the correction factor to account for the nonabsorptive disappearance of VACV.

*Analytical Methods*. VACV concentrations were quantitated using an HPLC method. The HPLC instrumentation included a Waters solvent delivery module (model 600E), a multi-wavelength UV detector (model 490E), and a WISP (model 712, 717) (Waters, Milford, MA). Separations were performed on a Supelcosil LC-18S column (25 cm × 4.6 mm) protected by a guard column Supelguard LC-18S (2 cm) (Supelco, Bellefonte, PA). The flow rate was 1 mL $min^{-1}$ and the detection wavelength was 252 mm. The buffer used for the analysis consisted of 0.1 M potassium phosphate monobasic containing 25% (v/v) methanol which was adjusted to pH 6.7 using 1 N sodium

hydroxide. The buffer was filtered through 0.45 μm HPLC certified membrane filters (Gelman Sciences, Ann Arbor, MI) and degassed under vacuum and sonication. The mobile phase consisted of buffer–water (80:20) mixture which was accomplished by on-line mixing. When VACV was co-perfused with formycin B, thymidine, NBTI, or d4T, the mobile phase consisted of buffer–water (50:50) mixture. Similarly, for the perfusion studies of VACV in the presence of $\beta$-lactam antibiotics (ampicillin or cephradine) the mobile phase was buffer–water (90:10) mixture. For all other inhibitor studies the mobile phase consisted of buffer–water (80:20). These variations were required to ensure that there was no coelution of VACV with the inhibitors used in the experimental study. For net water flux measurements, $^{14}$C PEG-3350 was measured using liquid scintillation counting (model 1409, Wallac, Gaithersburg, MD) by adding 0.8 mL perfusate sample to 5 mL of the liquid cocktail ScintiVerse BD.

*Valacyclovir Assay Validation*. The standard curve for VACV was obtained by dissolving known amounts of VACV in MES buffer and performing the HPLC analysis described in the preceding section. The concentration range of VACV studied was 0.001–25 mM to cover the entire range of experimental studies. Intra-day assay variability was assessed by analysing six injections from each of the six concentrations (0.001, 0.01, 1, 5, 10, and 25 mM VACV). Inter-day variability was also studied by repeating the triplicate injections for all six concentrations on different days. There was less than 1% intra- and inter-day variability detected. The standard curve for VACV in unperfused MES buffer was found to be linear with the square of correlation coefficient value of 0.9999. The sensitivity of the HPLC analysis was also verified by taking a solution of VACV in perfused MES buffer at initial concentrations from 0.01 to 25 mM and then diluting these samples to 95% of the initial concentration using the buffer. Both sets of samples were injected in triplicate to determine whether the 5% difference in concentration could be reliably detected.

*Data Analysis*. The perfusion data for VACV was analysed using the numerical aqueous resistance and nonlinear regression (NAR–NLR) analysis [10]. The NAR–NLR method allows for the use of a wider range of flow conditions than the modified boundary layer (MBL) analysis [11,12], resulting in more reliable and less variable estimates of intestinal transport parameters as well as intestinal wall permeability.

*Statistical Analysis*. One-way analysis of variance was used to test the difference in the mean values among the treatment groups followed by a *post hoc* Dunnett's test to examine significance within a treatment group. Weighted nonlinear regression analysis (BMDP Statistical Software, Los Angeles, CA) was used to determine the intrinsic transport parameters. $p < 0.05$ was used as the significance level for all tests.

## Results

Stability studies were carried out in perfused MES buffer at pH 5.0, 6.5, and 7.5 at 25°C. As shown in Figure 1, VACV was most stable at pH 5.0 and least stable at pH 7.5. The initial disappearance rate constant ($k_{diss}$, $min^{-1}$) was estimated from the initial slope on the semilogarithmic plot of the VACV remaining versus incubation time. The apparent first-order half-lives were determined using $t = 0.693/k_{diss}$. The apparent half-lives were 577.5, 38.5, and 19.3 h, for pH 5.0, 6.5, and 7.5, respectively, indicating adequate stability of VACV in the perfusion buffer during the procedure. The stability of VACV in intestinal homogenates followed a similar trend although VACV was less stable in the presence of the intestinal homogenate. As shown in Figure 2, the stability of VACV at pH 7.5, 25°C, did not vary significantly with changes in VACV concentration. The apparent first-order half-lives of VACV were 1.5 and 1.4 h for initial VACV concentrations of 1 and 0.1 mM, respectively. VACV in the intestinal homogenate at pH 7.5, 37°C, was found to have half-lives of 0.27 and 0.25 h for initial concentrations of 1 and 0.1 mM, respectively. As a result of the instability of VACV in the presence of intestinal homogenate, the *in situ* conversion of VACV was investigated at concentrations corresponding to the inlet perfusate concentrations. The perfused concentration and correction factors were as follows:

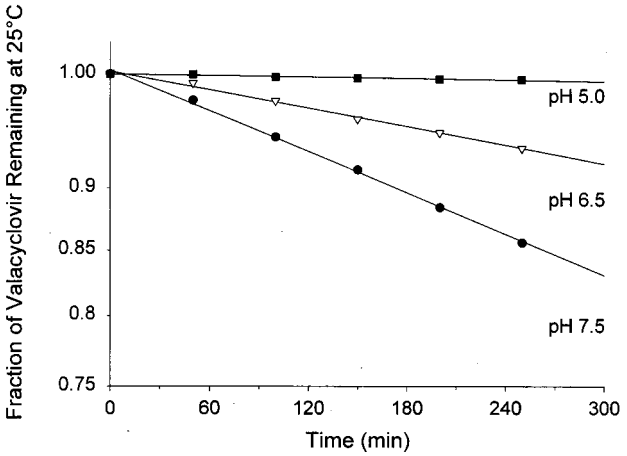


Figure 1. A plot of the stability of valacyclovir (0.1 mM) in perfused MES buffer observed at 25°C at pH 5.0, 6.5, and 7.5. Valacyclovir was most stable at pH 5 with the rate of degradation approximately 10 times faster at pH 6.5 and around 25 times faster at pH 7.5

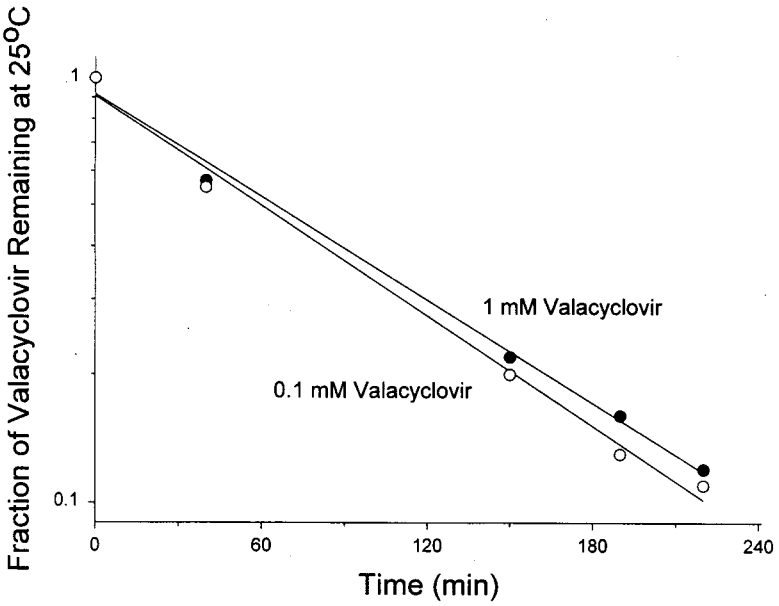


Figure 2. The stability of valacyclovir (0.1 and 1 mM) was studied in rat intestinal homogenates at 25°C. The homogenate was incubated in a water bath maintained at 25°C for 15 min. An aliquot of the enzyme solution was spiked with the VACV stock solution. A sample was injected into the HPLC every 30 min for 3 h. The stability of VACV did not vary significantly with the change in concentration and the apparent first-order half-life was found to be approximately 1.5 h for both initial VACV concentrations

0.01 mM, 1.27; 1 mM, 1.14; 5 mM, 1.07; 10 mM, 1.03; 25 mM, 1.00. Given the length of intestine, the flow rate, and the previously reported permeability of ACV ($P^*_w < 0.15$) in rats [13], the amount of ACV absorbed from the intestinal segment was considered negligible. Therefore, the amount of ACV measured in the perfusate accounted for nearly all of the ACV formed presystemically.

### *VACV Intestinal Transport Studies*

The intestinal transport of VACV was investigated using a steady-state uptake procedure in rats. Steady state was achieved prior to taking the first sample at 40 min (Figure 3). Coperfusion of VACV with putative transport inhibitors demonstrated varied results. Coperfusion of 0.01 mM VACV with 0.5 mM formycin B, thymidine, or d4T or 0.1 mM NBTI did not reduce the mean dimensionless effective permeability, $P^*_e$ (Figure 4). Five millimolar amoxicillin, ampicillin, cefadroxil, cephradine, and *p*-amino hippuric acid and 3 mM quinine significantly reduced $P^*_e$ while 5 mM valine reduced $P^*_e$ but not significantly (Figure 5). The concentration dependence of $P^*_e$ is shown in Figure 6. The intrinsic intestinal transport parameters were calculated

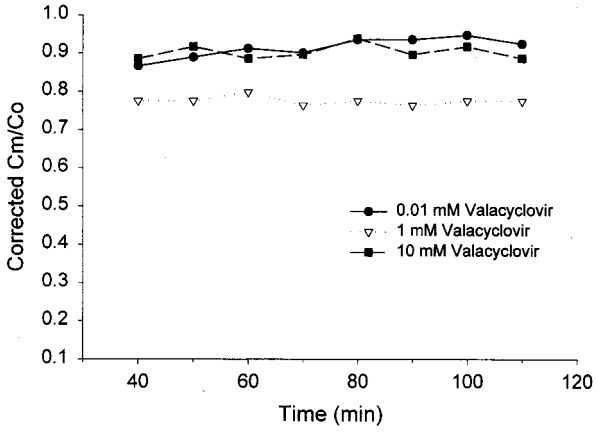


Figure 3. A plot of the corrected $C_m/C_0$ versus time demonstrating the achievement of steady state after equilibration of the intestinal segment with iso-osmotic MES buffer (pH 6.5, 37°C) for 30 min. $C_m/C_0$ are corrected for net fluid flux using $^{14}$C-PEG-3350 as a nonabsorbable marker. A total of eight perfusion samples were collected every 10 min after equilibration

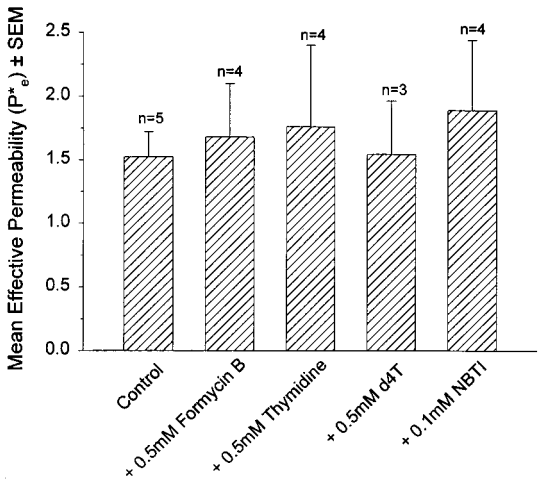


Figure 4. A plot of the effective permeability ($P^*_e$) of the valacyclovir in the presence of nucleoside inhibitors—formycin B, thymidine, NBTI, and d4T. One-way ANOVA showed that the differences in the mean $P^*_e$ values were not significant enough to exclude the possibility that the differences were due to random sample variability

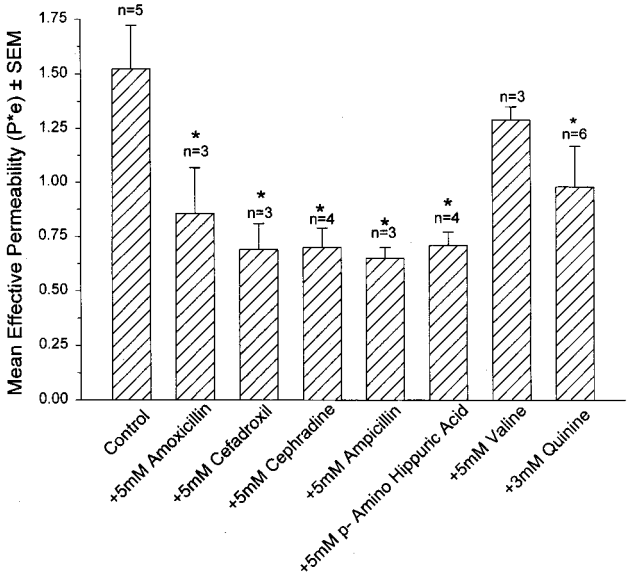


Figure 5. A plot of the effective permeability ($P_e^*$) of valacyclovir in the presence of peptide inhibitors (amoxicillin, ampicillin, cefadroxil, and cephradine), an organic anion transport inhibitor (*p*-amino hippuric acid), and an organic cation transport inhibitor (quinine). One-way ANOVA showed that the difference in the mean values among the treatment groups were statistically significant ($p < 0.004$). Dunnett's test demonstrated that all of the inhibitors significantly reduced the $P_e^*$ of valacyclovir with the exception of valine

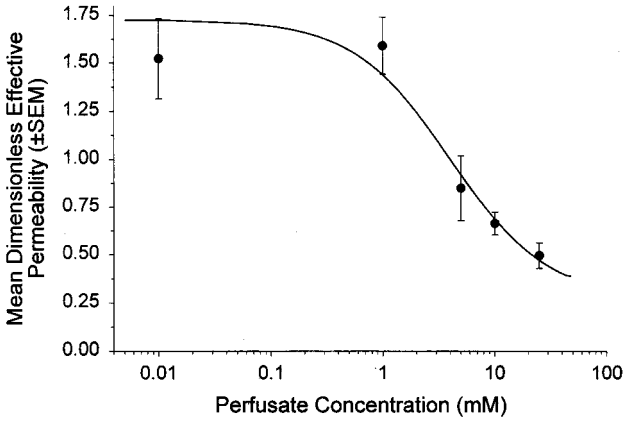


Figure 6. A plot of the concentration dependence of valacyclovir effective permeability. Michaelis–Menten transport parameters were determined using weighted nonlinear regression and are reported in the text. The intrinsic and apparent hybrid $K_m$ values are consistent with those previously reported in the literature for peptide analogues

using the NAR–NLR analysis [10] and were found to be $P_c^* = 3.75 \pm 0.91$, $K_m = 1.22 \pm 0.74$ mM, $P_m^* = 0.37 \pm 0.17$. The $P_e^*$ of VACV was insensitive to changes in convective fluid flux (Figure 7) induced by varying glucose concentrations (control, 25 or 56 mM) in the perfusate. The permeability of VACV was reduced by 50% at pH 5.0 ($n = 4$). VACV (0.01 mM) permeability in the colon ($n = 4$) was low ($0.09 \pm 0.01$). The results of the statistical analysis are given in Table 1.

## Discussion

Since the SPIP method measures drug disappearance as an indicator of intestinal drug uptake, the nonabsorptive disappearance of VACV was determined under perfusion conditions. It has been reported that VACV is extensively and almost completely converted to ACV during first-pass intestinal and hepatic metabolism in rats, primates, and humans [4–7]. VACV hydrolysis is mediated chemically and enzymatically; however, its conversion to ACV has been demonstrated in rats to be more rapid in blood and tissue homogenates, suggesting that enzymatic hydrolysis is the primary hydrolytic pathway *in vivo* [6]. Our results in perfused buffer and rat intestinal homogenates were consistent with these findings. For example, at pH

Table 1. One-way ANOVA table for effective permeability of valacyclovir in presence of various inhibitors. Dunnett's test for statistical analysis after ANOVA resulted in a statistically significant difference at $p < 0.004$

| Treatments | Mean ($\pm$S.E.M.) $P_e^*$ | Significance at $p < 0.05$ |
|---|---|---|
| Control (0.01 mM VACV) | 1.522 (0.206) | — |
| + amoxicillin | 0.856 (0.213) | Yes |
| + cefadroxil | 0.694 (0.124) | Yes |
| + cephradine | 0.700 (0.094) | Yes |
| + ampicillin | 0.654 (0.057) | Yes |
| + PAH | 0.711 (0.064) | Yes |
| + valine | 1.290 (0.060) | No |
| + quinine | 0.948 (0.141) | Yes |

| Sources of variation | DF | SS | MS | *F* |
|---|---|---|---|---|
| Treatments | 7 | 2.782 | 0.397 | 4.339 |
| Residuals | 21 | 1.923 | 0.091 | |
| Total | 28 | 4.705 | | |

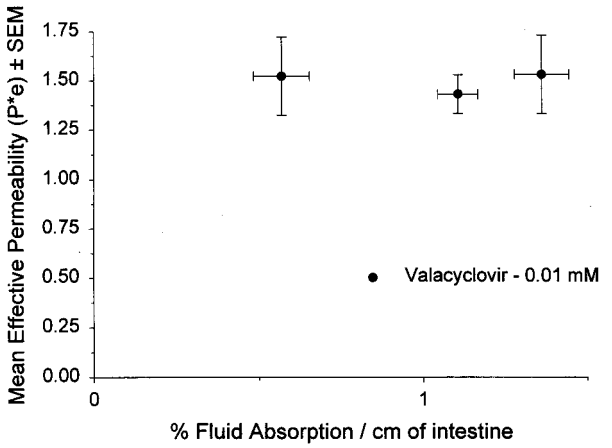


Figure 7. A plot depicting the lack of effect of glucose-induced fluid absorption on the mean effective permeability ($P_e^*$) of valacyclovir in rat small intestine. Valacyclovir concentration in MES buffer (pH 6.5, iso-osmotic at 37°C) was 0.01 mM

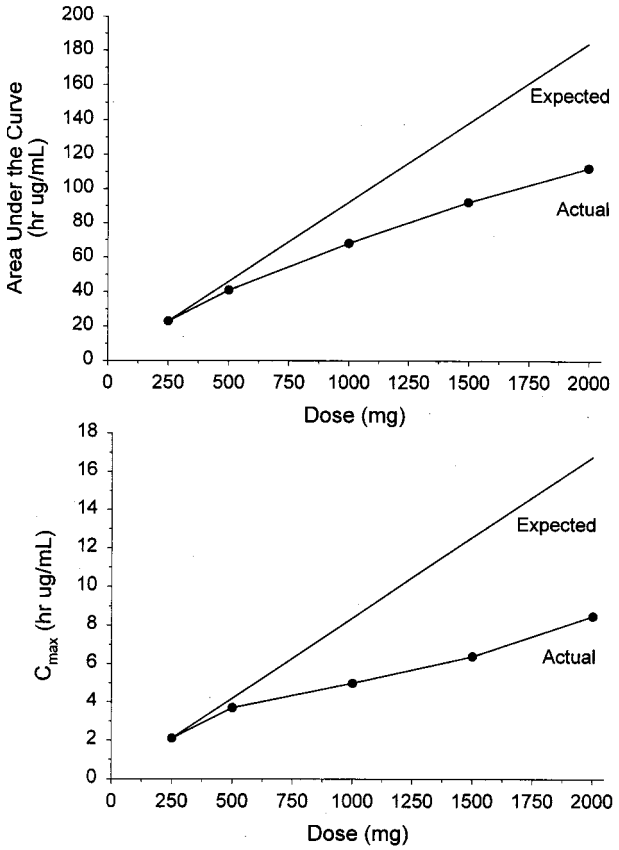


Figure 8. A plot of $C_{max}$ and AUC versus the VACV dose administered to human patients. The results are adapted from [1]. If the VACV absorption pharmacokinetics were linear, dose proportional increases in $C_{max}$ and AUC would be expected; however the actual values are significantly less than proportional, suggesting the involvement of a nonlinear (carrier mediated) intestinal transport mechanism consistent with the present study results in rats. Both rats and humans are known to express the $H^+$–oligopeptide transporter in their respective small intestines

7.5 VACV was 13 times less stable in intestinal homogenate than in perfused buffer. The stability of VACV decreased with increasing pH in either buffer or tissue homogenates and was consistent with a previous report that showed the half-lives of VACV at pH 6.0 and 7.4 (37°C) were 95 and 13 h, respectively [4]. Extensive presystemic conversion of VACV to ACV would increase the value of VACV permeability since it is assumed that VACV disappearance from the perfusing solution indicates absorption. The presystemic conversion of VACV to ACV ranged from 0% at high concentrations to as much as 25% at low concentrations. By correcting the outlet concentrations for ACV appearance, the confidence level in the calculated permeabilties is high.

The $P_e^*$ of VACV was concentration dependent, saturable, and inhibited by several compounds including peptide analogues, *p*-amino hippuric acid (PAH), an organic anion, and quinine, an organic cation. The permeability of VACV, an acyclic nucleoside analog, was not inhibited by prototypical nucleoside transport inhibitors such as formycin B, thymidine, or NBTI which inhibit the N1, N2 and *es* nucleoside transporters, respectively [14–18] even though the concentrations of inhibitors used in the current studies have been shown to significantly inhibit the transport of other nucleoside analogues such as d4T [19]. The penicillin and cephalosporin antibiotics significantly reduced the permeability of VACV compared to the control. This is consistent with previously published results regarding the interaction between cephalosporins and the intestinal $H^+$–oligopeptide transporter [20–22]. In the kidney, these peptide analogs also interact to varying degrees with the organic anion and cation transporters as well [23]. Further, significant overlap in the specificity of the renal organic anion and organic cation transporters has been demonstrated [24]. There is a paucity of literature concerning the intestinal organic anion and organic cation [25] transporters. However, other transporters found in the liver and kidney (e.g. $H^+$–oligopeptide, $Na^+$–nucleoside) have been described in the intestine as well. Therefore, the organic cation, quinine, and the organic anion, PAH, were coperfused with VACV as putative inhibitors. Quinine and PAH significantly inhibited VACV uptake, suggesting an interaction between VACV and those transporters. Given the potential overlap in substrate specificities, it is difficult to determine the exact nature of the interactions of VACV with the organic anion and cation transporters. In order to confirm the involvement of intestinal carriers with the absorption of VACV, concentration dependence was studied. VACV uptake was found to be concentration dependent and saturable with a hybrid $K_m$ of $1.2 \pm 0.7$ mM. The intrinsic hybrid $K_m$ of VACV is in the range typically observed for peptides and peptide analogs in the intestine, about 1–10 mM. The inhibi-

tion and concentration dependent uptake results strongly indicate the involvement of three carriers in the uptake of VACV from rat small intestine. The inhibition of VACV permeability in the presence of a significant inwardly driven proton gradient is a result of the interplay among the three transporters since the oligopeptide transporter is an $H^+$ symporter and the organic cation transporter is an $H^+$ antiporter. The intestinal organic anion transporter has not been well characterized. The permeability of VACV in the proximal colon was very low, also typical of a compound absorbed primarily by specialized transporters which are located in the small intestine.

The mean absolute oral bioavailability of VACV (54%) is three to five times that of ACV in humans [1,5]; however, the rate and extent of VACV absorption has been shown to be dose dependent [1] in human patients. A plot of the data (Figure 8) reported by Jacobson [1] clearly demonstrates the concentration dependence of $C_{max}$ in humans. A direct link between $C_{max}$ and intestinal permeability exists since $C_{max}$ is directly related to the absorption rate. The absorption rate is the product of the absorption rate constant ($k_a$) and the intestinal VACV concentration, and $k_a = 2/rP$, where $r$ is the intestinal radius and $P$ is permeability. If it is assumed that the doses of VACV (250, 500, 1000, 1500 and 2000 mg) are administered with a half glassful of water ($\sim$150 mL), then the initial intestinal concentration range of VACV ranges from about 5 mM to about 40 mM. This range of concentrations equals or exceeds the $K_m$ reported in the current studies. Based on these calculations, at the 1000 mg dose ($>4-5$ $K_m$), saturation of absorption is expected if the rat transport parameters are indicative of human transport behaviour. This is reflected in humans in the $C_{max}$ plot in Figure 8, suggesting that the transport kinetics are similar to those in rats. Although the estimation of the initial intestinal concentration of VACV may vary depending on the volume selected, choosing significantly lower or higher volumes doesnot qualitatively affect the conclusion. The dose dependence of AUC is also consistent with the absorption mechanism results of the current studies. As shown in Figure 8, the AUC increased less than proportionally and represented only 61% of the proportional value at the highest dose. Since most transport proteins are located in the small intestine an 'absorption window' exists for compounds such as VACV whose predominant absorption mechanism is carrier mediated. Therefore, the reduction in AUC is probably a result of the greatly diminished absorption rate due to the saturation of the intestinal carriers and the subsequent passage of VACV into the colon, where it appears to be extremely poorly absorbed. The reduction in permeability alone cannot account for the entire reduction in extent of absorption at high VACV doses. Therefore, a more likely scenario is that a reduction in permeability (absorption rate) would increase the residence time of VACV in the intestine, thereby increasing the potential for preabsorptive conversion to the more poorly absorbed ACV.

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