Pharmacokinetics of Novel Dipeptide Ester Prodrugs of Acyclovir after Oral Administration: Intestinal Absorption and Liver Metabolism

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ABSTRACT
The amino acid prodrug of acyclovir (ACV), valacyclovir (VACV), is an effective antiviral drug. Systemic availability of ACV in humans is 3 to 5 times higher after oral administration of VACV. Enhanced bioavailability of VACV has been attributed to its carrier-mediated intestinal absorption via hPEPT1 peptide transporter followed by rapid and complete conversion to ACV. An earlier report suggested that the dipeptide ester prodrugs of ACV possess high affinity toward the intestinal oligopeptide transporter hPEPT1 and therefore seem to be promising candidates in the treatment of oral herpes virus infections. In the present study, we have examined the bioavailability of a series of dipeptide prodrugs of ACV after oral administration in Sprague-Dawley rats with cannulated jugular and portal veins. The area under plasma-concentration time curves expressed as minutes microgram milliliter−1 for total concentration of VACV (208.4 ± 41.2), and the dipeptide prodrugs Gly-Val-ACV (GVACV) (416.1 ± 140.9), Val-Val-ACV (VVACV) (147.7 ± 89.3), and Val-Tyr-ACV (VYACV) (180.7 ± 81.2) were significantly higher than that of ACV (21.2 ± 5.2) upon intestinal absorption. Interestingly, the bioavailability of ACV after administration of GVACV was approximately 2-fold higher than VACV. There was significant metabolism by hepatic first pass effect of the dipeptide prodrugs as evident by the higher levels of ACV obtained after systemic absorption compared with intestinal absorption of GVACV and VVACV. The dipeptide prodrugs of ACV exhibited higher systemic availability of regenerated ACV upon oral administration and thus seem to be promising drug candidates in treatment of genital herpes infections.

Peptide transporters PepT1 and PepT2 are perhaps the drug transporters that have captured the most recent attention in drug delivery. Small peptides such as di- and tripeptides are transported by PepT1 and PepT2 in intestinal and renal epithelial cells, respectively. Structure, function, mechanism, and substrate specificity of the peptide transporters have been extensively studied (Ganapathy and Leibach, 1982, 1986; Dantzig and Bergin, 1990; Ganapathy et al., 1995; Hidalgo et al., 1995; Hu et al., 1995; Liang et al., 1995; Tanaka et al., 1998; Oghara et al., 1999; Zhu et al., 2000). Due to their broad substrate specificity, PepT1 and PepT2 contribute to the intestinal absorption of several drug compounds such as β-lactam antibiotics, cephalosporins, angiotensin-converting enzyme, and renin inhibitors (Dantzig and Bergin, 1990; Inui et al., 1992; Hashimoto et al., 1994; Han et al., 1998a; Kiss et al., 2000). Even compounds without a peptide bond such as δ-amino levulinic acid (Temple et al., 1998) and α-amino fatty acid (Doring et al., 1998) are known to be absorbed with the help of these transporters. A surprisingly diverse substrate specificity of the peptide transporters has been used very successfully to improve the bioavailability of the nucleoside analogs acyclovir (Steingrimsdottir et al., 2000) and zidovudine by designing 5′-amino ester prodrugs (Han et al., 1998b). A third peptide transporter, peptide/histidine transporter, translocates histidine and small peptides with high affinity and in a proton gradient-dependent manner (Yamashita et al., 1997). Although the presence of the peptide/histidine transporter has been confirmed in brain and eye, it is not found in intestine or kidney and shows little homology (<20%) with PepT1 and PepT2. The exact physiological role of this transporter is yet to be ascertained.

Strategies have been used to design prodrugs of various poorly absorbed drugs targeted toward receptors/transport-
ers to improve systemic bioavailability (Lupia et al., 1993; Weller et al., 1993; Guo and Lee, 1999; Sakaeda et al., 2001; Anand et al., 2002; Manfredini et al., 2002). Valacyclovir (VACV) is such a prodrug that is derived from acyclovir (ACV) by esterifying 3'-hydroxyl group of ACV with L-valine. Acyclovir, an antiviral nucleoside, possesses activity against human herpesviruses. Owing to its limited bioavailability, ACV has shown moderate antiviral efficacy after oral administration (Steinigrimsdottir et al., 2000). After oral administration, valacyclovir VACV is rapidly absorbed from the gastrointestinal tract and is converted to acyclovir and L-valine by first pass intestinal and/or hepatic metabolism. VACV has been reported to increase the oral bioavailability of acyclovir by 3- to 5-fold in humans (Beauchamp et al., 1992; Lupia et al., 1993; Weller et al., 1993). Enhanced oral absorption (Balimane et al., 1998; de Vrueh et al., 1998; Han et al., 1998b) of acyclovir after administration of valacyclovir has been attributed to the hPEPT1-mediated intestinal translocation of the amino acid prodrug. Recently, a protein with significant hydrolytic activity toward valacyclovir was identified and purified from Caco-2 cells and its identity with the biphenyl hydrolase-like (BPHL) protein, previously cloned from human breast cancer tissue was established (Kim et al., 2003). The high expression of BPHL in the human intestine, liver, and kidney (Puente and Lopez-Otin, 1995) suggests an important role for BPHL in the activation of VACV in human tissues.

A series of novel water-soluble dipeptide ester prodrugs of acyclovir (U.S. patent pending) were synthesized previously (Nashed and Mitra, 2003). These compounds were designed to target the peptide transporters on the cornea and intestinal epithelial cells for improved ocular and oral absorption of acyclovir, respectively. Results indicated that the dipeptide ester prodrugs of ACV exhibited high affinity toward the intestinal oligopeptide transporter, and the uptake of these prodrugs was efficiently mediated by hPEPT1 as suggested by significant inhibition of uptake of glycylsarcosine. These prodrugs hydrolyzed readily to regenerate the active parent drug, acyclovir (Anand et al., 2003). In this report, we describe pharmacokinetics and the bioavailability of these prodrugs after oral administration in Sprague-Dawley rats. The role of intestinal and hepatic first pass effect on metabolism of these dipeptide prodrugs is also discussed. The transport characteristics of these prodrugs across Caco-2 monolayers were compared with that of VACV to establish whether these compounds may be transported across enterocytic cell membranes owing to their recognition by the peptide transporter.

**Materials and Methods**

**Materials**

[3H]Glycylsarcosine (4 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA), and [14C]mannitol (50 mCi/mmol) was supplied by Amersham Biosciences Inc. (Piscataway, NJ). Valacyclovir was a gift from GlaxoSmithKline (Research Triangle Park, NC). Human colon carcinoma derived Caco-2 cells were obtained from American Type Culture Collection (Rockville, MD). The growth medium Dulbecco’s modified Eagle’s medium was obtained from In-vitrogen (Carlsbad, CA). Minimal essential medium nonessential amino acids, penicillin, streptomycin, sodium bicarbonate, HEPES, and unlabeled glycylsarcosine were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS) was obtained from JRH Biosciences (Lenexa, KS). Culture flasks (75-cm² growth area) and polyester membranes (pore size 0.4 μm) were procured from Costar (Bedford, MA). The buffer components and solvents were obtained from Fisher Scientific (St. Louis, MO). All the dipeptide prodrugs of acyclovir (Fig. 1) were synthesized in our laboratory according to previously published procedures (Nashed and Mitra, 2003). Four dipeptide prodrugs were used in this study: valine-valine acyclovir (VVA CV), glycine-valine acyclovir (GYACV), GYACV, glycine-tyrosine acyclovir (GYACV), and valine-tyrosine acyclovir (VYACV).

**Animals**

Portal and jugular vein cannulated male Sprague-Dawley rats weighing 200 to 250 g were obtained from Charles River Laboratories (Wilmington, MA). Animal care and treatment in this investigation were in compliance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

**Metabolism Studies**

**Plasma Hydrolysis.** Eight hundred microliters of the rat plasma was incubated with 200 μl of 1 mM solutions of prodrugs at 37°C in a shaking water bath for the length of the study. Fifty-microliter samples were withdrawn at predetermined time intervals and were immediately diluted with 50 μl of chilled acetone/methanol (4:5 mixture) to precipitate the proteins and supernatant was stored at −80°C until further analysis. Apparent first order rate constants were calculated and corrected for any chemical hydrolysis observed with the control.

**Intestinal Homogenate Studies.** Noncannulated male Sprague-Dawley rats were euthanized by lethal injection of sodium pentobarbital through the tail vein. Intestinal segments were isolated and stored at −80°C before use. Tissues were homogenized in 5 ml of chilled (4°C) DPBS for about 4 min with a tissue homogenizer (Tissue Tearor model 985-370; Biospec Products Inc., Bartlesville, OK) in an ice bath. Subsequently, the homogenates were centrifuged at 14,000g for 25 min at 4°C to remove cellular debris, and the supernatant was used for hydrolysis studies. Protein content of the supernatant was determined by the method of Bradford (Bradford, 1976) with bovine serum albumin as the standard (Bio-Rad protein estimation kit; Bio-Rad, Hercules, CA). The supernatant was equilibrated at 37°C for about 30 min before an experiment. Hydrolysis was initiated by the addition of 0.2 ml of a 1 mM prodrug solution to 0.8 ml of the supernatant. The control consisted of 0.8 ml of DPBS instead of the supernatant. Aliquots (50 μl) were withdrawn at appropriate time intervals for up to 24 h. The samples were immediately diluted with 50 μl of chilled acetone/methanol (4:5 mixture) to precipitate the proteins and stored at −80°C until further analysis. Apparent first order rate constants were calculated and corrected for any chemical hydrolysis observed with the control.

**Cell Culture**

All cell cultures were maintained in humidified incubator at 37°C with a 5% carbon dioxide in air atmosphere. Caco-2 cells were obtained at passage 25 from American Type Culture Collection and grown in plastic tissue culture flasks. Conventional culture medium containing Dulbecco’s modified Eagle’s medium, 10% FBS (heat-inactivated), 1% nonessential amino acids, 4 mM l-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 14 mM HEPES at pH 7.4 was used according to the protocol established in our laboratory for maintaining the cell line. Upon reaching 80% confluence, cells were removed by trypsin/EDTA treatment and plated at a density of 100,000 cells/cm² on collagen-coated plastic dishes containing clear polyester membranes (0.636 cm², 3.0–μm mean pore size). Cells were then grown in medium containing 10% FBS (heat-inactivated). Caco-2 cells used in our studies were grown for 21 to 23 days. [14C]Mannitol transport was determined as a marker of cellular integrity, which was <0.5% per hour in representative cell monolayers. Cell layers exhibiting greater than 0.3% [14C]mannitol penetration were discarded.
Transport Studies

Transport experiments were carried out with using side-by-side diffusion cells (type VSC-1; Crown Glass Company Inc., Somerfield, NJ). Before an experiment, Caco-2 cell monolayer grown on the clear polyester membranes was washed with DPBS (pH 6.0) and incubated at 37°C. Freshly prepared drug solution (1 mM) in DPBS (pH 6.0) was placed in the donor chamber, and the receiver chamber was filled with DPBS (pH 7.4). The volumes of donor and receptor chambers were 3 ml each. Receiver chamber was sampled at predetermined time intervals, and an equal volume of fresh DPBS solution was added to maintain sink conditions in the receiver chamber. All samples were stored at −80°C until further HPLC analysis. All experiments were performed at least in triplicates at 37°C.

In Vivo Studies with Sprague-Dawley Rats

Oral absorption studies of ACV and its prodrugs were carried out at an equivalent dose of 20.0 mg/kg. Animals were fasted overnight.

Fig. 1. Structures of dipeptide prodrugs of acyclovir.
(12–18 h) with free access to water. Freshly prepared drug solutions in water were administered by oral gavage (0.8 ml). Blood samples (200 μl) were collected from the jugular and portal veins at predetermined time intervals over a period of 4 h. Heparinized saline (200 μl) was injected through both the veins to maintain a fairly constant fluid volume. Plasma was immediately separated by centrifugation and then stored at –80°C until further analysis.

Plasma samples were thawed at room temperature and 0.2 ml of methanol was added to 0.2 ml of plasma in an Eppendorf tube. The mixture was vortexed for 30 s and centrifuged at 8960 g at 4°C. The supernatant was then separated, and an aliquot was directly injected onto the column for HPLC analysis.

Analytical Procedures

All samples were assayed with HPLC. The system was comprised of a Rainin Dynamax pump SD-200 (Rainin Instruments, Woburn, MA), Rainin Dynamax UV detector UV-C at 254 nm, an HP 1100 series fluorescence detector (Hewlett Packard, Palo Alto, CA) at an excitation wavelength of 285 nm and an emission wavelength of 370 nm, and an Alcott autosampler model 718 AL HPLC. The column used was a C18 Luna column 4.6 × 250 mm (Phenomenex, Torrance, CA). The mobile phase consisted of a mixture of buffer and an organic modifier. The percentage of organic phase was varied to elute compounds of interest. This method gave rapid and reproducible results. HPLC conditions for the other prodrugs have been reported previously (Anand et al., 2003). The limits of quantification were found to be ACV, 25 ng/ml; VACV, 50 ng/ml; GVACV, 100 ng/ml; VVACV, 125 ng/ml; VYACV, 250 ng/ml; and YACV, 250 ng/ml. The intra- and interday precision (measured by coefficient of variation, CV%) was less than 3 and 5%, respectively.

Calculation of Pharmacokinetic Parameters

All relevant pharmacokinetic parameters were calculated using noncompartmental analyses of plasma-time curves after oral administration of ACV, VACV, and the dipeptide prodrugs of ACV using a pharmacokinetic software package WinNonlin, version 2.1 (Pharsight, Mountain View, CA). Maximum plasma concentrations (C_{max}) were obtained from the plasma-concentration time curves and the area under the plasma concentration time curves (AUC_{0-last} and AUC_{int}) were determined by the linear trapezoidal method with extrapolation. The slopes of the terminal phase of plasma profiles were estimated by log-linear regression and the terminal rate constant (λ_{c}) was derived from the slope. The terminal plasma half-lives were calculated from the equation t_{1/2} = 0.693/λ_{c}. Clearance (CL/F) and mean residence time (MRT) were calculated as ratio of dose/AUC and area under the first moment curve (AUMC/AUC, respectively. The total concentration parameters were calculated by adding the concentrations of the administered drug and the regenerated intermediates in terms of ACV.

Statistical Analysis

All experiments were conducted at least in triplicate, and results are expressed as mean ± S.D. Student’s t-test was used to detect statistical significance between the parameters of the prodrugs and ACV, and p < 0.05 was considered to be statistically significant. Statistical comparisons between the parameters of the prodrugs and ACV were performed using the analysis of variance (SPSS for Windows, release 10.0.7; SPSS Inc., Chicago, IL).

Results

Metabolism Studies

Plasma. Dipeptide ester prodrugs of ACV exhibited rapid hydrolysis in plasma. VACV exhibited the highest half-life of 346.5 ± 133.3 min compared with the dipeptide prodrugs. With YACV, no intact prodrug was observed after 1-min sampling. Among the dipeptide prodrugs, GVACV generated a half-life of 20.9 ± 5.3 min (Table 1). It was observed during the experiments that all the dipeptide prodrugs (except GYACV) cleaved to their amino acid ester conjugate intermediate followed by complete hydrolysis to ACV.

Intestinal Homogenate. The prodrugs hydrolyzed to yield the parent drug ACV in intestinal homogenates. The half-lives of the dipeptide prodrugs GVACV and VVACV were calculated as 17.32 and 24.5 min, respectively, in comparison with 28.8 min for VACV. The dipeptide prodrugs VYACV and GYACV rapidly hydrolyzed (no intact prodrug detected after 1 min) after incubation with the homogenate (Table 1). GVACV and VVACV (except VYACV and GYACV) cleaved to their amino acid ester intermediate followed by hydrolysis to ACV.

Transport across Caco-2 Monolayers

Transepithelial transport of 1 mM VACV and GVACV has been reported previously (Anand et al., 2003). Therefore, in this study transport of 1 mM VACV, GVACV, and VYACV was investigated across Caco-2 monolayers. Cumulative amount of drug transported (the sum of the prodrug and the regenerated parent drug) was plotted as a function of time. P_{app} values were determined from the linear portion of the cumulative amount transported versus time plot. The P_{app} values of VACV, GVACV, VVACV, VYACV, and YACV were calculated at pH 6.0 (Table 2).

In Vivo Oral Absorption

Analyses of the metabolites after administration of VACV revealed that the prodrug was rapidly cleaved to the parent drug, ACV, whereas dipeptide conjugates hydrolyzed to an amino acid ester conjugate which was subsequently cleaved to generate the parent drug, ACV, in the case of GVACV and VVACV. After administration of VYACV, the amino acid

### TABLE 1

<table>
<thead>
<tr>
<th>Drug</th>
<th>Plasma&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Intestinal Segments&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>k_{int}</td>
<td>t_{1/2}</td>
</tr>
<tr>
<td></td>
<td>min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>min</td>
</tr>
<tr>
<td>VACV</td>
<td>0.002 ± 0.001</td>
<td>346.5 ± 133.3</td>
</tr>
<tr>
<td>GVACV</td>
<td>0.034 ± 0.009</td>
<td>20.9 ± 5.3</td>
</tr>
<tr>
<td>VYACV</td>
<td>0.13 ± 0.009</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>YACV</td>
<td>0.53 ± 0.48</td>
<td>2.1 ± 1.2</td>
</tr>
<tr>
<td>GVYACV</td>
<td>---&lt;sup&gt;c&lt;/sup&gt;</td>
<td>---&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Measured using rat plasma.

<sup>b</sup> Measured using intestinal segments.

<sup>c</sup> First order rate constant.

<sup>d</sup> No intact prodrug detected after 1 min.

### TABLE 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>Permeability, P_{app}</th>
<th>10&lt;sup&gt;6&lt;/sup&gt; cm/s&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Val-ACV</td>
<td>3.01 ± 0.21</td>
<td>2.89 ± 0.59</td>
</tr>
<tr>
<td>Gly-Val-ACV</td>
<td>1.96 ± 0.14</td>
<td>0.71 ± 0.47</td>
</tr>
<tr>
<td>Val-Tyr-ACV</td>
<td>2.09 ± 0.41</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Anand et al. (2003).
conjugate intermediate tyrosine-ACV could not be detected at any time points, possibly due to extensive metabolism by intestine and/or plasma. No intact dipeptide prodrug was visible at any time during the experiment.

**Intestinal Absorption.** The intestinal absorption of the drugs and prodrugs upon oral administration was determined by sampling the portal vein. The intestinal absorption plasma-concentration time profiles of ACV, VACV, GVACV, VVACV, and VYACV are depicted in Figs. 2 to 6. Figures 2, 3A–5A, and 6 depict the total concentration of the drug absorbed in terms of ACV. Pharmacokinetic parameters have been summarized in Table 3. Oral administration of VACV and the dipeptide prodrugs led to an increase in intestinal absorption of ACV compared with ACV alone. GVACV led to approximately 2-fold elevation over VACV, whereas VVACV and VYACV had lower AUC values than VACV. C\text{max} values for total concentration of ACV after administration of ACV, VACV, GVACV, VVACV, and VYACV were observed to be 0.89 \pm 0.18, 4.53 \pm 0.95, 4.07 \pm 1.03, 1.43 \pm 0.32, and 1.64 \pm 0.5 \mu g \text{ ml}^{-1}, respectively, with VACV exhibiting the highest C\text{max} value. Time to reach maximum concentration (T\text{max}) for ACV, VACV, GVACV, VVACV, and VYACV did not vary significantly (p < 0.05). The mean residence time for regenerated ACV [MRT\text{ (ACV)}] for VACV and the dipeptide prodrugs was significantly higher than that of ACV, and the highest MRT\text{ (ACV)} was observed with VYACV. The mean residence time value of the amino acid conjugate MRT\text{ (AA)} of GVACV and VVACV were similar and significantly higher than VACV. The elimination rate constants of regenerated ACV [\lambda\text{Z (ACV)}] for ACV, VACV, GVACV, VVACV, and VYACV were calculated as 0.015 \pm 0.001, 0.004 \pm 0.0001, 0.005 \pm 0.001, 0.01 \pm 0.005, and 0.008 \pm 0.004 \text{ min}^{-1}, respectively. Also the elimination rate constants of regenerated amino acid intermediate [\lambda\text{Z (AA)}] from GVACV and VVACV were observed to be 0.005 \pm 0.003 and 0.001 \pm 0.0005 \text{ min}^{-1}, respectively (Table 3). ACV clearance [Cl\text{ (ACV)}] (161.3 \pm 36.2 ml \text{ min}^{-1}) after parent drug administration was higher relative to regenerated ACV clearance after administration of VACV (17.6 \pm 6.6 ml \text{ min}^{-1}), GVACV (12.5 \pm 5.5 ml \text{ min}^{-1}), VVACV (15.6 \pm 1.12 ml \text{ min}^{-1}), and VYACV (8.5 \pm 2.5 ml \text{ min}^{-1}). The clearance of the regenerated VACV after administration of GVACV (31.9 \pm 5.3 ml \text{ min}^{-1}) was higher compared with VVACV (6.91 \pm 0.92 ml \text{ min}^{-1}).

**Systemic Absorption.** The systemic absorption of the drugs and prodrugs upon oral administration was determined by sampling the jugular vein. The systemic absorption plasma-concentration time profiles of ACV, VACV, GVACV, VVACV, and VYACV are depicted in Figs. 2 to 6. As mentioned above, Figs. 2, 3A–5A, and 6 depict the total concentration of the drug absorbed in terms of ACV. The pharmacokinetic parameters obtained after administrations of parent ACV and the prodrugs are listed in Table 4. Highest systemic exposure was obtained upon administration of GVACV relative to ACV, VACV, VVACV, and VYACV. The
AUC obtained after oral administration of GVACV was approximately 2-fold higher relative to VACV administration. 

$C_{\text{max}}$ values for total ACV concentration after administration of ACV, VACV, GVACV, VVACV, and VYACV were observed to be $0.83 \pm 0.19, 5.44 \pm 2.33, 7.2 \pm 3.3, 1.27 \pm 0.22,$ and $1.85 \pm 0.3 \mu g\text{ml}^{-1}$, respectively, with GVACV exhibiting the highest $C_{\text{max}}$ value. Time taken to reach maximum concentration ($T_{\text{max}}$) for ACV, VACV, GVACV, VVACV, and VYACV was not significantly different from each other ($p < 0.05$). 

AUCs of the parent drug, ACV, obtained after systemic administration of GVACV and VVACV were calculated as $571.68 \pm 99.22$ and $96.8 \pm 24.4 \text{min}\mu g\text{ml}^{-1}$, respectively, and were found to be higher than from intestinal absorption, $347.8 \pm 108.2$ and $59.04 \pm 4.13 \text{min}\mu g\text{ml}^{-1}$, possibly due to a significant liver metabolism of the amino acid metabolite VACV. VACV and VYACV did not show such a phenomenon because they rapidly hydrolyzed to yield the parent drug, ACV. The clearance of ACV ($150.5 \pm 12.6 \text{ml}\text{min}^{-1}$) after ACV administration was found to be higher compared with clearance of regenerated ACV after administration of VACV ($15.3 \pm 3.1 \text{ml}\text{min}^{-1}$), GVACV ($13.3 \pm 3.5 \text{ml}\text{min}^{-1}$), VVACV ($13.9 \pm 1.36 \text{ml}\text{min}^{-1}$), and VYACV ($7.5 \pm 0.7 \text{ml}\text{min}^{-1}$). The clearance of the regenerated VACV after administration of GVACV ($26.8 \pm 4.5 \text{ml}\text{min}^{-1}$) was higher compared with VVACV ($12.2 \pm 2.6 \text{ml}\text{min}^{-1}$).
Pharmacokinetic parameters after intestinal absorption

TABLE 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ACV</th>
<th>VACV</th>
<th>GVACV</th>
<th>VVACV</th>
<th>VYACV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-last} (TC) (min µg ml^{-1})</td>
<td>21.2 ± 5.2</td>
<td>208.4 ± 41.2</td>
<td>416.1 ± 140.9</td>
<td>147.7 ± 89.3</td>
<td>180.7 ± 81.2</td>
</tr>
<tr>
<td>AUC_{inf} (TC) (min µg ml^{-1})</td>
<td>27.9 ± 6.1</td>
<td>237.9 ± 51.8</td>
<td>598.7 ± 164.1</td>
<td>266.1 ± 34.3</td>
<td>292.2 ± 59.7</td>
</tr>
<tr>
<td>C_{max} (TC) (µg ml^{-1})</td>
<td>0.89 ± 0.18</td>
<td>4.53 ± 0.95</td>
<td>4.07 ± 1.03</td>
<td>1.43 ± 0.32</td>
<td>1.86 ± 0.6</td>
</tr>
<tr>
<td>T_{max} (TC) (min)</td>
<td>24.0 ± 8.9</td>
<td>22.5 ± 5.0</td>
<td>21.4 ± 5.7</td>
<td>13.1 ± 5.7</td>
<td>15.0 ± 5.7</td>
</tr>
<tr>
<td>C_{last} (TC) (µg ml^{-1})</td>
<td>0.05 ± 0.01</td>
<td>0.3 ± 0.003</td>
<td>0.81 ± 0.12</td>
<td>0.54 ± 0.4</td>
<td>0.52 ± 0.12</td>
</tr>
<tr>
<td>AUC_{0-4h} (AACV) (min µg ml^{-1})</td>
<td>21.2 ± 5.2</td>
<td>246.2 ± 82.2</td>
<td>347.8 ± 108.2</td>
<td>59.04 ± 4.13</td>
<td>180.7 ± 81.2</td>
</tr>
<tr>
<td>AUC_{0-4h} (AAA) (min µg ml^{-1})</td>
<td>19.07 ± 2.5</td>
<td>143.6 ± 51.4</td>
<td>100.8 ± 9.7</td>
<td>140.9 ± 147.7</td>
<td>140.9 ± 147.7</td>
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<tr>
<td>C_{max} (AACV) (µg ml^{-1})</td>
<td>0.89 ± 0.18</td>
<td>4.88 ± 1.51</td>
<td>4.06 ± 1.44</td>
<td>1.11 ± 0.38</td>
<td>1.66 ± 0.6</td>
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<tr>
<td>C_{max} (AAA) (µg ml^{-1})</td>
<td>0.41 ± 0.2</td>
<td>0.59 ± 0.54</td>
<td>0.54 ± 0.14</td>
<td>1.27 ± 0.46</td>
<td>1.92 ± 0.66</td>
</tr>
<tr>
<td>CI/F (AACV) (ml min^{-1})</td>
<td>161.3 ± 36.2</td>
<td>17.6 ± 6.6</td>
<td>17.0 ± 5.5</td>
<td>15.6 ± 1.2</td>
<td>8.5 ± 2.5</td>
</tr>
<tr>
<td>CI/F (AAA) (ml min^{-1})</td>
<td>31.9 ± 5.3</td>
<td>6.91 ± 0.92</td>
<td>12.90 ± 9.5</td>
<td>12.90 ± 9.5</td>
<td>12.90 ± 9.5</td>
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<tr>
<td>MRT_{AACV} (min)</td>
<td>44.8 ± 5.6</td>
<td>62.6 ± 6.6</td>
<td>64.1 ± 5.35</td>
<td>69.2 ± 3.9</td>
<td>94.7 ± 3.3</td>
</tr>
<tr>
<td>MRT_{AAA} (min)</td>
<td>0.005 ± 0.001</td>
<td>0.004 ± 0.0001</td>
<td>0.005 ± 0.001</td>
<td>0.001 ± 0.005</td>
<td>0.008 ± 0.004</td>
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<tr>
<td>λ_{2} (AACV) (min^{-1})</td>
<td>0.015 ± 0.001</td>
<td>12.6 ± 2.5</td>
<td>110.9 ± 18.7</td>
<td>129.1 ± 9.5</td>
<td></td>
</tr>
<tr>
<td>λ_{2} (AAA) (min^{-1})</td>
<td>0.005 ± 0.001</td>
<td>0.001 ± 0.005</td>
<td>0.005 ± 0.001</td>
<td>0.001 ± 0.005</td>
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</tbody>
</table>

AA, amino acid intermediate; TC, total concentration in terms of ACV.

Discussion

Although it is not yet possible to cure herpes virus infections, the management of genital herpes infections has improved considerably since the introduction of antiviral drugs in the early 1980s (Balfour, 1999). The incidence of genital herpes infections caused by HSV-1 and 2 has increased significantly in the past 20 years (Fleming et al., 1997). Although genital herpes is self-limiting in healthy adults, the disease is painful and distressing, with severe psychosocial impact (Manne and Sandler, 1984; Goldmeier et al., 1988). Acyclovir was the first effective antiviral drug approved for widespread use and is still extensively prescribed, particularly in the treatment of immunocompromised patients with genital HSV disease (Perry and Faulds, 1996). Although acyclovir is a well tolerated and effective antiviral drug, its bioavailability after oral administration is low. As a result, up to 5 times administration per day is often necessary for the management of genital HSV disease. Valacyclovir, L-valyl ester prodrug of acyclovir, enhances the bioavailability of ACV after oral administration significantly over ACV due to the recognition of VACV by intestinal peptide transporter hPEPT1 that mediates its transport across intestinal epithelium to blood.

Plasma hydrolysis and intestinal homogenate hydrolysis studies were carried out to evaluate the regeneration characteristics of the ACV dipeptide ester prodrugs to the parent drug. All the prodrugs hydrolyzed to regenerate the active parent drug, ACV. The half-lives of the prodrugs ranged from 2.1 to 346.5 min (Table 1) in plasma and from 17.2 to 28.8 min in intestinal homogenates, demonstrating varied susceptibility of the prodrugs to the hydrolyzing enzymes. In plasma, VACV was found to be the most stable compound with a half-life of 346.5 ± 133.3 min and GVACV the least, because no intact prodrug was detected 1 min after the beginning of an experiment. Hydrolysis kinetics of the prodrugs in Caco-2 cell suspensions have been previously studied, and these prodrugs were also observed to undergo hydrolysis by enterocytic enzymes (Anand et al., 2003), which could limit their bioavailability upon oral administration. The schematic representation of mechanism of bioconversion of VACV and the dipeptide prodrugs to ACV upon in vitro vivo metabolism has been depicted in Fig. 7. GVACV and VVACV are sequentially metabolized to the parent drug, ACV via the amino acid intermediate, whereas VVACV, GVACV, and VYACV are rapidly metabolized to regenerate the parent drug, ACV.

The dipeptide prodrugs of ACV have been recently studied for their affinity toward hPEPT1 expressed in colon carcinoma cell line Caco-2. These compounds exhibited high affinity toward the transporter (Anand et al., 2003). In general terms, inhibition studies may not be a good predictor for the actual cellular uptake of drug candidates, because the substrates might only bind to the transporter without being translocated by it. Hence, the affinity of these prodrugs for hPEPT1 may not be translated into hPEPT1-mediated trans epithelial transport and oral delivery. Therefore, transport experiments with VACV and the dipeptide prodrugs GVACV, VVACV, and GVACV were carried out across Caco-2 monolayers at pH 6.0 (Table 2). The permeabilities of VACV and GVACV have been reported previously (Anand et al., 2003).

Oral absorption studies of ACV, VACV, and the dipeptide
prodrugs GVACV, VVACV, and VYACV were carried out in Sprague-Dawley rats with cannulated jugular and portal veins. After oral administration, VACV is rapidly absorbed from the gastrointestinal tract and nearly completely converted to ACV and L-valine by first pass intestinal and/or hepatic metabolism. A small amount of ACV is converted to inactive metabolites by aldehyde oxidase and by alcohol and hepatic metabolism. Neither VACV nor ACV is metabolized by cytochrome P450 enzymes. Therefore, to assess the role of first pass effect due to intestine and liver, intestinal and systemic absorption of the prodrugs was determined by sampling the portal and jugular vein, respectively. The samples collected from the portal vein were compared for their role in drug and the metabolite levels with that from the jugular vein to establish the role of intestinal and hepatic metabolism.

Upon oral administration of GVACV and VVACV, formation of the amino acid intermediate was observed, which was further metabolized to yield ACV. However, VYACV rapidly metabolized to ACV because no intact amino acid intermediate metabolite YACV could be detected. However, intermediate metabolite VACV formed after administration of GVACV, and VVACV further underwent significant metabolism in the liver as evident by higher levels of ACV generated after systemic absorption relative to intestinal absorption (Tables 3 and 4). It is highly likely that the enzyme BPHL, principally responsible for hydrolysis of VACV, is present in the liver as well as intestine (Puente and Lopez-Otin, 1995). AUC(TC) values obtained after oral administration of VACV and the dipeptide ester prodrugs of ACV were significantly higher (p < 0.05) than ACV itself. This increase in bioavailability of ACV upon oral administration has been attributed to the recognition of VACV by intestinal peptide transporter hPEPT1 that mediates its transport across intestinal epithelium to blood. Also, because the dipeptide prodrugs show appreciable affinity toward hPEPT1 (Anand et al., 2003), the oral absorption of these prodrugs is believed to be mediated through the intestinal peptide transporter hPEPT1. GVACV yielded the highest AUC(TC), which is at least 2-fold higher than VACV after intestinal as well as systemic absorption. Such enhancement in absorption could be attributed to the similar affinity of VACV and GVACV toward hPEPT1 (Anand et al., 2003), which allows it to be absorbed efficiently across the intestinal mucosa. Also, upon metabolism of GVACV, VVACV is formed, which itself is a substrate of hPEPT1, whereas on the other hand, VACV hydrolyzes rapidly to form ACV, which is not a substrate of hPEPT1. The same is not the case with VYACV, which hydrolyzes to VACV and has a similar AUC(TC), as that of VACV, due to rapid metabolism of VYACV to ACV. As previously reported, there is no accumulation of ACV after the administration of VACV at the recommended dosage regimens of 250 mg, 500 mg, and 1 g of VALTREX (valacyclovir hydrochloride) administered four times daily for 11 days in volunteers with normal renal function. In our studies, the plasma elimination half-life of ACV after administration of VACV ranged from 2.2 to 2.8 h. Upon intestinal and systemic absorption. The plasma clearance of ACV after prodrug administration was significantly reduced (p < 0.05) compared with clearance of ACV after administration of ACV alone due to the formation of the ACV upon metabolism of the dipeptide prodrugs as well as the amino acid intermediate.

In conclusion, oral administration of dipeptide ester prodrugs of ACV led to an increase in intestinal and systemic absorption of ACV compared with direct administration of ACV. The dipeptide prodrugs of ACV except VYACV are rapidly metabolized to the amino acid intermediate metabolite.

![Fig. 7. Mechanism of bioconversion (in vitro and in vivo metabolism) of Val-ACV and ACV dipeptide ester prodrugs Gly-Val-ACV, Val-Val-ACV, and Val-Tyr-ACV to acyclovir. Gly-Val-ACV and Val-Val-ACV are sequentially hydrolyzed via Val-ACV to yield the parent drug ACV, whereas Val-Tyr-ACV is rapidly hydrolyzed to ACV without the formation of the intermediate amino acid metabolite.](image-url)
lite VAC due to intestinal first pass effect. Despite their rapid metabolism, the dipeptide prodrugs are efficiently absorbed by the intestinal peptide transporter hPEPT1, leading to an increase in intestinal absorption of ACV relative to oral ACV itself. Therefore, the dipeptide prodrugs of ACV partially or totally GVACV may provide a significant therapeutic advantage in the treatment of oral and genital HSV infections and may be considered a considerable improvement over VACV.

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References


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