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In Vivo Ocular Pharmacokinetics of Acyclovir Dipeptide Ester Prodrugs by Microdialysis in Rabbits

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Abstract: In vivo corneal absorption of the dipeptide prodrugs of acyclovir (ACV) was evaluated using microdialysis in rabbits. A corneal well was placed on the cornea of the anesthetized New Zealand White rabbits with implanted linear probes into the aqueous humor. Two hundred microliters of a 1% solution of L-valine-ACV (VACV), glycine-valine-ACV (GVACV), valine-valine-ACV (VVACV), and valine-tyrosine-ACV (VYACV) was placed in the corneal well and was allowed to diffuse for a period of 2 h, following which the drug solution was aspirated and well removed. Samples were collected every 20 min throughout the infusion and postinfusion phases and were analyzed by HPLC to obtain the aqueous humor concentrations. Absorption rate constants of all the compounds were found to be lower than the elimination rate constants. GVACV exhibited highest absorption rate (k_a) compared with other prodrugs, but all the prodrugs showed similar terminal elimination rate (λ_z). The time of maximum absorption (T_{max}) of ACV after administration of VACV and the dipeptide prodrugs did not vary significantly ($p < 0.05$). GVACV exhibited the highest concentration (C_{max}) and area under curve (AUC) upon absorption ($p < 0.05$) compared to VACV, VVACV, and VYACV. Dipeptide prodrugs of ACV were absorbed through the cornea at similar rates but to varying extents. The dipeptide prodrug GVACV owing to its enhanced absorption of ACV seems to be a promising candidate for the treatment of ocular HSV infections.

Keywords: Acyclovir; dipeptide prodrugs; ocular absorption; microdialysis

Introduction

Herpes simplex keratitis is the leading cause of blindness in the United States as well as the most frequent cause of corneal opacities in developed countries.[1] Nucleoside analogues developed initially for the treatment of herpes simplex virus (HSV) infections (HSV keratitis) include trifluridine (TFT), idoxuridine (IDU), and cytosine arabinoside (Ara-A), all of which were found to be too toxic for systemic use and were, therefore, restricted to topical use for herpetic keratitis.[2] Acyclovir (ACV), also a nucleoside analogue, has been shown to be clinically effective against herpes viruses, but due to poor aqueous solubility and low corneal permeability, the drug is not very effective against ocular herpes infections.[3]

Ocular availability of drugs is restricted due to pharmacological, pharmacokinetic, or pharmaceutical barriers. Chemical approach to designing bioreversible prodrugs can be useful in the optimization of drug absorption properties.[4] Prodrugs are designed to overcome the undesirable properties of drugs but are themselves biologically inactive. Further

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prodrug strategy can be utilized to specifically target membrane transporters expressed on epithelial cells. In that direction transporter-targeted prodrug derivatization strategy seems to be one of the most exciting of all the current drug delivery strategies.[5]

Recently peptide transporters attracted a lot of attention as drug delivery targets. Due to their broad substrate specificity, peptide transporters contribute to the intestinal absorption of several drug compounds.[6−12] In the past, peptide transporters have been utilized successfully to improve the bioavailability of the nucleoside analogues acyclovir[13] and zidovudine (AZT) by designing 5′-amino acid ester prodrugs.[9,14] Previously in our lab PepT1 was identified on the corneal epithelium for the first time and successfully targeted using valacyclovir.[15]

In an earlier report the ACV dipeptide prodrugs were shown to be substrates for peptide transporter (PEPT1) on the rabbit cornea.[16] Also the prodrugs exhibited excellent solution stability relative to valacyclovir (VACV), a drug of choice for oral and genital herpes.[16] The dipeptide prodrugs also showed significantly lower cytotoxicty on the Statens Seruminstitut rabbit corneal cell line and rabbit primary corneal epithelial cells in comparison with TFT and ACV itself and exhibited excellent in vitro antiviral efficacy against HSV-1 in comparison with ACV. Finally the prodrugs were highly soluble and permeable across the cornea in comparison with ACV.[17] The dipeptide ACV prodrugs can be formulated into 1−3% eye drops and seem, therefore, to be promising drug candidates for the treatment of HSV keratitis with stromal involvement. In this study we have utilized the dipeptide prodrugs to target the PepT1 transporter on the corneal epithelium for enhanced absorption of ACV.

Topical administration is the preferred mode to treat diseases that affect the anterior chamber of the eye. Unfortunately, the disposition of drugs administered in this manner is not well understood. Several pharmacokinetic models of varying complexity have been proposed to predict absorption and disposition of drugs applied topically to the eye.[18−20] Pharmacokinetics of topically applied pilocarpine in the albino rabbit eye has been described using a four-compartment classical model represented by a four exponential equation yielding eight equation parameters.[19] Another pharmacokinetic model has been applied to pilocarpine pharmacokinetics that uses a physiologically based model.[18,20] However, both modeling approaches are complex with regard to numerical analyses.

Two basic problems in determining anterior chamber kinetics are as follows. (i) Determination of k_a is difficult due to the presence of precorneal kinetic events. (ii) Absorption across the cornea is often a slower process than elimination from the eye, and an erroneous assignment of slopes is possible. To simplify the approach and correctly estimate ocular absorption rate constant, a "topical infusion"

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model has been described previously.[21] In this model, a constant concentration of the drug is maintained on the cornea so that the effect of tear dynamics is minimized and simpler equations can be applied independent of compartment modeling. During constant input of the drug through the cornea, absorption, distribution, and elimination can be determined independent of the number of peripheral compartments that are operative. Constant concentration was maintained through the use of a plastic cylindrical well containing the drug solution.

A major constraint in the determination of ocular pharmacokinetics of drugs is the inaccessibility of ocular fluids such as aqueous humor and vitreous humor for continuous serial sampling. Furthermore, adding to the problem in assessing ocular pharmacokinetics is the fact that a single rabbit must be used for a single time point. Complete pharmacokinetic profiles are usually constructed by sacrificing 6−20 rabbits at each time point. Microdialysis has been proven to be beneficial over conventional sampling techniques in determining ocular pharmacokinetics by both reducing the number of subjects and providing statistically robust data. It has been applied in aqueous and vitreous drug disposition and delivery studies.[3,22−25] In this study we have employed the use of microdialysis for sampling the aqueous humor. We conceptualized the use of a combination of the topical well infusion model and aqueous humor microdialysis sampling for precise prediction of ocular absorption.

In this report, we have examined the in vivo corneal absorption of the dipeptide prodrugs through a topical infusion model along with aqueous humor microdialysis in New Zealand White rabbits. The aqueous humor kinetics of the dipeptide prodrugs GVACV, VVACV, and VYACV was compared with that of VACV, which is transported across cornea owing to its recognition by the oligopeptide transporter on the corneal epithelium.[15]

Materials and Methods

Materials. VACV was a gift from GlaxoSmithKline Inc., Research Triangle Park, NC. All other chemicals were obtained from Sigma Chemical Company (St. Louis, MO). The solvents were of analytical grade and obtained from Fisher Scientific (St. Louis, MO). The dipeptide prodrugs, namely, Val-Val-ACV (VVACV), Gly-Val-ACV (GVACV), and Val-Tyr-ACV (VYACV) (Figure 1), were synthesized in our laboratory.[16] Linear microdialysis probes (MD-2000, 0.32 × 10 mm, polyacrylonitrile membrane and 0.22 mm tubing) employed for aqueous humor sampling were purchased from Bioanalytical Systems (West Lafayette, IN). A microinjection pump (CMA/100) for perfusing the isotonic buffer saline was procured from CMA/Microdialysis (Acton, MA). Ketamine HCl was supplied by Fort Dodge animal health and xylazine by Bayer animal health. Nembutal sodium was purchased from Abbott Laboratories (Abbott Park, Chicago, IL). Topical wells (Figure 2A) were custom made by Hansen Ophthalmic Development Corporation (Iowa City, IA) according to special instructions.

Animals. New Zealand White male rabbits weighing between 5 and 5.5 lb were obtained from Myrtle's Rabbitry (Thompson Station, TN). Animal care and treatment in this investigation was in compliance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

In Vivo Absorption Experiments. (1) Probe Implantation. Aqueous humor sampling to assess the ocular absorption of the dipeptide prodrugs was carried out using microdialysis. The animals were anesthetized prior to the surgery by administration of ketamine (50 mg/kg) and xylazine (5 mg/kg) intramuscularly. Pupils were dilated by topical instillation of 1% tropicamide prior to the probe implantation. The linear microdialysis probe was placed in the anterior chamber using a 25G needle. It was inserted across the cornea preventing any damage to the iris-ciliary body, and the outlet of the linear probe was placed into the needle at the bevel edge. Then the needle was slowly withdrawn such that the probe remained fixed in the anterior chamber (Figure 2B). The microdialysis probe was perfused with isotonic phosphate buffer saline at a flow rate of 2 μL/min by a microinjection pump. The animals were kept under anesthesia throughout an experiment with ketamine HCl and xylazine given intramuscularly every 40 min. After probe implantation, the animals were allowed to stabilize for 2 h before the initiation of any study. This duration has been shown to be sufficient for the restoration of intraocular pressure and replenishment of the aqueous humor originally lost during probe implantation.[26]

(2) Microdialysis. Subsequent to probe implantation and recovery of the animal, the eyelids of the rabbits were mechanically retracted with Colibri retractors, and the precorneal well (Hansen Ophthalmic Development Corporation, Iowa City, IA), designed to fit over the sclera of the rabbit eye, was mounted. Care was taken to avoid contact with the entry and exit ports of the aqueous humor microdialysis probe (Figure 2B). The base of the device fitted over the eye with the central portion forming a well, thereby

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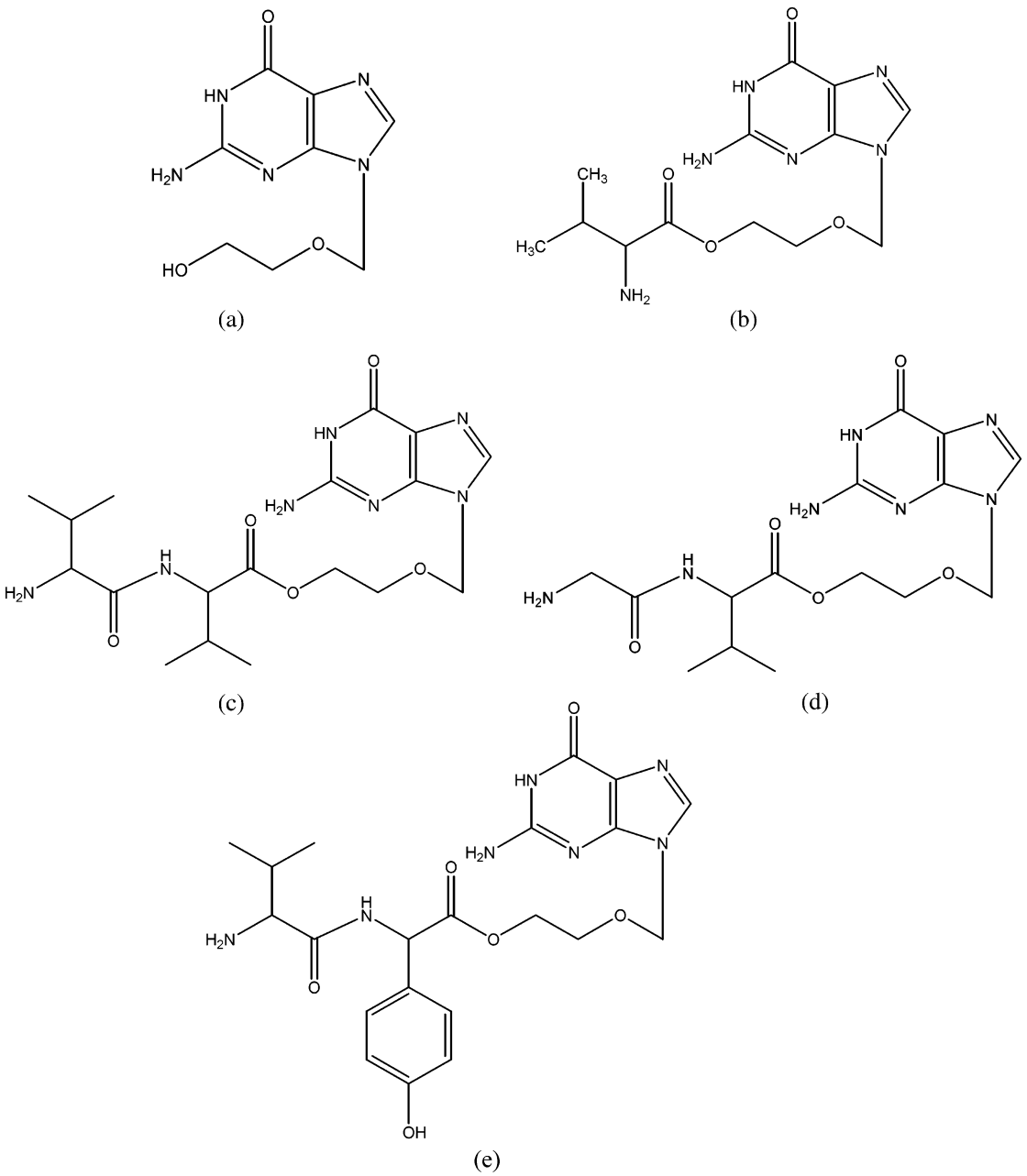

Figure 1. Structure of acyclovir and prodrugs of acyclovir: (a) ACV; (b) VACV; (c) VVACV; (d) GVACV; (e) VYACV.

allowing the drug/prodrug solution to remain in direct contact with the cornea. The outer edge of the precorneal well was coated with silicone grease to prevent its movement. The rabbit was placed on its left side, and the base of the well was positioned on the sclera of the right eye such that the empty well was above the cornea. Subsequent to placement of the well, the animals were allowed to stabilize for another 45 min to maintain proper intraocular pressure. After this time period, 200 μL of isotonic phosphate-buffered saline containing drug/prodrug was added to the well at time zero and samples were collected at predetermined time points by microdialysis. The compounds were allowed to diffuse for a period of 120 min, after which the drug solution was aspirated from the well, which was subsequently removed. The corneal surface was washed clean with a few drops of distilled water. Samples were collected every 20 min throughout the infusion and postinfusion phases over a period of 8 h. At the end of an experiment, euthanasia was performed under deep anesthesia with an intravenous injection of sodium pentobarbital through the marginal ear vein. Samples obtained in the study were analyzed by HPLC.

In Vitro Probe Calibration. In vitro probe calibration was performed by placing the probe in isotonic phosphate buffer saline (IPBS) solution, pH 7.4, of the appropriate prodrug/drug of known concentration. The probe was perfused at a flow rate of 2 μL/min with IPBS, and the dialysate was collected every 20 min. Relative recovery of the respective prodrug was calculated by eq 1. C_d is the dialysate

$$\text{recovery} = C_d/C_s \quad (1)$$

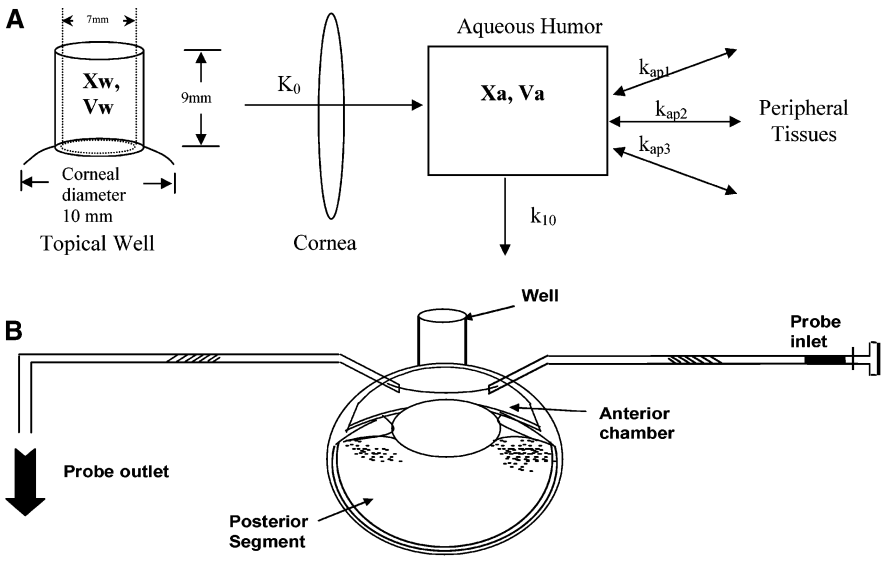

Figure 2. (A) Schematic model representing the absorption barriers and elimination pathways when drug was administered at a constant rate to the surface of the cornea. The dimensions of the plastic cylindrical well are also given. The cornea is assumed to act more like a barrier than a compartment. Elimination was assumed to be occurring primarily through the central (aqueous humor) compartment. From aqueous humor, drug may reversibly distribute to peripheral tissues. (B) Schematic representation of the well model in anterior chamber ocular microdialysis.

concentration, and C_s is the known concentration of prodrug/drug in IPBS. The concentration of the respective drug in aqueous humor during the pharmacokinetic experiment was calculated by dividing the dialysate concentration with the in vitro recovery above obtained.

The recovery of the linear probe was between 15% and 18% for both acyclovir and the dipeptide prodrugs studied. There was no significant variation (<1−2%) in the recovery of the probes with time over the experimental time period, but for all experiments the average of the recovery determined before and after the experiment was considered for assessing the intraocular fluid concentrations.

Absorption Kinetics. In Figure 2A, K_0 represents the overall input rate into the corneal tissue, X_a is the amount of drug in the aqueous humor, X_w is the amount of drug in the precorneal well, V_a is the physiological volume of the aqueous humor, and V_w is the volume of drug solution in the precorneal well. k_{ap} and k_{pa} are the first-order rate constants for the transfer of the drug/prodrug from the aqueous humor to the peripheral compartments and vice versa, and k_{10} represents the overall elimination from the aqueous humor. The subscripts a and p refer to the aqueous and peripheral compartments, respectively (Figure 2A).

Assuming sink conditions during initial times of the infusion (i.e., $X_w \gg X_a$ since $C_w \gg C_a$), the absorption rate k_a can be calculated according to eq 2,

$$k_a = \frac{\left(\frac{dC_a}{dt}\right)_I V_a}{C_w V_w} \tag{2}$$

where k_a is the corneal absorption first-order absorption rate constant, C_w is the concentration of the drug/prodrug in the well, V_w the volume of the drug/prodrug solution in the well, and V_a is the physiological volume of aqueous humor (250 μL), which was assumed constant during the infusion period, which is further indicated by negligible volume of loss from the corneal well after the infusion period. Subscript I refers to the initial rate, which can be determined from the initial slope of C_a versus t as determined by aqueous humor microdialysis.

Assuming that steady state is reached before the postinfusion period ($\lim_{t\to\infty} C_A \equiv C_{ss}$), the elimination rate k_{10} can be calculated according to eq 3,

$$k_{10} = \frac{\left(\frac{dC_a}{dt}\right)_I}{C_{ss}} \tag{3}$$

where C_{ss} represents the steady state concentration in aqueous humor and other terms are as previously defined. The disposition mean residence time for the prodrugs in the aqueous humor, MRT_d, is defined by

$$MRT_d = \frac{AUMC}{AUC} - \frac{t}{2} \tag{4}$$

where AUMC is the area (0−∞) under the aqueous humor concentration × time calculations plotted versus time, whereas AUC is the area (0−∞) under the aqueous humor versus time, and t is the time of topical infusion.

The topical infusion method along with eqs 2−4 permits a rational and reliable determination of ocular pharmacokinetics whereby absorption, distribution, and elimination can be characterized using noncompartmental analysis without the need for complex compartmental analysis. Detailed explanation for the derivation of the above pharmacokinetic parameters was given by Eller et al.[21]

Analytical Procedures. All samples were assayed using RP-HPLC. The system comprised a Rainin Dynamax Pump SD-200, a Rainin Dynamax UV detector UV-C at 254 nm, and an Alcott autosampler model 718 AL HPLC. The column used was a C18 Luna column, 4.6 × 250 mm (Phenomenex). The mobile phase consisted of a mixture of buffer and an organic modifier. The percentage of organic phase was varied in order to elute compounds of interest. This method gave rapid and reproducible results. HPLC conditions for these prodrugs have been reported previously.[27] The limits of quantification were found to be ACV, 25 ng/mL; VACV, 50 ng/mL; GVACV, 100 ng/mL; VVACV, 125 ng/mL; and VYACV, 250 ng/mL. The intra- and interday precision (measured by coefficient of variation, CV%) was less than 3% and 5%, respectively.

Statistical Analysis. All experiments were conducted at least in triplicate, and the results are expressed as mean ± SD. 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 were performed using the analysis of variance (SPSS for Windows, release 10.0.7; SPSS Inc., Chicago, IL).

All relevant pharmacokinetic parameters were calculated using noncompartmental analyses of plasma concentration−time curves of VACV and the dipeptide prodrugs of ACV with a pharmacokinetic software package, Win Nonlin, v2.1 (Pharsight, CA). Data was fitted into a noncompartmental model, with a constant infusion over a period of time. Dose normalized maximum plasma concentrations (C_{max}) were obtained from the plasma concentration−time profiles, and the areas under the plasma concentration−time curves (AUC_{0-last} and AUC_{0-inf}) 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_z) was derived from the slope. The terminal plasma half-lives were calculated from the equation $t_{1/2} = 0.693/\lambda_z$.

Results

In Vivo Ocular Absorption. The absorption profiles of the compounds exhibited linear accumulation of the drug until the removal of the well. The postinfusion phase showed first-order decline of drug concentrations in aqueous humor (Figures 3−6). Pharmacokinetic profiles for the absorption of the compounds have been shown in Figures 3−7, whereas pharmacokinetic parameters have been summarized in Table 1. The area under curve (AUC_{inf}) values of the total ACV concentrations plot after administration of VACV, GVACV, VVACV, and VYACV were calculated as 4334.7 ± 443.5, 7967.3 ± 774.1, 1303.8 ± 929.4, and 3508.6 ± 1626.4 min μg mL^{-1}, respectively. GVACV administration exhibited an approximately 2-fold increase in AUC relative to VACV administration. The C_{max} (maximum concentration) value for total concentration of ACV also exhibited a 2-fold increment for GVACV compared to VACV. VVACV exhibited significantly lower AUC and C_{max} values compared to all the prodrugs. The values of time to reach maximum concentration (T_{max}) for all the prodrugs did not vary significantly ($p < 0.05$) and were observed to range from 165 to 173 min. The absorption rate constants (k_a) of all the compounds obtained from the linear portion of the cumulative concentration profile were found to be lower than the elimination rate constants (λ_z) obtained from the terminal portion of the profiles. The mean residence time values for regenerated ACV ($MRT_{(ACV)}$) for all the prodrugs did not vary significantly, and mean residence time values of the amino acid conjugate ($MRT_{(AA)}$) after administration of VACV, GVACV, VVACV, and VYACV were calculated to be 98.1 ± 6.7, 118.9 ± 15.9, 116.4 ± 24.8, and 50.5 ± 10.6 min, respectively. $MRT_{(AA)}$ of VYACV was significantly lower

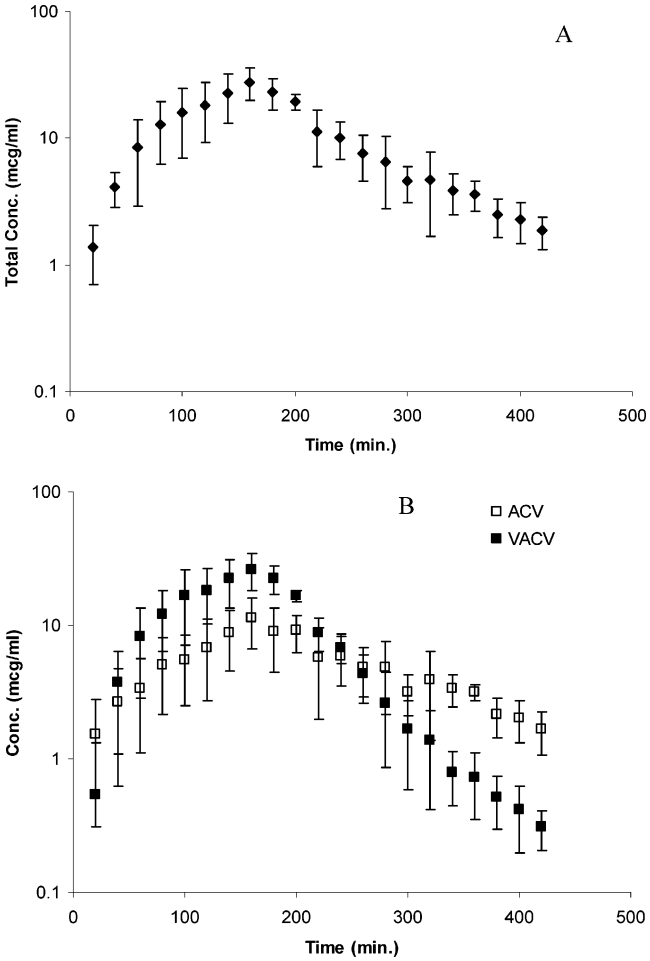

Figure 3. (A) Aqueous humor concentration−time profile of absorption of cumulative ACV (◆) upon topical administration of VACV. (B) Aqueous humor concentration−time curves of intact VACV (■) and regenerated ACV (□) upon corneal absorption.

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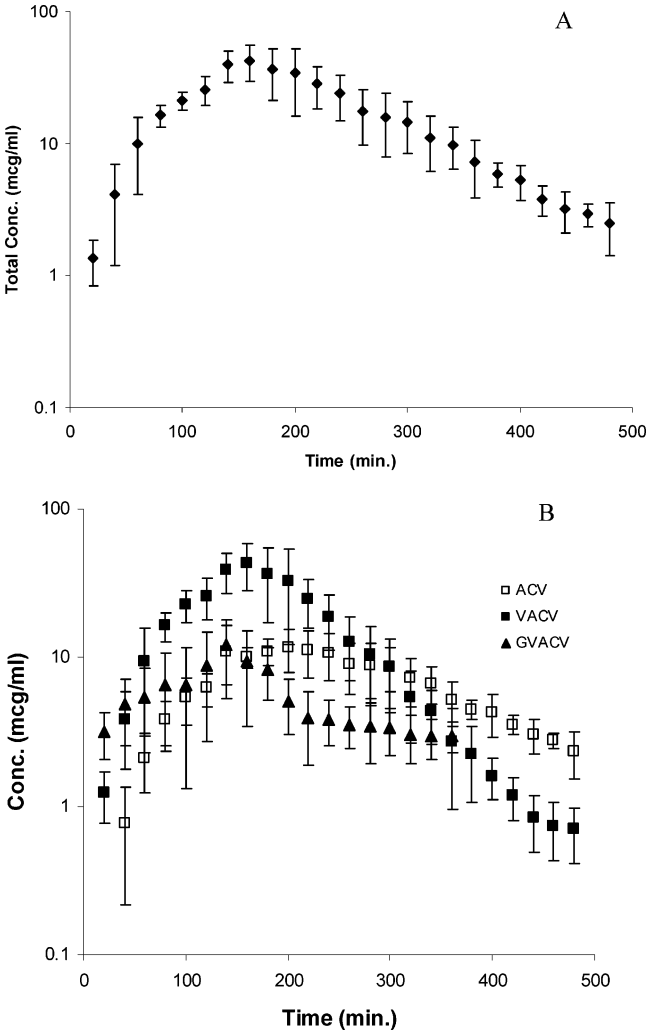

Figure 4. (A) Aqueous humor concentration−time profile of absorption of cumulative ACV (◆) upon topical administration of GVACV. (B) Aqueous humor concentration−time curves of intact GVACV (▲) and regenerated VACV (■) and ACV (□) upon corneal absorption.

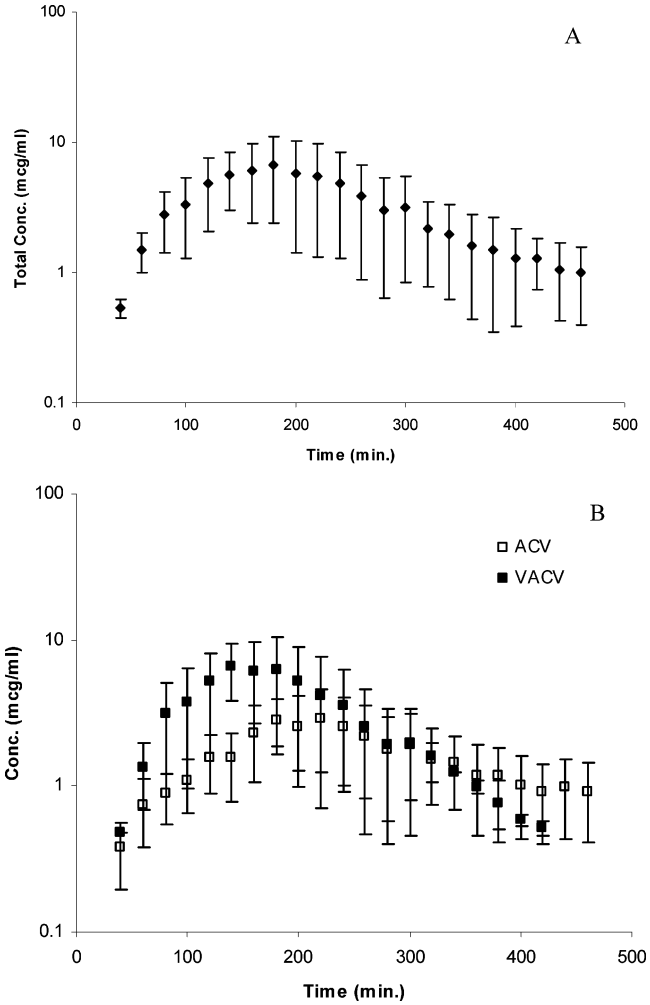

Figure 5. (A) Aqueous humor concentration−time profile of absorption of cumulative ACV (◆) upon topical administration of VVACV. (B) Aqueous humor concentration−time curves of regenerated VACV (■) and ACV (□) upon corneal absorption.

($p < 0.05$) than $MRT_{(AA)}$ of VACV, GVACV, and VVACV. The elimination rate constants of regenerated ACV ($\lambda_{z(ACV)}$) for all the prodrugs were similar. Also the elimination rate constants of regenerated amino acid intermediate ($\lambda_{z(AA)}$) from GVACV and VVACV were observed to be 0.015 ± 0.003 and 0.011 ± 0.004 min^{-1}, respectively. C_{max} values for regenerated amino acid intermediate ($C_{max(AA)}$) were almost similar to the C_{max} (maximum concentration) value for total concentration of ACV for all the prodrugs (Table 1).

Pharmacokinetic parameters of intact GVACV upon topical administration of GVACV have been listed in Table 2. The AUC and C_{max} were calculated as 1.56 ± 0.12 min μg mL^{-1} and 12.2 ± 3.1 min μg mL^{-1} respectively. These values indicate that AUC and C_{max} values for total concentration of ACV for GVACV constitute around 40% of the intact prodrug. Also MRT and λ_z were calculated as 89.1 ± 25.5 min and 0.011 ± 0.051 min^{-1}, respectively, indicating that they were similar to total concentration of ACV or regenerated amino acid intermediate for GVACV.

Discussion

The rates of elimination for all the compounds were found to be much higher than the rates of absorption (Table 1). Previously Eller et al. studied the absorption of carbonic anhydrase inhibitors using a well model and found out that the values of elimination rate constants were 2−3 orders of magnitude higher than the absorption rate constant.[21] GVACV exhibited the highest in vivo corneal absorption rate constant (k_a) compared to VACV and the dipeptide prodrugs (Table 1). The time to reach maximum concentration (T_{max}) for VACV and the dipeptide prodrugs was observed to be in the range of 165−173 min, which exceeds the time of removal (120 min) of the drug from the topical well. Therefore we can assume that dipeptide prodrugs once recognized by the corneal epithelial oligopeptide transporter[16] cross the lipoidal epithelium layer and are slowly diffused across stroma, which is hydrophilic. The dipeptide prodrugs, being more lipophilic than ACV and VACV, do not easily diffuse across the stromal layer of the cornea and therefore are slowly hydrolyzed to the parent drug via sequential

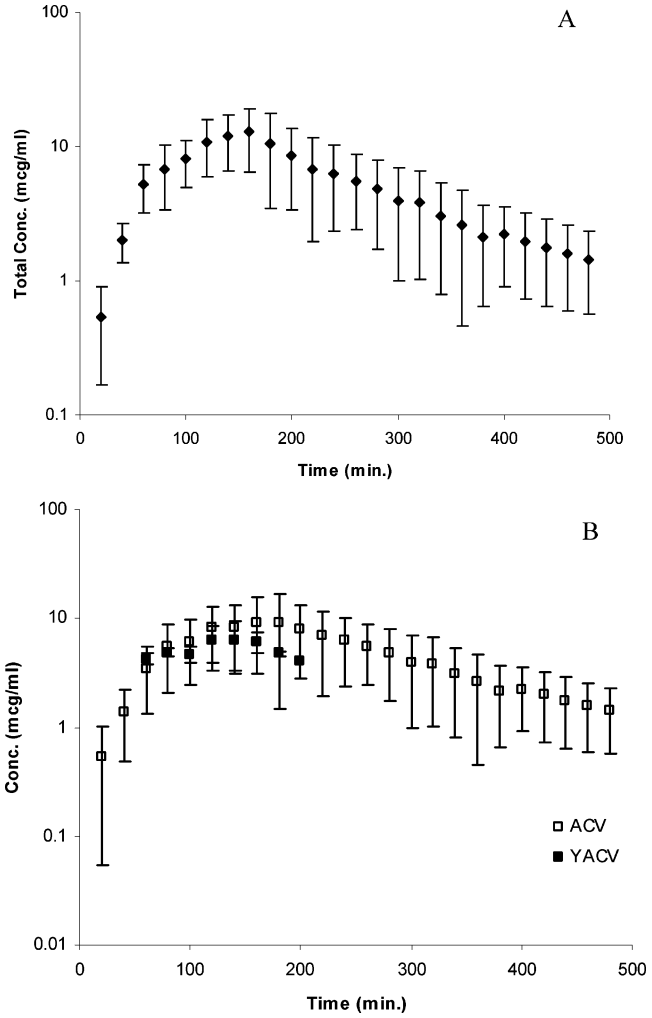

Figure 6. (A) Aqueous humor concentration−time profile of absorption of cumulative ACV (◆) upon topical administration of VYACV. (B) Aqueous humor concentration−time curves of regenerated YACV (■) and ACV (□) upon corneal absorption.

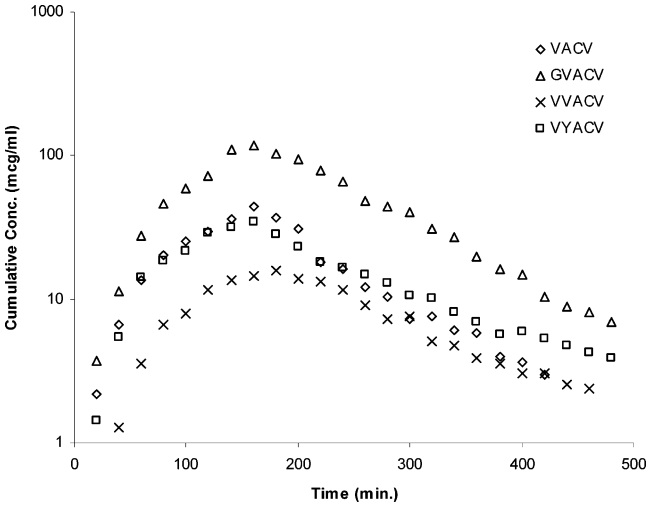

Figure 7. Dose normalized aqueous humor concentration−time profile of absorption of ACV upon topical administration of (◇) VACV, (△) GVACV, (×) VVACV, and (□) VYACV.

hydrolysis to the amino acid intermediate. For this reason intact dipeptide prodrugs VVACV and VYACV were not detected in the aqueous humor samples at any time points. A speculation on the mechanism of corneal absorption of the ACV dipeptide prodrugs is depicted in Figure 8. The corneal tissue half-lives of the prodrugs VACV, GVACV, VVACV, and VYACV have been reported as approximately 85, 256, 92, and 119 min,[16] which shows that the prodrugs are quite labile in the enzymatic environment of the cornea. Cornea expresses the highest amount of aminopeptidase activity compared to other ocular tissues,[28] and it is predominantly these enzymes which are responsible for the bioreversion of the dipeptide prodrugs of ACV as evident by the formation of amino acid metabolite.[16] Detectable levels of GVACV ranging from 2.9 to 12.1 μg mL^{-1} were observed in aqueous humor at 360 min as the last time point of detection. The above reason could be attributed to the longest half-life of GVACV compared to the other dipeptide prodrugs and also due to a lower lipophilicity of GVACV due to the more hydrophilic amino acid residue, glycine, compared to tyrosine and valine.[29] Despite the above limitations, GVACV generated approximately 2-fold, 6-fold, and 2-fold higher AUC of ACV than those of VACV, VVACV, and VYACV, respectively. Since these prodrugs are transported by a saturable carrier mediated process,[16,27] dose normalization would not yield the actual results due to dose dependence. However taking into account that all the drugs being compared here are absorbed actively and assuming similar affinities for the transporter, hPEPT1, the dose normalized AUC values for cumulative amounts of ACV absorbed were calculated as 3.46 ± 1.05 (VACV), 11.1 ± 1.01 (GVACV), 1.55 ± 1.11 (VVACV), and 4.69 ± 2.17 (VYACV) min μg mL^{-1}. Dose normalized AUC of ACV upon GVACV administration was approximately 3-fold, 7-fold, and 2-fold higher than those of VACV, VVACV, and VYACV administration, respectively.

Dipeptide prodrugs could be readily absorbed across corneal epithelium owing to their appreciable affinity to PEPT1.[16] Following absorption through the corneal epithelium the prodrugs can penetrate the deeper layers of the cornea, namely, stroma and simultaneously can undergo hydrolysis to yield ACV. This strategy would be helpful in treating HSV infections of the deeper tissues without having to administer the drug quite as frequently. Moreover the current drug of choice, trifluridine, is not indicated for the treatment of keratitis with deep stromal invasion, recurrent epithelial keratitis, and Epstein Barr virus keratitis. Also in addition to its cytotoxicity, trifluridine has a very short half-life (18−20 min) and must be administered every 2 h. The

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Table 1. Pharmacokinetic Parameters for Corneal Absorption of VACV and Dipeptide Prodrugs of ACV[a]

parameters	VACV[b]	GVACV	VVACV	VYACV
$AUC_{(0-t)(TC)}$ (min μg mL^{-1})	4237.4 ± 1366.4	7578.4 ± 617.8*	1218.4 ± 864.9	3136.9 ± 1303.7
$AUC_{inf(TC)}$ (min μg mL^{-1})	4334.7 ± 443.5	7967.3 ± 774.1*	1303.8 ± 929.4	3508.6 ± 1626.4
$AUC_{inf\_dose(TC)}$ (min μg mL^{-1})	3.46 ± 1.05	11.1 ± 1.01*	1.55 ± 1.11	4.69 ± 2.17
$C_{max(TC)}$ (μg mL^{-1})	27.9 ± 7.8	44.9 ± 2.9*	7.68 ± 4.36	17.5 ± 2.04
$T_{max(TC)}$ (min)	166.7 ± 11.5	173.3 ± 30.5	173.4 ± 11.5	165.0 ± 19.1
$k_{a(TC)} \times 10^3$ (min^{-1})	0.042 ± 0.022	0.11 ± 0.018*	0.021 ± 0.014	0.041 ± 0.016
$\lambda_{z(TC)}$ (min^{-1})	0.011 ± 0.001	0.008 ± 0.005	0.008 ± 0.0004	0.007 ± 0.002
$AUC_{(0-t)(ACV)}$ (min μg mL^{-1})	1632.41 ± 576.2	2703.6 ± 468.7	464.7 ± 218.5	2778.7 ± 1369.7
$AUC_{(0-t)(AA)}$ (min μg mL^{-1})		5051.6 ± 1470.3	1342.3 ± 607.9	743.4 ± 113.4
$C_{max(ACV)}$ (μg mL^{-1})	11.5 ± 4.8	15.61 ± 1.76	3.09 ± 1.84	13.7 ± 3.7
$C_{max(AA)}$ (μg mL^{-1})		44.06 ± 4.58	7.86 ± 4.5	6.1 ± 1.3
$MRT_{(ACV)}$ (min)	144.2 ± 24.7	181.6 ± 3.1	171.3 ± 32.5	138.5 ± 37.5
$MRT_{(AA)}$ (min)		118.9 ± 15.9	116.4 ± 24.8	50.5 ± 10.6
$\lambda_{z(ACV)}$ (min^{-1})	0.006 ± 0.003	0.006 ± 0.002	0.006 ± 0.0006	0.006 ± 0.003
$\lambda_{z(AA)}$ (min^{-1})		0.015 ± 0.003	0.011 ± 0.004	
C_{last}(ACV) (μg mL^{-1})	1.66 ± 0.6	2.33 ± 0.82*	0.98 ± 0.58	1.44 ± 0.87

[a] Values presented herewith are for ACV, amino acid intermediate, and total concentration. Values are mean ± SD (n = 3−6). TC: total concentration in terms of ACV. ACV: acyclovir. AA: amino acid intermediate. MRT: mean residence time. AUC: area under curve. C_{max}: maximum concentration. T_{max}: time to reach maximum concentration. λ_z: terminal elimination rate constant. k_a: absorption rate constant. [b] Control. Asterisks (*) designate $p < 0.05$ compared to control.

Table 2. Pharmacokinetic Parameters for Corneal Absorption of GVACV[a]

parameters	GVACV
$AUC_{inf(GVACV)}$ (min μg mL^{-1})	3124.4 ± 245.9
$AUC_{inf\_dose(GVACV)}$ (min μg mL^{-1})	1.56 ± 0.12
$C_{max(GVACV)}$ (μg mL^{-1})	12.2 ± 3.1
$T_{max(GVACV)}$ (min)	141.2 ± 10.6
$Cl_{(GVACV)}$ (mL min^{-1})	0.64 ± 0.05
$MRT_{(GVACV)}$ (min)	89.1 ± 25.5
$\lambda_{z(GVACV)}$ (min^{-1})	0.011 ± 0.051

[a] Values presented herewith are for intact GVACV absorbed.

half-life of the cumulative ACV from the dipeptide prodrugs was calculated as approximately 180 min (Table 1), which would allow the dosing frequency to be reduced, resulting in improved patient compliance and safety.

The concentrations of ACV at the conclusion of the experiment, C_{last}, upon topical administration of VACV, GVACV, VVACV, and VYACV were calculated as 1.66 ± 0.6, 2.33 ± 0.82, 0.98 ± 0.58, and 1.44 ± 0.87 μg mL^{-1}, which correspond to 7.3, 10.5, 4.4, and 6.6 μM. Among the dipeptides, C_{last} for GVACV exceeded the EC_{50} for HSV-1 (7.1 μM for ACV) isolates whereas C_{last} for VVACV and VYACV was below the EC_{50} for HSV-1. Therefore at the end of the experiment the concentration of ACV in the aqueous humor upon administration of GVACV was higher than the concentration necessary to inhibit viral cytopathogenicity by 50%. On the other hand the C_{last} values of the

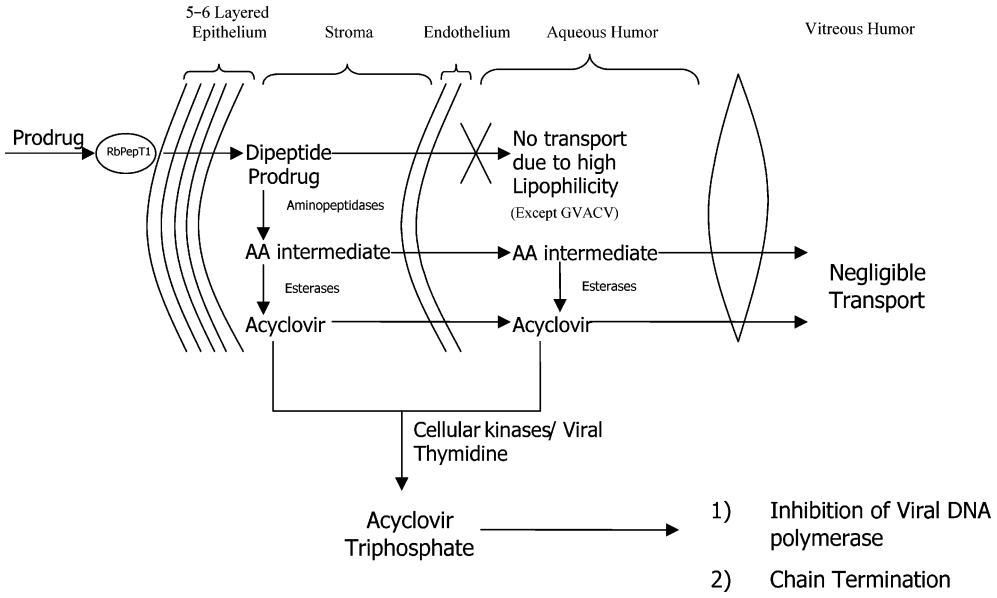

Figure 8. Schematic representation of corneal absorption of ACV dipeptide prodrugs. This figure depicts speculation on the barriers and pathways available for dipeptide prodrugs crossing cornea. The figure also depicts speculation on the localization of enzymes that are useful for the activity of these prodrugs.

regenerated amino acid intermediate, VACV, from GVACV and VVACV were observed to be 2.12 and 1.69 μM, respectively, which are much below the EC_{50} of VACV (9.1 μM[16]) for HSV-1. These concentrations are even lower than the C_{last} values of the regenerated ACV despite the higher C_{max} values (except VYACV), probably due to the higher rapid elimination of the amino acid intermediate, VACV, compared to ACV (Table 1) and also due to conversion to ACV. Although the C_{last} of YACV regenerated from VYACV calculated as 10.8 μM was much higher than the EC_{50} of YACV (6.17 μM[30]), it could only be detected up to 200 min.

This study indicates that topical administration of dipeptide prodrugs of ACV resulted in appreciable therapeutic concentrations of ACV in the aqueous humor. This study demonstrates for the first time that the ocular bioavailability of ACV can be elevated by approximately 2-fold upon topical administration of the dipeptide prodrug, GVACV, in comparison to VACV. This study also gives various pharmacokinetic parameters (C_{last}, half-life) indicating that GVACV constitutes a significant therapeutic advantage over the current drug of choice, trifluorothymidine, in the treatment of ocular herpes infections. In conclusion, a combination of the topical well infusion model and microdialysis can be a valuable tool to determine in vivo ocular pharmacokinetics of the topically applied drugs.

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Abbreviations Used

hPEPT1, human intestinal peptide transporter; ACV, acyclovir; VACV, valacyclovir; VVACV, valine-valine acyclovir; GVACV, glycine-valine acyclovir; YACV, tyrosine acyclovir; GYACV, glycine-tyrosine acyclovir; VYACV, valine-tyrosine acyclovir; IPBS, isotonic phosphate buffered saline; k_a, absorption rate constant.

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