Chemical Stability, Enzymatic Hydrolysis, and Nasal Uptake of Amino Acid Ester Prodrugs of Acyclovir

CHUN YANG, HONGWU GAO, ASHIM K. MITRA

Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri, 5005 Rockhill Road, Kansas City, Missouri 64110

Received 22 June 2000; revised 17 October 2000; accepted 25 October 2000

ABSTRACT: The objective of this work was to improve nasal absorption of relatively impermeable small drug molecules via an amino acid prodrug approach. Acyclovir was selected as a model drug. L-Aspartate β-ester, L-lysyl, and L-phenylalanyl esters of acyclovir were synthesized to investigate their effectiveness in enhancing nasal absorption of acyclovir. A stability study was conducted in phosphate buffer under various pH conditions at 25 and 37°C. Enzymatic hydrolysis in rat nasal washings and plasma was conducted at 37°C. A rat *in situ* nasal perfusion technique was utilized in this investigation to examine the rate and extent of nasal absorption of amino acid prodrugs. The remaining analyte concentrations in the nasal perfusate were quantitated by reversed-phase high-performance liquid chromatography. The results revealed that the L-lysyl and L-phenylalanyl esters were less stable than L-aspartate β-ester. The stability of all three esters decreased with increasing pH and temperature. L-phenylalanyl ester is highly susceptible to plasma esterases, with an *in vitro* half-life 1.33 min. The rat *in situ* nasal perfusion study revealed that the extent of nasal absorption of acyclovir, L-lysyl and L-phenylalanyl esters was not significant ($p < 1\%$). L-Aspartate β-ester was absorbed to the extent of ~8% over 90 min of perfusion at an initial drug concentration of 100 μM. Nasal absorption of L-aspartate β-ester of acyclovir was inhibited by L-asparagine but not by a dipeptide glycylsarcosine (Gly-Sar). The enhancement of acyclovir nasal absorption from the L-aspartate β-ester prodrug suggests that nasal uptake of this prodrug probably involves an active transport system. J Pharm Sci 90:617–624, 2001

Keywords: nasal drug delivery; acyclovir; prodrug; active transport; amino acid transporter; peptide transporter

INTRODUCTION

Systemic drug delivery by the nasal route is currently receiving considerable attention because this route offers many advantages, such as (a) rapid absorption and onset of pharmacological effect; (b) avoidance of liver first- pass metabolism and high systemic availability; and (c) an easy administration route particularly suitable for self-medication.[1] There are, however, limitations; for examples, low permeability across the mucosa, degradation of drug by enzymes in the nasal cavity, and drug loss by rapid mucociliary clearance. To improve systemic bioavailability through nasal administration, two strategies are commonly employed; these are, structural modification and formulation manipulation. Prodrug strategy usually involves transient modification by connecting particular functional group(s) of a drug with a promoiety to obtain favorable physiochemical and biological properties for absorption (i.e., attaching biocleavable lipophilic moieties, thereby thermodynamically favoring solute partitioning into the nasal membrane[2]). Previous studies reported from our laboratory demonstrated that

Correspondence to: A.K. Mitra (Telephone: 816-235-1615; Fax: 816-235-5190; E-mail: mitraa@umkc.edu)

Journal of Pharmaceutical Sciences, Vol. 90, 617–624 (2001)

aliphatic ester prodrugs of acyclovir resulted in improved nasal drug absorption.[3] In this study, an attempt has been made to demonstrate the feasibility of utilizing amino acids as a promoiety such that the conjugate may translocate a model drug across a mucosal membrane by a carrier-mediated transport process. The hypothesis behind this approach is that the amino acid or oligopeptide transport system in the nasal mucosa will recognize the amino acid-conjugated drug. Once the conjugate is absorbed into the systemic circulation, it will revert enzymatically and/or chemically to the parent drug.

Acyclovir, an acyclic analogue of the natural nucleoside 2'-deoxyguanosine, is clinically used in the treatment of herpes simplex, varicella zoster, cytomegalovirus, and Epstein–Barr virus infections.[4] Absorption of orally administered acyclovir is slow, variable, and incomplete, with a bioavailability of ~15–30%. It is impermeable across the nasal mucosa.[5] An *in vitro* study using porcine buccal tissue indicated that buccal transport of acyclovir occurs predominantly by a passive diffusion mechanism, probably through the paracellular route.[6] Therefore, this compound may serve as a good model drug to study nasal absorption enhancement via transporters. Previous reports from our laboratory have demonstrated that L-phenylalanine is absorbed primarily through an amino acid transporter expressed on the rat nasal mucosa.[7, 8] An electrogenic amino acid transporter is expressed on the apical membrane of cultured human bronchial epithelial cells and nasal polyp epithelial cells. Apical L-lysine was effective in increasing the short-circuit current.[9]

In this study, L-aspartate β-ester, L-lysyl, and L-phenylalanyl esters of acyclovir were synthesized as potential prodrugs. A rat *in situ* nasal perfusion technique was utilized to examine the nasal uptake of these esters, and their chemical stability and enzymatic hydrolysis were evaluated. Possible mechanisms involved in the nasal absorption of these prodrugs are discussed.

MATERIALS AND METHODS

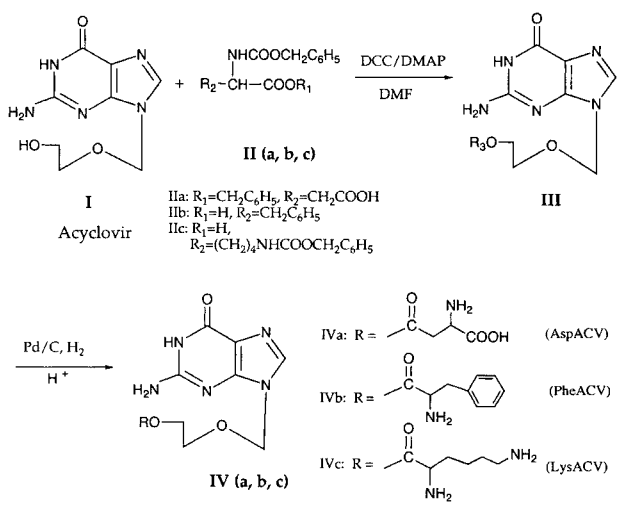
Materials

High-performance liquid chromatography (HPLC) grade acetonitrile (ACN) and NaH_2PO_4 were obtained from Fisher Scientific Company (St. Louis, MO). *N*-Carbobenzoxy (Cbz)-L-aspartic acid α-benzyl ester, *N*-Cbz-L-phenylalanine, and *N*-α-*N*-ε-di-Cbz-L-lysine were obtained from Novabiochem (San Diego, CA). Perchloric acid (70%) was purchased from Allied Chemical (Morristown, NJ). Acyclovir was a gift from Burroughs Wellcome, Inc. (Research Triangle Park, NC). L-Asparagine and glycylsarcosine (Gly-Sar) were obtained from Sigma Chemical Company (St. Louis, MO). All other chemicals were of special reagent grade and were received from Aldrich Chemical Company, Inc. (Milwaukee, WI). Reversed-phase thin layer chromatographic (TLC) plates ($MKC_{18}F$) were obtained from Whatman International Ltd. (Maidstone, U.K.).

Synthesis of Amino Acid Ester Prodrugs

Amino acid ester prodrugs were synthesized by following the procedure described by Beauchamp et al.[10] (Scheme I). Column chromatography was carried out using Grade 62 (60–200 mesh) silica gel, and elution was performed by methanol–methylene chloride (1:9) solvent system. Melting points were determined on a Thomas Hoover capillary melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were measured at 250 or 62 MHz (Bruker AC250). Electrospray ionization (ESI) mass spectra were obtained on a Finnigan TSQ-700 triple-quadrupole mass spectrometer equipped with an electrospray source.

Acyclovir (2.5 mmol) was dissolved in anhydrous dimethylformamide (DMF; 75 mL) by warming on a steam bath. Successively, 4-(dimethylamino) pyridine (DMAP; 0.3 mmol), protected amino acid (4 mmol), and dicyclohexylcarbodiimide (DCC; 4 mmol) were added to the



Scheme 1. Synthesis of amino acid ester prodrugs of acyclovir.

cooled solution. The solution was stirred under a nitrogen atmosphere at ambient temperature for 24 h. The mixture was recharged with additional DMAP (0.3 mmol), amino acid (4 mmol), and DCC (4 mmol), and stirring was continued at ambient temperature for 2 days. The mixture was filtered, DMF was removed from the filtrate *in vacuo*, and the residue was chromatographed on silica gel using 1:9 MeOH–CH_2Cl_2 as the eluent, to generate intermediate products (IIIa, IIIb, IIIc). The ^{1}H NMR and mass spectrometry (MS) were satisfactory for the desired structure. For deprotection, a solution of III (2 mmol) in MeOH (30 mL), tetrahydrofuran (THF; 15 mL), and water (5 mL) was added to 0.5 N aqueous HCl (4.5 mL) and 60 mg of 10% palladium on charcoal. The mixture was shaken in a Parr apparatus under an initial pressure of 50 psi of hydrogen at ambient temperature for 4 h or until no reactant spot was visible on TLC. The mixture was filtered, the catalyst was washed with MeOH, and the combined washings and filtrate were lyophilized. The residue of IVb and IVc was recrystallized following the method reported by Beauchamp et al.[10] The residue of IVa was dissolved in a small volume of water, and the pH of the solution was adjusted to ~5.0 with NaOH (1 N). Then the precipitate was recollected and washed with a small volume of water. After repeating the same procedure once more, the precipitate was dried *in vacuo* to yield the desired amino acid ester product.

Purity of final products was monitored by reversed-phase TLC and HPLC. All of prodrugs were at least 99% pure. Starting material (acyclovir) content was $< 0.5\%$.

IVa: decomposition 195°C; yield (two steps) 51.6%; ^{1}H NMR (DMSO-d_6 + CF_3CO_2D, 10:1): δ 2.89 (d, 2H, CH_2CH(NH_2)CO), 3.76 (m, 2H, CH_2O), 4.17 (m, 1H, αCH), 4.23 (m, 3H, $COCH_2$), 5.53 (s, 2H, NCH_2O), 7.23 (bs, 2-NH_2), 8.41 (bs, NH_3^+), 9.27 (s, 1H, H-8), 11.67 (bs, HN), 13.73 (COOH); ^{13}C NMR (DMSO-d_6 + CF_3COOD, 10:1): δ 34.23 (βC), 48.58 (αC), 63.95 (CH_2OCO), 67.61 (CH_2O), 74.20 (NCH_2O), 108.26 (C-5), 138.66 (C-8), 150.24 (C-4), 153.88 (C-2), 155.99 (C-6), 169.69 (βC=O), 169.93 (terminal C=O), MS (ESI): 341 $[M+1]^+$ (100), 363 $[M+Na]^+$ (25).

IVb: mp, 210–215; yield, 52.2% ^{1}HNMR (DMSO-d_6): δ 3.09 (m, 2H, *CH*2CH(NH_2)C), 3.61 (m, 2H, CH_2O), 4.17(s, 1H, αCH), 4.24 (m, 2H, $COOCH_2$), 5.34 (s, 2H, NCH_2O), 6.77(s, 2H, 2-NH_2), 7.20, 7.22, 7.26, and 7.29 (m, 5H, C_6H_5), 7.90 (s, 1H, H-8), 8.67 (brs, 2H, NH_3^+); 10.93 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 35.78 (βC), 53.11 (αC), 64.45 (CH_2OCO), 66.22 (CH_2O), 71.96 (NCH_2O), 115.99 (C-5), 127.30 (aromatic), 128.57 (aromatic), 129.50 (aromatic), 134.60 (aromatic), 137.64 (C-8), 151.33 (C-4), 154.25 (C-2); 156.61 (C-6), 169.00 (C=O); MS (ESI): 373 $[M+1]^+$ (100).

IVc: foamy solid (lit. 142–154°C); yield 72%; ^{1}H NMR (DMSO-d_6): δ1.42 (m, 2H, COCH(NH_3^+) CH_2 $CH_2CH_2CH_2NH_3^+$), 1.60 (m, 2H, OCH (NH_3^+)$CH_2CH_2CH_2CH_2$ NH_3^+), 1.79 (dm, 2H, COCH(NH_3^+)$CH_2CH_2CH_2CH_2NH_3^+$), 2.77 (bs, 2H, COCH(NH_3^+)$CH_2CH_2CH_2$ $CH_2NH_3^+$), 3.84 (bs, 2H, CH_2O), 3.95 (bs, 1H, CO*CH*(NH_3^+)CH_2CH_2 $CH_2CH_2NH_3^+$), 4.30 (s, 2H, OCH_2), 5.55 (s, 2H, NCH_2O), 7.39 (bs, 2H, 2-NH_2), 8.23 (s, 3H, NH_3^+), 8.76 (s, 3H, NH_3^+), 9.03 (s, 1H, H-8), 11.74 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 21.08, 26.05, 29.15 (COCH(NH_3^+) $CH_2CH_2CH_2CH_2NH_3^+$), 38.08 (COCH(NH_3^+) $CH_2CH_2CH_2CH_2NH_3^+$), 51.47 (CO*CH*-(NH_3^+) $CH_2CH_2CH_2CH_2NH_3^+$), 64.24 (CH_2OCO), 66.76 (CH_2O), 72.98 (NCH_2O), 110.74 (C-5), 137.80 (C-8), 150.22 (C-4), 154.41 (C-2), 155.19 (C-6), 169.28 (C=O); MS(ESI): 354 $[M+1]^+$ (14), 270 (22), 186 (45), 169 (100).

Stability Study

Aqueous stability of prodrugs was examined at pH 7.4 in isotonic phosphate buffer saline solution at 25 and 37°C, and at pH 5.5 and 3.0 in 50 mM sodium phosphate buffer at room temperature. Hydrolysis of the amino acid esters to acyclovir was detected by HPLC. The reaction was followed for 1 week. Apparent first-order rate constants for chemical hydrolysis were determined from the slopes of semilogarithmic plots of percent prodrug remaining versus time.

Analytical Procedure

The concentrations of acyclovir and its prodrugs were quantitated by reversed-phase HPLC. Prior to analysis, all of the biological samples were treated with 10% volume of perchloric acid (70% solution) to precipitate the proteins. Then, internal standard solution was added to a final concentration of 50 μM. After centrifugation at $100{,}000 \times g$ for 20 min, the supernatant was injected onto the HPLC column (Rainin Microsorb-MV C18 column, 5 μm, 250 mm).

The HPLC system was comprised of Varian 9012 Solvent Delivery System, 9100 AutoSampler, and 9050 Variable Wavelength UV-VIS Detector. The signal was monitored at 254 nm. The ratio of integrated peak areas of drug to

Table 1. HPLC Conditions for Analyses of Prodrugs

Compound	Mobile Phase and Flow Rate	Internal Standard;	Retention Time (min)		
			Prodrug	I.S.	ACV
ACV	3% ACN + 97% Buffer 1.0 mL/min	Thymine	—	6.3	7.5
Asp–ACV	1% ACN + 99% Buffer 1.2 mL/min	Thymine	9.5	7.9	12.1
Lys–ACV	3% ACN + 97% Buffer 0.8 mL/min	Thymine	6.7	8.3	10.4
Phe–ACV	12% ACN + 88% Buffer 1.0 mL/min	Caffeine	7.1	9.6	3.0

internal standard was used for quantitative purposes. Mobile phase was prepared with ACN and NaH_2PO_4 (20 mM, pH 3.0). Table 1 provides the mobile phase composition, flow rate, internal standard used, and retention times.

Method for Nasal Perfusion

The rat *in situ* nasal perfusion technique developed by Hirai et al.[11] and Huang et al.[12] was used. Male Sprague-Dawley rats, weighing 250–350 g, were anesthetized with an intraperitoneal injection (0.1 mL/100 g body weight) of a ketamine (90 mg/mL) and xylazine (10 mg/mL) mixture. After an incision was made in the neck, the trachea was cannulated with a polyethylene (PE-200) tube to maintain respiration. Another PE-200 tube was inserted through the esophagus toward the posterior part of the nasal cavity and ligated. The passage of the nasopalatine tract was sealed with an adhesive agent to prevent drainage of the solution from the nasal cavity to the month. The cannula served to deliver the solution to the nasal cavity. The perfusion medium (initial concentration 100 μM), which was prepared with isotonic phosphate buffer saline solution, pH 7.4, was circulated by means of a peristaltic pump at a flow rate of 2 mL/min. The perfusate was recollected into a reservoir, which was maintained at a temperature of 37 ± 0.5°C throughout the course of an experiment. A constant perfusate volume of 5 mL was maintained throughout with constant stirring and an aliquot (100 μL) was sampled at predetermined time intervals.

In Vitro Enzymatic Degradation Study with Rat Nasal Washings and Plasma

Isotonic phosphate buffer saline solution, pH 7.4, was perfused through the rat nasal cavity for 90 min. The perfusate at the end of the experiment was collected and stored at −80°C until further use. One volume of drug stock solution (1.0 mM, 4°C) was mixed with nine volumes of prewarmed nasal washing solution (37°C) and vortexed. A zero-time sample (100 μL) was taken immediately and mixed with 10 μL of an $HClO_4$ solution (70%) and vortexed for 30 s to precipitate the proteins. The mixture was incubated at 37°C, and 100-μL samples were withdrawn at predetermined time intervals and were subjected to the same treatment. Blood was collected from rat jugular vein. After centrifugation at 10,000 rpm for 10 min, plasma was collected and then stored at −80°C until further use. The incubation procedure with rat plasma was similar to that with rat nasal washings.

RESULTS

Apparent first-order rate constants for hydrolysis and nasal absorption were determined from the slopes of various semilogarithmic plots of percent remaining of prodrugs in buffer solutions, nasal washings, plasma and nasal perfusate versus time curves. Table 2 summarizes the half-lives of chemical hydrolyses of various prodrugs in different buffer solutions. A longer half-life ($t_{1/2}$) of L-aspartate β-ester than that of the other two esters indicated L-aspartate β-ester possessed a better stability. The stability of three esters decreased as the temperature and pH of aqueous solution were raised.

Two processes contribute to the prodrug loss from perfusate; they are, nasal uptake and hydrolysis, including chemical and enzymatic hydrolysis. The rate of prodrug loss from perfusate is expressed by eq.1:

$$-\frac{dC}{dt} = k_{app}C = k_{hyd} + k_{abs}C \qquad (1)$$

where C denotes drug concentration in perfusate, k_{app} represents apparent first-order rate constant of drug loss from perfusate, k_{hyd} denotes apparent first-order hydrolysis rate constant, and k_{abs}

Table 2. Half-Lives (h) of Chemical Hydrolyses of Prodrugs[a]

Compound	pH 7.4		pH 3.0	pH 5.0
	37°C	25°C	25°C	25°C
Asp–ACV	16.6 (0.3)	60.3 (0.7)	No measurable degradation during 1 week	Less than 10% degraded during 1 week
Lys–ACV	3.9 (0.09)	11.4 (0.2)	Less than 10% degraded during 1 week	Less than 10% degraded during 1 week
Phe–ACV	4.2 (0.2)	—	Less than 15% degraded during 1 week	136.6 (3.7)

[a]Data are mean (SD) ($n = 3$).

signifies apparent first-order absorption rate constant. The apparent first-order rate constants of prodrug hydrolysis and drug loss from rat nasal perfusate are given in Table 3. The $t_{1/2}$ values of prodrug hydrolysis in nasal washings were, respectively, 16.96, 2.93, and 0.80 h for the Asp, Lys, and Phe conjugates of acyclovir (Asp–ACV, Lys–ACV, and Phe–ACV, respectively). A comparison of hydrolysis $t_{1/2}$ in nasal washings with hydrolysis $t_{1/2}$ in isotonic phosphate buffer saline solution indicated that enzymatic hydrolysis of L-lysyl ester and L- phenylalanyl ester in rat nasal washings solution was significantly more rapid than in isotonic phosphate buffer saline solution, whereas that in L-aspartate β-ester exhibited no significant difference. Therefore, L-aspartate β-ester appears to be less labile to the esterases in rat nasal cavity (Table 3), an encouraging indication that may allow for enhanced nasal mucosal transport of the intact prodrug because of its longer possible residence time in contact with the mucosal epithelium.

The rate constants for hydrolysis of L-lysyl and L-phenylalanyl esters in rat nasal washing solution were 3.94 and 14.5×10^{-3} min^{-1}, respectively (Figure 1 and Table 3). These values were not significantly different from the rate constants of prodrug loss from nasal perfusate that were 3.99 and 14.5×10^{-3} min^{-1}, respectively (Figure 1 and Table 3). The absorption rate constants of L-lysyl and L-phenylalanyl esters were calculated according to eq. 1 and were found to be 5.00×10^{-5} min^{-1} and not measurable, respectively. These results indicate that nasal absorption of intact L-lysyl and L-phenylalanyl esters was negligible. However, L-aspartate β-ester prodrug was absorbed significantly to the extent of ~8% over a 90-min perfusion at an initial concentration of 100 μM. The absorption rate constant of L-aspartate β-ester prodrug was calculated to be 7.69×10^{-4} min^{-1} (Figure 2 and Table 3). Figure 3 depicts the loss of L-aspartate β-ester from nasal perfusate and simultaneous hydrolysis to acyclovir.

Table 3. Apparent First-Order Rate Constants ($\times 10^3$ min^{-1}) of Enzymatic Hydrolysis and Drug Loss from Perfusate and Percent Remaining after 90-min Perfusion[a]

Compound	Hydrolysis in Nasal Washings	Plasma Metabolism	% Remaining after 90-min Perfusion	Drug Disappearance from Perfusate
ACV	—	—	99.06 (5.65)	0.142 (0.075)
Asp–ACV	0.681 (0.072)	8.94 (0.88)	86.72 (1.32)	1.45 (0.26)
Lys–ACV	3.94 (0.86)	12.2 (0.25)	69.34 (1.23)	3.99 (0.25)
Phe–ACV	14.5 (1.8)	519 (37)	29.06 (10.87)	14.5 (5.0)

Data are mean (SD) ($n = 3$–5).
[a]Experiments were run at 37°C in IPBS pH7.4.

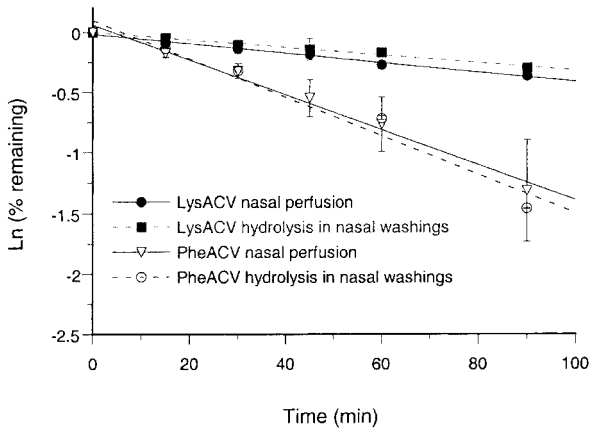


Figure 1. Time courses of disappearance of L-lysyl and L-phenylalanyl esters of acyclovir from incubation with nasal washing solution and perfusion through nasal cavity. Data are mean ± standard deviation (SD; $n = 3–5$).

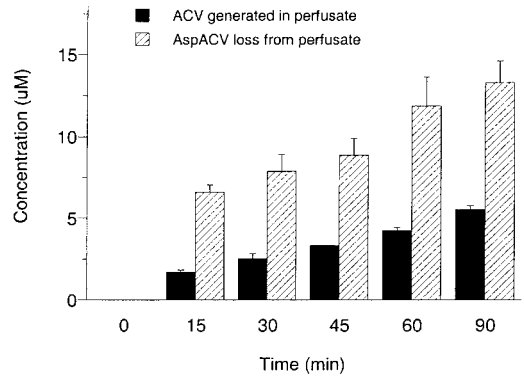


Figure 3. Loss of L-aspartate β-ester prodrug (Asp-ACV) in nasal perfusate increased as a function of time and simultaneous hydrolysis to acyclovir. Data are mean ± SD ($n = 3$). Acyclovir generated from hydrolysis was significantly lower than Asp-ACV lost ($p < 0.005$, two-tailed student t test).

Plasma hydrolysis study suggests that L-phenylalanyl ester of acyclovir is highly susceptible to degradation with a reversion $t_{1/2}$ of 1.33 min, whereas plasma bioconversion $t_{1/2}$ for L-lysyl ester and L-aspartate β-ester are 56.8 and 77.5 min, respectively (Table 3).

To delineate the possible mechanism for nasal uptake of L-aspartate β-ester of acyclovir, a competitive inhibition study was conducted in the presence of L-asparagine (2 mM) and the dipeptide Gly-Sar (2 mM). The inhibitors were dissolved in isotonic phosphate buffer saline solution, pH 7.4, and the concentration of L-aspartate β-ester of acyclovir was kept at 100 μM. Figure 4 illustrates the inhibitory effect of L-asparagine and Gly-Sar on nasal absorption of L-aspartate β-ester. After 90 min of perfusion, the extent of L-aspartate β-ester loss from nasal perfusate in the presence of L-asparagine showed significant difference from that in the absence of L-asparagine (one-tail student t test, $p < 0.05$). However, there is no significant difference in prodrug loss from nasal perfusate in the presence and absence of Gly-Sar. These preliminary

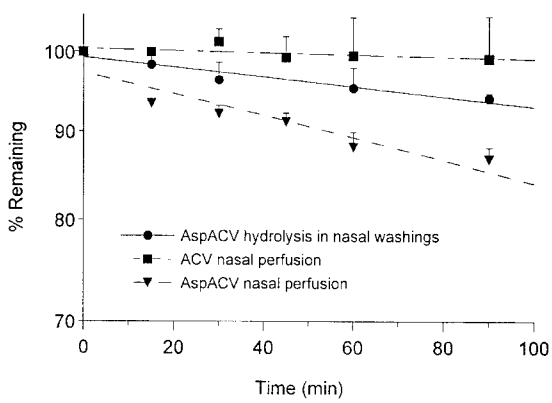


Figure 2. Time courses of disappearance of L-aspartate β-ester of acyclovir from incubation with nasal washing solution and perfusion through nasal cavity. Comparison with the time course of nasal perfusion of acyclovir. Data are mean ± SD ($n = 3–5$).

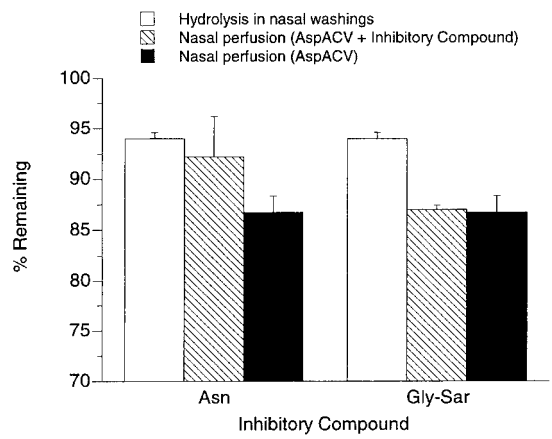


Figure 4. Inhibitory effects of L-asparagine (Asn) and Gly-Sar on nasal absorption of L-aspartate β-ester of acyclovir. Percent remaining of prodrug in nasal perfusate after a 90-min perfusion. Data are mean ± SD ($n = 3$). L-Asparagine inhibited prodrug absorption significantly ($p < 0.05$, one-tailed student t test).

results indicate that L-asparagine inhibits nasal uptake of the prodrug, whereas Gly-Sar exerts no inhibitory effect on the nasal uptake of Asp–ACV.

DISCUSSION

In recent years, different nutrient transporters (e.g., peptide, amino acid, and glucose transporters) have been identified and cloned. The active nutrient transport systems have become a key target for prodrug design. The preferential delivery of drugs or their derivatives to a target site may be improved by using the active transport system present at the target site. For example, prodrug designed in the form of di/tripeptide analogues can be absorbed across the intestinal brush border membrane via the oligopeptide transport system.[13]. Valacyclovir, the L-valyl ester prodrug of acyclovir, has been demonstrated to be absorbed in the intestinal tract by the PepT1 peptide transporters.[14, 15] Amino acid transport systems have also been utilized to improve the absorption of amino acid-conjugated drugs or amino acid derivatives.[16, 17] Walker et al.[17] demonstrated that the phosphonoformate–L-tyrosine conjugate is actively transported by means of active amino acid transporters through monolayers of porcine brain microvessel endothelial cells. Surendran et al.[18] reported that PD 158473, an NMDA antagonist and a phenylalanine derivative with a phosphoric acid and naphthyl substitutent on positions 5 and 3, respectively, is a substrate for large neutral amino acid (LNAA) transporter and dipeptide (hPEPT1) transporter.

In this study, two different classes of amino acid esters, α-amino acid ester and amino acid β-ester, were synthesized to investigate their ability to improve nasal absorption of acyclovir. The results suggest that L-lysyl ester and L-phenylalanyl ester are not sufficiently absorbed through nasal pathway, although there is evidence to show that L-lysine and L-phenylalanine can be taken up by an amino acid transport system in the nasal cavity.[8,9] However, L-aspartate β-ester can be absorbed significantly by the nasal route. Preliminary results from the inhibition of nasal absorption of L-aspartate β-ester by L-asparagine but not by Gly-Sar suggest that nasal uptake of this amino acid ester prodrug is probably mediated by an amino acid transport system in the rat nasal mucosa. In another report, using an *in vitro* cell culture model of human airway epithelial cell line, we have demonstrated that L-aspartate β-ester of acyclovir inhibited L-tyrosine uptake in a competitive manner.[19] The amino acid transport system expressed on nasal epithelial membrane may require that substrate possesses certain structural features (i.e., free α-amino and terminal carboxyl groups). The β-esters were not absorbable probably because of their lack of free terminal carboxyl group. Our previous study on nasal absorption of an L-tyrosine-like model compound also showed that the L-tyrosine conjugate, which was formed through ether linkage between hydroxyl group of parent compound and aromatic hydroxyl group of tyrosine, was absorbed, whereas its parent compound was not absorbed.[20] Amino acids are absorbed in the intestine by distinctly different mechanism from the peptides.[21] The L-valine ester prodrug of acyclovir was taken up in the intestine by the intestinal PepT1 peptide transporter. L-Lysyl ester and L-phenylalanyl ester might be substrates for peptide transport system. However, the peptide transport systems may not be highly expressed on the nasal epithelial cells. Previous studies from our laboratory showed that nasal absorption of the dipeptide Tyr-Gly and [-Arg^2]-kyotorphin was negligible.[22]

Rational design of prodrugs that can be taken up by a specific transporter requires more information on the substrate structural specificity of the transport system. It is currently unknown as to which specific amino acid transport system is involved in the nasal absorption of the L-aspartate β-ester of acyclovir.

In conclusion, the L-aspartate β-ester prodrug can be absorbed nasally and is least labile to enzymatic hydrolysis in the nasal mucosa. This report demonstrates that improvement in nasal absorption of nonabsorbable acyclovir could be achieved by attaching the drug to an amino acid such that the amino acid transporter can recognize the conjugated drug. This strategy appears to have wide applicability in the design of potential prodrugs of poorly permeable drugs for efficient transmucosal delivery.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Paul Brown, Quintiles, Kansas City, Missouri, for his help in obtaining mass spectra. This work was supported by NIH Grants 2R01 EY 09171-06 and 2R01 EY10659-05.

REFERENCES

1. Hussain AA. 1998. Intranasal drug delivery. Adv Drug Delivery Rev 29:39–49.
2. Krishamoorthy R, Mitra AK. 1998. Prodrugs for nasal drug delivery. Adv Drug Delivery Rev 29: 135–146.
3. Shao Z, Park GB, Krishnamoorthy R, Mitra AK. 1994. The physicochemical properties, plasma enzymatic hydrolysis, and nasal absorption of acyclovir and its 2'-ester prodrugs. Pharm Res 11:237–242.
4. O'Brien JJ, Campoli-Richards DM. 1989. Acyclovir. An updated review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy. Drug 37:233–309.
5. Shao Z, Mitra, AK. 1994. Bile salt-fatty acid mixed micelles as nasal absorption promoters: III. Effects on nasal transport and enzymatic degradation of acyclovir prodrugs. Int J Pharm 11:243–250.
6. Shojaei AH, Berner B, Li X. 1998. Transbuccal Delivery of acyclovir: *In vitro* determination of routes of buccal transport. Pharm Res 15:1182–1188.
7. Tengamnuay P, Mitra AK. 1988. Transport of tyrosine and phenyalanine across the rat nasal mucosa, Life Sci 43:585–593.
8. Tengamnuay P. 1989. Ph.D. Thesis, Purdue University, West Lafayette, IN.
9. Galietta LJV, Musante L, Romio L, Caruso U, Fantasia A, Gazzolo A, Romano L, Sacco O, Rossi GA, Varesio L, Zegarra-Moran O. 1998. An electrogenic amino acid transporter in the apical membrane of cultured human bronchial epithelial cells. Am J Physiol 275: L917–L923.
10. Beauchamp LM, Orr GF, de Miranda P, Burnette T, Krenitsky TA. 1992. Amino acid ester prodrugs of acyclovir. Antiviral Chem Chemother 3:157–164.
11. Hirai S, Yashiki T, Matsuzawa T, Mima H. 1981. Absorption of drugs from the nasal mucosa. Int J Pharm 7:317–325.
12. Huang CH, Kimura R, Bawarshi-Nassar R, Hussain A.1985. Mechanism of nasal absorption of drugs I: Physicochemical parameters influencing the rate of *in situ* nasal absorption of drugs in rats. J Pharm Sci 74:608–611.
13. Yang CY, Dantzig AH, Pidgeon C. 1999. Intestinal peptide transport systems and oral drug availability. Pharm Res 16:1331–1343.
14. Han HK, Devrueh RLA, Rhie JK, Covitz KMY, Smith PL, Lee CP, Oh DM, Sadee W, Amidon GL. 1998. 5'-Amino acid ester of antiviral nucleosides, acyclovir, and AZT are absorbed by the intestinal PEPT1 peptide transporter. Pharm Res 15:1154–1159.
15. Guo AL, Hu PD, Balimane PV, Leibach FH, Sinko PJ. 1999. Interactions of a nonpeptidic drug, valacyclovir, with the human intestinal peptide transporter (hPEPT1) expressed in a mammalian cell line. J Pharmacol Exp Ther 289:448–454.
16. 16. *.Bonina FP, Arenare L, Palagiano F, Saija A, Nava F, Trometta D, de Caprariis P. 1999. Synthesis, stability, and pharmacological evaluation of nipecotic acid prodrugs. J Pharm Sci 88: 561–567.
17. Walker I, Nicholls D, Irwin WJ, Freeman S. 1994. Drug delivery via active transport at the blood-brain barrier: Affinity of a prodrug of phosphonoformate for the large amino acid transporter. Int J Pharm 104:157–167.
18. Surendram N, Covitz, KY, Han H, Sadee W, Oh D, Amidon GL, Williamson RM, Bigge CF, Stewart BH. 1999. Evidence for overlapping substrate specificity between large neutral amino acid (LNAA) and dipeptide (hPEPT1) transporters for PD 158473, an NMDA antagonist. Pharm Res 16:391–395.
19. Yang C, Mitra AK. 2000. Mechanistic study on nasal absorption of amino acid ester prodrug of acyclovir: competitive inhibition of L-tyrosine uptake by L-aspartic acid β-ester of acyclovir, manuscript in preparation.
20. Yang C, Mitra AK. 2000. Nasal absorption of tyrosine linked model compounds. J Pharm Sci, (in press)
21. Ganapathy V, Brandsch M, Leibach FH. 1994. Intestinal transport of amino acids and peptides. In: Johnson, LR, Editor. Physiology of the gastrointestinal tract, 3rd ed. New York: Raven Press, pp 1773–1794.
22. Tengamnuay P, Mitra AK. 1990. Bile salt-fatty acid-mixed micelles as nasal absorption promoters of peptides I—Effects of counterions, adjuvant composition of lipid structure on the nasal absorption of [-Arg^2]-Kyotorphin. Pharm Res 7:129–135.