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5'-O-D-VALYL *ara* A, A POTENTIAL PRODRUG FOR IMPROVING ORAL BIOAVAILABILITY OF THE ANTIVIRAL AGENT VIDARABINE

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Abstract

In order to improve the oral bioavailability of Adenine 9- β -D-arabinofuranoside (Vidarabine, also called *ara* A), an antiviral drug which is active against herpes simplex and varicella zoster viruses and the first agent to be licensed for the treatment of systematic herpes virus infection in man, the corresponding 5'-O-D-valyl ester derivative has been synthesized. Based on their physicochemical properties, 5'-O-valyl *ara* A has emerged as a potential prodrug candidate to improve the oral bioavailability of vidarabine. We describe in this paper a facile synthesis route for the prodrug and its physicochemical properties.

Keywords

Herpes virus; small pox virus; adenine 9- β -D-arabinofuranoside vidarabine; *ara* A; prodrug; selective protection of secondary hydroxyl groups; levulinate ester

INTRODUCTION

1- β -D-Arabinofuranosyladenine (vidarabine or *ara* A) is an antiviral drug with activity against herpes viruses, poxviruses, and certain rhabdoviruses, hepadnaviruses, and RNA tumor viruses.[1–4] However, it is more toxic and less metabolically stable than other current antivirals such as acyclovir and ganciclovir. It is readily deaminated by adenosine deaminase to *ara*-hypoxanthine (*ara*-H).[5] This metabolite possesses weak antiviral activity, which is at least 10-fold less potent than vidarabine.[6] Our recent interest in prodrugs of vidarabine was triggered by the discovery that vidarabine was 3- to 5-fold more active against vaccinia and cow pox viruses than cidofovir in plaque reduction assays.[7] Cidofovir is an anti-pox virus agent[8–11] with serious limitations.[12,13–15] Furthermore, vidarabine's activity against these viruses was enhanced approximately 10-fold when combined with 2'-deoxycoformycin (pentostatin, a potent inhibitor of adenosine deaminase; ADA), thus providing significant superiority to cidofovir. Based on these results and earlier studies on 5'-substituted vidarabine analogs,[16] we hypothesized that minimizing the conversion of vidarabine to its hypoxanthine analog could yield a significantly more potent anti-pox virus agent. With this goal in mind, we have developed a prodrug strategy that protects the vidarabine from metabolic conversion by making 5'-D-valyl esters derivative of vidarabine. We report here the chemical syntheses of the prodrug, as well as some physicochemical characteristics of 5'-O-D-valyl *ara* A.

CHEMISTRY

In order to efficiently synthesize 5'-O-amino acid ester derivative of vidarabine in scalable synthetic process, it is crucial to selectively protect 2' and 3' hydroxyl groups of vidarabine. Such blocking groups had to be easily and quickly removed under non-basic conditions (otherwise concomitant cleaving of the acyl groups in the amino acid ester moiety would be expected). After several unsuccessful attempts with various protecting groups, the final candidate for protection of the 2', 3' hydroxyl positions was the levulinate group. The levulinate group can survive the synthesis conditions for these prodrugs while it can be easily removed by treating with 2M hydrazine in pyridine-acetic acid buffer for 10 minutes.[17a,b] Normal esters are not cleaved under these conditions.[17c,d] In addition, the levulinate is less prone to migrate than the benzoate and acetate.[18] The synthesis route is illustrated in Scheme 1.

EXPERIMENTAL

General Procedures

Reagents, solvents and solutions: All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. All chemicals were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). For anhydrous reactions, solvents were dried according to Vogel's Textbook of Practical Organic Chemistry. [28] Removal of solvent was performed under reduced pressure using a Büchi rotary evaporator, followed by evacuation (<0.1 mm Hg) to constant sample weight. Deionized water was obtained from a Milli-Q reagent water system (Millipore Co., Milford, MA, USA). All reactions were carried out under argon atmosphere in dried glassware.

Purification Techniques—All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using plates with a UV fluorescent indicator (normal SiO₂, Merck 60 F254). One or more of the following methods were used for visualization: UV absorption by fluorescence quenching; iodine staining. Flash chromatography was performed according to the method of Still[29] using Merck type 60, 230–400 mesh silica gel.

Instrumentation for Compound Characterization—Low resolution FAB mass spectra (FAB-MS) were recorded on a Quattro II mass spectrometer (Waters Corp., Milford, MA, USA). Nuclear magnetic resonance (NMR) spectra were obtained on Bruker 400 spectrometer (Bruker BioSpin Ltd., Billerica, MA, USA) from Spectral Data Service (Champaign, IL, USA). ¹H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual proton resonance of solvents as reference: D₂O δ 4.8 and DMSO-d₆ δ 2.50. ¹³C NMR chemical shifts are reported relative to TMS or DMSO-d₆ δ 39.5. ³¹P NMR chemical shifts are reported relative to trimethylphosphate δ 3.0. ¹H NMR data are reported in the following order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet and m, multiplet), and coupling constant (J) in Hertz (Hz). When appropriate, the multiplicity is preceded by br, indicating that the signal was broad. Elemental analysis (C, H, N) were performed by the Robertson Microlit Laboratories (Madison, NJ, USA). Yields refer to isolated yields of compounds estimated to be > 95% pure as determined by ¹H and ¹³C NMR, analytical HPLC and elemental analysis.

9-[5-O-(tert -Butyldimethylsilyl)-β-D-arabinofuranosyl]adenine (2): The described procedure[19] was modified. To a stirred suspension of 2.0 g (7.5 mmol) of dry 9-(β-D-arabinofuranosyl)adenine (1) in 20 mL of anhydrous N,N-dimethylformamide was added 1.2 g (18 mmol) of imidazole, followed by 1.13 g (7.48 mmol) of tert-butyldimethylchlorosilane. The mixture was stirred with protection of argon at room temperature for 20 hours, at the end of which time the solvent was removed at 50°C in vacuo. The residue was dissolved in 50 mL

of ethyl acetate, the solution was washed with two times of 40 mL of water, one time of 30 mL brine and the extract was dried (anhydrous Na₂SO₄) and evaporated to give a white solid which is pure enough for next step. Yield: 2.76 g (96%).

¹H NMR (DMSO-d₆) δ 0.100 (6H, 2s, SiMe₂), 0.926 (9H, s, t-butylSi); arabino: 3.822–3.915 (3H, m), 4.193–4.206 (1H, m), 4.238–4.251 (1H, m), 5.5–6.0 (2H, br, OH2' and OH3'), 6.305 (1H, d, ³J = 5.35Hz, H1'); Adenine: 7.298 (2H, s, NH₂), 8.168 (1H, s, H2 of adenine), 8.179 (1H, s, H8 of adenine). ¹³C NMR (DMSO-d₆) δ 5.351 (Me from SiMe₂), 18.125 (Quaternary Carbon from t-butylSi), 25.855 (Me from t-butylSi); arabino: 62.478 (C5'), 74.262 (C3'), 75.627 (C2'), 83.000 (C4'), 83.056 (C1'); adenine: 121.681 (C5), 138.128 (C8), 139.909 (C4), 152.437 (C2), 155.882 (C6). Mass spectrum: calculated for C₁₆H₂₇N₅O₄Si: 381.5. FAB-MS: m/z 382.30 (M+1). Anal. Calcd. For C₁₆H₂₇N₅O₄Si: C, 50.37; H, 7.13; N, 18.36. Found: C, 50.67; H, 7.33; N, 18.26

9-[5-O-(tert-Butyldimethylsilyl)-2,3-O-dilevulinyl- β -D-arabinofuranosyl]-adenine (3):

Levulinic acid (3.77 g, 3.33 mL, 32 mmole) was dissolved in anhydrous ethyl acetate (30 mL), and solution of DCC (6.7 g, 32.6 mmol) in 10 mL anhydrous ethyl acetate was added. The reaction mixture was stirred under protection of argon at room temperature for 1.5 hours. The mixture was then filtered through a filter tube into a suspension of **2** (2.56 g, 6.7 mmol) and DMAP (0.26 g, 2.1 mmol) in anhydrous ethyl acetate (20 mL), washing the filter pad (DCU) with more anhydrous ethyl acetate (20 mL). This reaction was stirred under protection of argon at room temperature for another 1.5 hours after which time anhydrous EtOH (4 mL) was added. After an additional 30 minutes, the precipitate (DCU) was filtered off, and solvent was removed from filtrate in vacuo. The residue was redissolved in 50 mL ethyl acetate and extracted with saturated ammonium chloride aqueous solution 40 mL once, water 40 mL twice and 40 mL brine once, the organic layer was dried with anhydrous Na₂SO₄ before being evaporated to dryness. The resulted syrup was further purified by silica flash chromatograph (6% MeOH in DCM as eluent) to obtain 3.48 g pure product (89.7%).

¹H NMR (DMSO-d₆) δ 0.050 (6H, 2s, SiMe₂), 0.880 (9H, s, t-butylSi); Levulinate: 1.946 (3H, s CH₃), 2.119 (3H, s, CH₃), 2.345–2.379 (2H, t, CH₂), 2.500–2.547 (4H, m, two CH₂), 2.744–2.777 (2H, t, CH₂); arabino: 3.875–4.121 (3H, m), 5.557–5.633 (2H, m), 6.486 (1H, d, ³J = 5.19Hz, H1'); adenine: 7.333 (2H, s, NH₂), 8.148 (1H, s, H2 of adenine), 8.190 (1H, s, H8 of adenine).

¹³C NMR (DMSO-d₆) δ 5.869 (Me from SiMe₂), 18.273 (Quaternary Carbon from t-butylSi), 26.027 (Me from t-butylSi); Levulinate: 28.463 and 28.624 (two CH₃), 29.532 and 29.847 (two CH₂ α to the ester bonds), 37.183 and 37.526 (two CH₂ β to the ester bonds), 170.829 and 171.947 (two ester carbonyl carbon), 206.308 and 207.027 (two ketone carbo) arabino 61.386 (C5'), 74.037 (C3'), 75.290 (C2'), 82.068 (C4'), 82.296 (C1'); adenine: 120.385 (C5), 138.776 (C8), 149.269 (C4), 152.832 (C2), 155.711 (C6). Mass spectrum: calculated for C₂₆H₃₉N₅O₈Si: 577.7. FAB-MS: m/z 579.13 (M+1). Anal. Calcd. For C₂₆H₃₉N₅O₈Si: C, 54.06; H, 6.80; N, 12.12. Found: C, 54.07; H, 7.03; N, 12.07.

9-[2,3-O-dilevulinyl- β -D-arabinofuranosyl]adenine (4): The foam of 3.0 g (5.21 mmol) of **3** was dissolved in a solution of 1 M TBAF in THF (23 mL) containing glacial acetic acid (2 mL). The reaction mixture was stirred under protection of argon at room temperature for 1.5 hours and was then directly filtered through a silica gel plug (4 $\times$ 5 cm) with THF as eluent. The collected fractions were evaporated to dryness in vacuo. The residue was redissolved in DCM and purified by silica gel flash chromatography with 3% methanol in DCM as eluent to obtain 2.1 g (87% yield) gum like product, which was crystallized from ethyl acetate as white solid.

¹H NMR (DMSO-d₆) δ Levulinate: 1.916 (3H, s, CH₃), 2.127 (3H, s, CH₃), 2.380–2.413 (2H, t, CH₂, 3J = 6.7), 2.532–2.564 (2H, t, CH₂, 3J = 6.4Hz), 2.748–2.780 (2H, t, CH₂, 3J = 6.6Hz), 3.167–3.177 (2H, d, CH₂); arabino: 3.659–3.730 (2H, m), 4.071–4.099 (1H, m), 5.163–5.192 (1H, t, 5' OH, ³J = 5.51), 5.499–5.528 (2H, m), 6.475 (1H, d, ³J = 5.29Hz, H1'); adenine: 7.324 (2H, s, NH₂), 8.144 (1H, s, H2 of adenine), 8.287 (1H, s, H8 of adenine).

¹³C NMR (DMSO-d₆) δ Levulinate: 27.243 and 27.592 (two CH₃), 29.195 and 29.466 (two CH₂ α to the ester bonds), 36.925 and 37.383 (two CH₂ β to the ester bonds), 170.538 and 171.541 (two ester carbonyl carbon), 205.990 and 206.745 (two ketone carbo); arabino 60.307 (C5'), 74.406 (C3'), 74.866 (C2'), 81.185 (C4'), 81.743 (C1'); adenine: 118.286 (C5), 139.610 (C8), 149.040 (C4), 152.688 (C2), 155.972 (C6). Mass spectrum: calculated for C₂₀H₂₅N₅O₈: 463.44. FAB-MS: m/z 464.86 (M+1). Anal. Calcd. For C₂₀H₂₅N₅O₈: C, 51.83; H, 5.44; N, 15.11. Found: C, 51.81; H, 5.47; N, 15.31.

9-[5-O-D-valyl- β -D-arabinofuranosyl]adenine (7): 435 mg (2 mmole) of ^αN-Boc-D-valine, 24.5 mg (0.2 mmole) of DMAP and 310 mg (0.669 mmole) of compound **4** was dried in a 250 ml single-necked round bottom flask under high vacuum overnight. 5 ml anhydrous DMF was added. After stirred for 5 minutes at 0°C, 414 mg (2.00 mmole) of DCC in 5 ml DMF was added dropwise at 0°C. After 1.5 hours, TLC indicated completion of the reaction. 5 ml anhydrous ethanol was added and the reaction mixture was stirred overnight. Then DMF was removed under high vacuum by rotavapor at 40°C. The residue was dissolved in 50 ml ethyl acetate. The insoluble white precipitate was removed by filtration. The filtrate was consecutively washed with 20 ml saturated ammonium chloride, 20 ml water, and 20 ml brine. After the solvent was dried with anhydrous sodium sulfate, it was removed in vacuo. The residue was dissolved in dichloromethane and eluted from a silica gel column using 92:8 dichloromethane and methanol as the eluting solvent. Proper fractions were combined and evaporated to dryness. The fully blocked compound **5** obtained was treated with 1 ml 2 M hydrazine hydrate in pyridine-acetic acid buffer for 10 minutes to produce an uncharged compound **6**. The excess hydrazine was eliminated through reaction with pentane-2,4-dione for another 10 minutes. After evaporating the volatile components, the residue was dissolved in EtOAc, washed with 20 ml saturated ammonium chloride, water, and brine. The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated to dryness. The residue was dissolved in dichloromethane and eluted from a silica gel column using 85:15 dichloromethane and methanol as the eluting solvent. Proper fractions were combined and evaporated to dryness. 6 ml of 1:1 TFA and dichloromethane was added to the residue and stirred for 2 hours. Solvent was removed *in vacuo*. The residue was washed with cold diethyl ether. The resulting white solid was purified by reverse phase preparative HPLC to obtain 240 mg compound **7** (60% from compound **4**). HPLC condition: gradient, 0 to 85% methanol in water containing 0.02% TFA within 30 minutes.

¹H NMR (D₂O) δ Valyl: 0.881–0.912 (6H, t, CH₃), 2.205–2.286 (1H, m, CH), 3.949–3.961 (1H, d, CH, ³J = 4.63Hz); arabino: 4.185–4.225 (1H, m), 4.289–4.315 (1H, m), 4.448–4.501 (2H, m), 4.590–4.650 (1H, m), 6.405 (1H, d, 3J = 5.25Hz, H1'); adenine: 8.315 (1H, s, H2 of adenine), 8.331 (1H, s, H8 of adenine).

¹³C NMR (DMSO-d₆) δ Valyl: 16.827 and 17.031 (two CH₃), 29.017 (CH), 58.163 (CH), 169.394 (ester carbonyl carbon); arabino: 65.42 (C5'), 75.024 (C3'), 75.193 (C2'), 80.481 (C4'), 84.868 (C1'); adenine: 114.694 (C5), 143.480 (C8), 144.368 (C4), 148.106 (C2), 149.897 (C6).

Mass spectrum: calculated for C₁₅H₂₂N₆O₅: 366.37. FAB-MS: m/z 367.95 (M+1)

Anal. Calcd. For C₁₅H₂₂N₆O₅•2TFA: C, 38.39; H, 4.07; N, 14.14. Found: C, 38.65; H, 4.15; N, 14.36.

PHYSICOCHEMICAL PROPERTIES

Aqueous solubility of 5'-O-D-valyl ara A has been determined in comparison with the solubility of the parent nucleoside ara A. 5'-O-D-valyl ara A is highly soluble in aqueous media (>10mg/ml) while ara A has aqueous solubility ranging from 0.143 mg/ml (pH = 7) to 0.605 mg/ml (pH = 2). Stability of 5'-O-D-valyl ara A in the presence of adenosine deaminase (ADA) is also improved.[7] The different physicochemical properties in between vidarabine and 5'-O-D valyl ara A are illustrated in details below.

Antiviral Activity of Vidarabine and Its Prodrugs

We examined the antiviral activity of vidarabine and D-valyl ara A using two small pox viruses, vaccinia, and cowpox virus. Drug effects were calculated as a percentage of the reduction in plaque number in the presence of each drug concentration compared to the numbers obtained in the absence of drug. As seen in Table 1, vidarabine has significant anti-pox virus activity with EC50 values that are 4–5 times lower than cidofovir, the positive control for this virus. It is intriguing that coadministration of the vidarabine with the adenosine deaminase inhibitor 2'-deoxycoformycin (at a concentration of 1 μ M) gave EC50 values that are >5-(vaccinia) to 10-(cowpox) fold lower than those seen with vidarabine alone. At this low level (1 μ M), the 2'-deoxycoformycin alone had no effect on virus replication; however, with a Ki of ~50 nM as an ADA inhibitor, its effect is likely at the level of adenosine deaminase.[20] The D-valyl ester prodrugs of vidarabine showed EC50 values comparable to vidarabine alone. It is unclear at this stage whether the antiviral activity of the prodrugs resulted from the prodrug itself or vidarabine derived by the hydrolysis of the amino acid ester moiety. However, as discussed below, these compounds show enhanced enzymatic stability and high potential for oral delivery.

Stability Studies with Adenosine Deaminase

One aspect of our prodrug strategy for vidarabine is to minimize the activity of adenosine deaminase (ADA) activity that converts the vidarabine to 9-(β -D-arabinofuranosyl) hypoxanthine (ara-H), which is at least 10-fold less potent than vidarabine (Scheme 2). Significant levels of this enzyme are found in the intestinal mucosa,[20,21] which limits the effectiveness of oral vidarabine delivery. Previous studies suggested that 5'-hydroxy substituted vidarabine analogs were resistant to adenosine deaminase.[22] Thus, we examined the stability of vidarabine and the prodrugs in the presence of adenosine deaminase to evaluate the effect of the amino acid promoieties on deamination by the enzyme. Briefly, 230 μ L adenosine deaminase 1 (0.2 mg/mL) were placed in wells of 96-well plates, the reactions initiated by the addition of substrates (200 μ M) in pH 7.4 phosphate buffer, and incubated at 37°C for 90 minutes. A minimum of three wells were used for each compound tested. At various time points, 40 μ L aliquots were removed and added to 40 μ L 10% cold TFA. The mixtures were centrifuged and filtered through a 0.45 μ m filter for 10 minutes at 2000 rcf and 4°C. The filtrate was then analyzed by HPLC-Mass. The disappearance of vidarabine or prodrug as well as the appearance of the deaminated product was monitored to determine stability profiles. As seen in Figure 1, vidarabine was rapidly hydrolyzed with a commensurate rise in the concentration of the deaminated product, ara-H (Figure 1, top panel). However, the 5'-D-valyl-ara A was resistant to the enzyme, with >90% remaining after a 90-minute incubation with excess enzyme (Figure 1, bottom panel). These results indicate that the prodrug was capable of withstanding the metabolic effects of the ADA.

Caco-2 Monolayer Transport Studies

Our next step was to look at the transepithelial transport of the prodrugs through Caco-2 monolayers. The permeability of vidarabine and the 5-D-valyl ara A was evaluated as previously described.[23] In these experiments, transport studies were performed 21 days post-

seeding. The assay was initiated by adding drug transport solution (0.2 mM drug in MES buffer, pH 6.0 containing 5 mM D-glucose, 5 mM MES, 1 mM CaCl₂, 1 mM MgCl₂, 150 mM NaCl, 3 mM KCl, 1 mM NaH₂PO₄) to the apical chamber of the Caco-2 insert. Two hundred microliter aliquots were withdrawn from the basolateral chamber at predetermined intervals and replaced with fresh HEPES pH 7.4 buffer. The epithelial integrity of representative cell monolayers was assessed by monitoring transepithelial resistance. As seen in Table 2, the permeabilities of the 5'-O-D-valyl ara A were enhanced by >3-fold.

In Vivo Absorption Studies

One important aspect of our prodrug strategies is to improve the absorption of the drug through the intestinal tract. In previous work, we have shown that amino acid ester prodrugs of a variety of antiviral and anticancer agents is improved over that of the parent compound, in part because the transport of the prodrug is mediated by nutrient transporters (e.g., the dipeptide transporter PepT1) that reside in the intestinal membrane.[24–27] We examined the absorption of the 5'-O-D-valyl-ara A in rats. As a test of intestinal uptake without the complication of gastric dilution or degradation, the duodenal intestinal segment of an anesthetized rat was exposed and the prodrug or vidarabine parent compound was directly injected into the intestinal lumen. Blood was sampled from a jugular vein cannula over a 4-hour period and analyzed for the prodrug, vidarabine, and the metabolite ara-H. In the case of ara-A (vidarabine) dosing (Figure 2, top panel), over 95% of the drug was converted to ara-H. However, for the D-valyl ara A (Figure 2, bottom panel), significant levels of vidarabine were detected. Approximately 13% of the total dose was present as vidarabine, 12% of the total dose was found to be ara-H, and 75% was the prodrug, based on AUC_{0–4hr} calculations (Table 3). The 5'-O-D-valyl ara A resulted in 13-fold increase in circulating vidarabine levels over dosing with Vidarabine.

CONCLUSION

Our results have shown that 5'-O-D-valyl ester prodrugs of vidarabine maintain antiviral activity against the surrogate pox virus, vaccinia. Further, using purified enzyme and intact cell systems, we show that the prodrugs are resistant to inactivation by deamination. The prodrug also shows enhanced transport potential. In our experiments, delivery of the prodrug to the intestine results in a 20-fold increase in the vidarabine plasma levels when compared with intestinal delivery of vidarabine.

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FIGURE 1.

Deamination of vidarabine and its prodrugs by adenosine deaminase1. Top: Concentration (μM) profiles of the disappearance of vidarabine and the appearance of Ara-H in the presence of adenosine deaminase. Bottom: Percentage of vidarabine or prodrug remaining following incubation with adenosine deaminase1 for 90 minutes.

FIGURE 2.

Plasma profile of vidarabine (top) or D-valy-Ara A (bottom) after duodenal injection in the rat. Animals were dosed via duodenal injection into anesthetized rats ($n = 3$ per compound) with either Vidarabine (ara A) or 5'-O-D valyl ester of vidarabine prodrug. Plasma samples were taken at 0, 0.25, 0.5, 1, 2, and 4 hours from the jugular vein and were assayed for prodrug ($\square$), vidarabine ($\blacksquare$), and ara H ($\blacklozenge$) by LC/MS/MS. The dose for vidarabine (1.5 mg/rat) and D-valyl vidarabine (2.05 mg/rat) were such that the same molar amount of vidarabine was delivered in both groups.

(a). Tert-butyldimethylsilyl chloride and imidazole in DMF, (b). Levulinic acid, DCC, DMAP in ethyl acetate, (c). TBAF-acetic acid (1:2 mole ratio) in tetrahydrofuran, (d). Boc-D-valine, DCC, DMAP in DMF, (e). 1M hydrazine hydrate in pyridine-acetic acid buffer for 10 minutes, (f). 1:1 TFA/Dichloromethane 2 hours

SCHEME 1.
Synthesis of 5'-O-D-valyl *ara* A.

SCHEME 2.

Enzymatic hydrolysis of vidarabine to its hypoxanthine analog.

TABLE 1

Antiviral activity of amino acid prodrugs of vidarabine

Promoiety (Compound #)	EC ₅₀ ^a (μM)		CC ₅₀ ^b (μM)
	Cowpox	Vaccinia	HFF
Vidarabine ^c	12.6	6.2	>100
Vidarabine + dCF ^c	1.4	1.0	90
5'-O-D-valyl ara A	35	8.3	>100
Cidofovir	26	30	>100

^a Fifty percent inhibitory concentration by plaque assay against the two viruses grown in human foreskin fibroblasts (HFFs).

^b Cytotoxicity determined in HFFs not affected by virus replication expressed as 50% inhibitory concentrations.

^c Data for vidarabine and vidarabine plus 1 μM deoxycoformycin (dCF) are averages from three to five experiments. All other data are averages of at least two experiments.

TABLE 2Transport of vidarabine and 5'-O-D valyl *ara* A in Caco-2 cell monolayer

Compound	Permeability ($\times 10^{-7}$, cm/s)
Vidarabine	1.70
5'-O-D-valyl <i>ara</i> A	6.00

TABLE 3AUC_{0-4hr} values for vidarabine and 5'-O-D-valyl ara A (n = 3)

Compounds	AUC _{0-4hr} values (ng/mL·hr)		
	Vidarabine	Ara-H	Prodrug
Vidarabine	8.3	181.5	—
D-Val-ara A	109 ± 13	98 ± 16	637 ± 74