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Acyclovir diphosphate dimyristoylglycerol: A phospholipid prodrug with activity against acyclovir-resistant herpes simplex virus

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ABSTRACT **Infection with herpes simplex viruses (HSVs) resistant to treatment with acyclovir {9-[(2-hydroxyethoxy)-methyl]guanine, Zovirax} is a growing clinical problem in patients with AIDS and other immunosuppressed states. Most virus isolates resistant to acyclovir are deficient or defective in virally coded thymidine kinase (TK), which converts acyclovir to acyclovir monophosphate in virus-infected cells. To restore acyclovir efficacy, we synthesized acyclovir diphosphate dimyristoylglycerol, an analog of a naturally occurring phospholipid, CDP-diacylglycerol. Its biological activity was tested in WI38 human lung fibroblasts infected with the acyclovir-resistant DM21 strain of HSV, which is TK negative due to an 816-base-pair deletion in the TK coding region. Acyclovir diphosphate dimyristoylglycerol has substantial activity in DM21-infected cells (IC_{50} = 0.25 μM), whereas acyclovir and acyclovir monophosphate were ineffective (IC_{50} > 100 μM). Similar results were obtained in TK-altered and TK-deficient strains of HSV-1 and in acyclovir-resistant isolates of HSV-2 obtained from two AIDS patients. The phospholipid prodrug is active by means of TK-independent metabolic pathways that liberate acyclovir monophosphate inside the host cell. Acyclovir phosphates were 56 times greater in WI38 human lung fibroblasts incubated for 24 hr with [8-^{3}H]acyclovir diphosphate dimyristoylglycerol relative to acyclovir. Acyclovir monophosphate added to the culture medium (outside the cell) did not circumvent the acyclovir resistance of the TK-negative DM21 mutant, presumably due to its conversion to acyclovir by phosphatases. Acyclovir diphosphate diacylglycerol prodrugs may be useful in treating TK-deficient mutant and wild-type strains of HSV.**

Acyclovir {9-[(2-hydroxyethoxy)methyl]guanine, Zovirax (ACV)} an acyclic analog of guanosine, is a selective antiviral agent with activity against herpes group viruses (1, 2). In cells infected with herpes simplex virus (HSV), the drug is converted to acyclovir monophosphate (ACV-MP) by viral thymidine kinase (TK) (3) followed by anabolic phosphorylation catalyzed by cellular enzymes to acyclovir triphosphate (4), which inhibits viral DNA polymerase (5).

ACV is effective orally for the treatment of first episodes of genital herpes and as chronic suppressive therapy for recurrent disease (6, 7). It is used for treating recurrent herpes simplex infections in immunocompromised patients (8), for prevention of cytomegalovirus infection after allogeneic bone marrow transplantation (9), and for infections caused by varicella zoster virus (10).

In patients with normal immune status, ACV-resistant HSV has not been associated with progressive disease, but in immunocompromised patients, ACV-resistant HSV may produce clinically significant disease (11–13). The vast majority of these isolates have mutations in the virally coded TK (14), whereas mutations in the HSV DNA polymerase are much rarer (12). TK mutations may result in TK-deficient, TK low producer, or TK-altered phenotypes (11).

HSV is a common pathogen in AIDS patients and may produce persistent ulcerative disease, which usually responds to ACV treatment. However, chronic ACV therapy in AIDS patients may lead to recurrent infection with ACV-resistant HSV mutants; this is generally perceived as a growing clinical problem (15). In the past several years there have been reports of HSV pneumonia (16), progressive whitlow (17), meningoencephalitis (18), and mucocutaneous dissemination (19) in AIDS patients infected with ACV-resistant viruses (HSV-1 and HSV-2).

To restore ACV effectiveness against TK-deficient (defective) mutants of HSV, we designed and synthesized a lipid prodrug of ACV that is an analog of CDP-diacylglycerol, a naturally occurring phospholipid.

EXPERIMENTAL PROCEDURES

Synthesis of Acyclovir Diphosphate Dimyristoylglycerol (ACVDP-DMG). ACVDP-DMG (acyclovir diphosphate-*sn*-1,2-dimyristoylglycerol) was prepared by coupling *sn*-1,2-dimyristoylglycerol-3-phosphomorpholidate to the ACV-MP (tributylammonium salt) in dry pyridine, and the product was purified by silica gel and anion-exchange chromatography as described for 3′-azido-3′-deoxythymidine (AZT) diphosphate dimyristoylglycerol (AZTDP-DMG) (20). Methodological details of the synthesis and properties of ACVDP-DMG have been reported elsewhere (21). The structure of ACVDP-DMG is shown in Fig. 1.

Viruses. Wild-type HSV-1 (strain F) and HSV-2 (Wolfe strain) were propagated as described (22). The DM21 strain of HSV-1, the TK low producer RSC-11, and the TK-altered S1-C mutants of HSV-1 were the generous gifts of Graham

Abbreviations: ACV, acyclovir; ACVDP-DMG, acyclovir diphosphate dimyristoylglycerol; ACV-MP, acyclovir monophosphate; ACV-DP, acyclovir diphosphate; AZT, 3′-azido-3′-deoxythymidine; AZTDP-DMG, 3′-azido-3′-deoxythymidine diphosphate dimyristoylglycerol; ddC, dideoxycytidine; pfu, plaque-forming unit; DMSO, dimethyl sulfoxide; HPLC, high-pressure liquid chromatography; HPTLC, high-performance thin-layer chromatography; HSV, herpes simplex virus; IC_{50}, inhibitory concentration of drug required to reduce viral replication by 50%; TC_{50}, toxic concentration of drug required to reduce viable cell number by 50%; TK, thymidine kinase; 3dT, 3′-deoxythymidine; 3dTDP-DMG, 3′-deoxythymidine diphosphate dimyristoylglycerol; HIV, human immunodeficiency virus; TCA, trichloroacetic acid.

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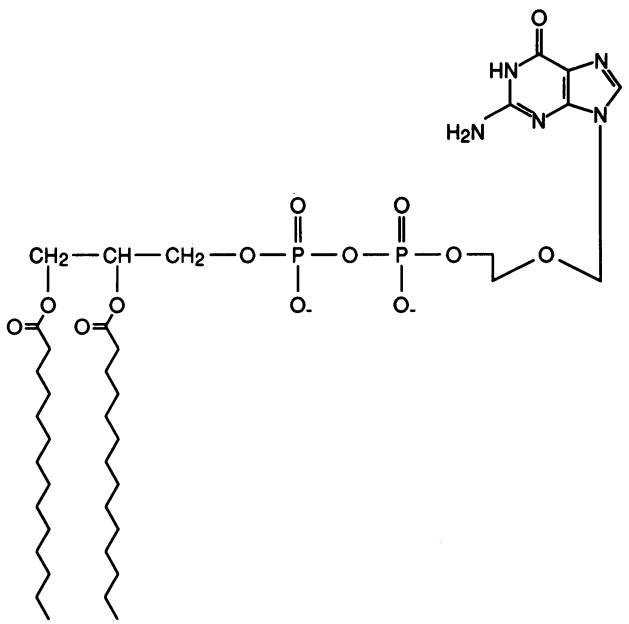

FIG. 1. Structure of ACVDP-DMG. The structure has been drawn to emphasize the amphipathic nature of the molecule. It should be noted that the acyl chains are shown without hydrogens and the structure does not reflect the stereochemistry of the glycerol moiety. The compound was named by analogy to CDP-diacylglycerol, but an alternative name by IUB–IUPAC nomenclature would be *sn*-1,2-dimyristoylglycero-3-diphospho-acyclovir.

Darby and Peter Collins (Wellcome Foundation, Beckenham, Kent, U.K.).

Isolation of ACV-Resistant HSV from AIDS Patients. ACV-resistant isolates of HSV-2 were obtained from perineal swabs of two different adult men with AIDS. Both patients had <20 CD4 lymphocytes per mm^3 of blood and chronic perineal ulceration despite therapy with oral ACV at 1 g daily. Patient 1 (Q512) failed to respond to therapy with intravenous ACV, 5 mg/kg every 8 hr for 14 days, but did have a clinical response to intravenous foscarnet, as previously reported for ACV-resistant HSV in patients with AIDS (23). Patient 2 (P729) declined parenteral therapy. Virus isolates were obtained after isolation of these cultures in diploid human fibroblasts (line 350Q) and incubation for 48 hr. The frozen-thawed culture fluid clarified by centrifugation at 1000 × *g* was used to prepare virus stocks.

Antiviral Assays. ACVDP-DMG was incorporated into sonicated vesicles containing dioleoylphosphatidylcholine/dioleoylphosphatidylglycerol/cholesterol/ACVDP-DMG (50:10:30:10 molar ratio). Liposomes are a convenient means for preparing aqueous dispersions of liponucleotides for *in vitro* antiviral assays (20). Blank liposomes at matched lipid concentrations have no effect on human immunodeficiency virus (HIV) replication (20) or HSV plaque formation up to the equivalent of 100 μM liponucleotide, representing a total lipid concentration of 1 mM. The ACVDP-DMG-containing liposomes were applied at the indicated concentrations to WI38 human lung fibroblasts infected with either HSV-1, HSV-2 (wild-type), or the ACV-resistant HSV-1 mutant DM21, which lacks TK activity due to an 816-base-pair deletion in the TK coding region of HSV-1 (SC16) (24), or other ACV-resistant isolates including RSC-11 (TK low producer), S1-C (altered-specificity mutant TK), and two clinical isolates of HSV-2, P729 and Q512.

The antiviral activity of ACV, ACV-MP, and ACVDP-DMG against wild-type HSV-1 and HSV-2 was evaluated *in vitro* in a standard 96-well plaque reduction assay. ACV-MP was the generous gift of Thomas Krenitsky (Burroughs Wellcome). WI38 human lung fibroblast cells were obtained from the American Type Culture Collection and seeded in a 96-well flat-bottom tissue culture plate (Nunclon, Naperville, IL) at 6×10^4 cells per well and grown to confluency at 37°C, in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. The confluent monolayers were infected with 50 plaque-forming units (pfu) of HSV-1 per well. After a 1-hr adsorption period at 37°C, the drugs were added to the wells at the indicated concentrations in overlay medium consisting of DMEM supplemented with 2% fetal bovine serum and 0.5% methylcellulose. The cells were incubated for an additional 18–24 hr followed by fixation with a solution of 2% crystal violet in 20% methanol, and the plaque number per well was determined. The data are represented as a function of drug concentration as the percentage of plaques observed relative to infected control without added drug.

The cellular toxicity of ACV, ACV-MP, and ACVDP-DMG was determined by incubation with subconfluent uninfected WI38 cells grown for 72 hr at 10, 31.6, 100, 316, and 1000 μM drug. The cells were removed from the culture well after treatment with trypsin and cell number was determined by manual counting in a hemocytometer. Cell viability was determined by trypan blue exclusion or by fluorescence-activated cell sorting using the fluorescent dye propidium iodide, as reported by Dangl *et al.* (25).

Metabolism of [8-^{3}H]ACVDP-DMG in Human Skin Fibroblasts. [8-^{3}H]ACVDP-DMG was prepared by catalytic labeling (Moravek Biochemicals, Brea, CA) of sonicated ACVDP-DMG and purified by lipid extraction and preparative thin-layer chromatography. Details will be reported elsewhere. [8-^{3}H]ACV-MP was purchased from Moravek Biochemicals. Human lung fibroblasts (WI38) were cultured to near confluence in modified Eagle's medium (MEM) containing 10% fetal bovine serum (Gemini Biological Products, Calabasas, CA). The medium was replaced with either [8-^{3}H]ACVDP-DMG or [8-^{3}H]ACV in MEM containing 6.7 μM nucleoside (30 μCi; 1 Ci = 37 GBq), 15% fetal bovine serum, and 0.2% dimethyl sulfoxide (DMSO). After 24 hr at 37°C, the medium was removed and the cells were washed quickly with ice-cold phosphate-buffered saline (PBS). The cells were frozen and thawed twice in 3 ml of distilled water, scraped, and collected, at which point trichloroacetic acid (TCA) was added to a final concentration of 8 g %. The mixture was spun in a Vortex for 30 sec followed by centrifugation for 10 min at 4°C at 1000 × *g*, and the TCA supernatant was withdrawn and extracted with 5 ml of triocytylamine/Freon-II, 3:1 (vol/vol), as described (26) and reserved for high-pressure liquid chromatography (HPLC). The TCA pellet was extracted by the method of Bligh and Dyer (27) and the lipid extract was subjected to high-performance thin-layer chromatography (HPTLC) and radioscanning as noted above. The TCA-free water-soluble metabolites were analyzed by Partisil SAX HPLC. After application of the sample, the following elution scheme was used: 0–8 min, 25 mM KH_2PO_4 (pH 3.5) (isocratic); 8–40 min, 25–250 mM KH_2PO_4 (pH 3.5)/0–500 mM KCl (linear gradient); 40–55 min, 250 mM KH_2PO_4 (pH 3.5)/500 mM KCl (isocratic). One-milliliter fractions were collected and analyzed by liquid scintillation spectroscopy.

RESULTS

ACVDP-DMG was compared with ACV and ACV-MP in a plaque reduction assay in human lung fibroblasts (WI38) infected with wild-type HSV-1 (Fig. 2). All three compounds exhibited comparable antiviral activities (Table 1). Ninety percent inhibition was observed at 0.7 μM for ACV and 1.0–1.3 μM for ACVDP-DMG and ACV-MP.

In WI38 cells infected with ACV-resistant DM21-HSV, ACV had very little antiviral effect, $IC_{50} > 100$ μM (Fig. 3).

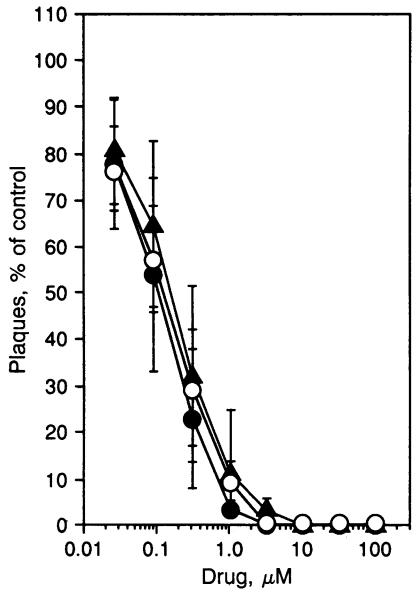

FIG. 2. Effect of ACV, ACV-MP, and ACVDP-DMG on HSV-1 replication in WI38 cells. Data represent the average ± SD of four individual experiments. ●, ACV; ○, ACV-MP; ▲, ACVDP-DMG.

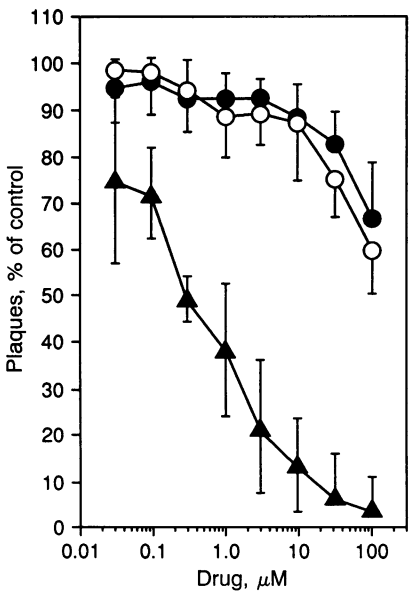

FIG. 3. Effect of ACV analogs on the replication of ACV-resistant, TK-deficient DM21 strain of HSV. WI38 human lung fibroblasts were infected with the DM21 strain of HSV-1 and incubated in the presence of drugs as indicated. The presentation of data and symbols is as indicated in Fig. 2.

ACV-MP also had little effect, presumably due to the lack of penetration into the cell and to cleavage of the phosphate moiety by phosphatases present in fetal bovine serum. However, the lipid prodrug, ACVDP-DMG, exhibited substantial antiviral activity in DM21-infected cells, with an IC_{50} of 0.25 μM. Liposomes at matched lipid concentrations but without ACVDP-DMG had no effect on HSV-1 or DM21 plaque formation (data not shown). None of the agents decreased the viable cell number below 50% of control when incubated with subconfluent WI38 cells for 24 or 72 hr at concentrations up to 1000 μM. The selectivity indexes (TC_{50}/IC_{50}, where TC_{50} indicates toxic concentration of drug required to reduce viable cell number by 50%) were as follows: ACV, >16,600; ACV-MP, >7140; and ACVDP-DMG, >14,300 (HSV-1) or >4000 (DM21).

Similar results were obtained with two other TK mutants of HSV-1: RSC-11, a TK low producer, and S1-C, a TK-altered HSV-1 (Table 1). In HSV-2 clinical isolates obtained from two AIDS patients on chronic ACV suppression, ACVDP-DMG was effective (IC_{50} = 0.22–0.27 μM), whereas ACV and ACV-MP were ineffective *in vitro* (IC_{50} values > 100 μM) (Table 1). The antiviral results with ACVDP-DMG suggested that cells take up and metabolize the prodrug to ACV-MP, bypassing phosphorylation by HSV TK, delivering ACV-MP to the interior of the cell.

To evaluate the cellular metabolism of ACVDP-DMG, we incubated [8-^{3}H]ACVDP-DMG and [8-^{3}H]ACV with WI38 human lung fibroblasts for 24 hr at 37°C and analyzed the TCA-soluble metabolites by Partisil SAX HPLC (Fig. 4). After incubation of [8-^{3}H]ACVDP-DMG with human fibroblasts, the cellular TCA-soluble radioactive material was analyzed by HPLC (Fig. 4 *Lower*) and substantial amounts of ACV-MP and acyclovir diphosphate (ACV-DP) were measured. However, when the fibroblasts were incubated with [8-^{3}H]ACV, only a small ACV-MP peak was detected and ACV-DP could not be identified (Fig. 4 *Upper*). HPTLC of the lipid-soluble material extracted from the [8-^{3}H]ACVDP-DMG pellet revealed only intact ACVDP-DMG (data not shown).

We calculated the total pmol of ACV and each metabolite (Table 2). With [8-^{3}H]ACV, only 15.4 pmol of ACV-MP was detected versus 726 pmol with [8-^{3}H]ACVDP-DMG; no ACV-DP was detected in the ACV incubation versus 136 pmol with ACVDP-DMG. Thus, total phosphorylated metabolites (ACV-MP plus ACV-DP) were increased 56-fold with ACVDP-DMG versus ACV. Under these experimental conditions we were unable to detect ACV triphosphate (data not shown).

Table 1. Effects of ACV, ACV-MP, and ACVDP-DMG on plaque formation by HSV-1, HSV-2, and various ACV-resistant mutants in WI38 human lung fibroblasts

HSV type	IC_{50}, μM		
	ACVDP-DMG	ACV	ACV-MP
Wild-type virus			
HSV-1	0.07 ± 0.02 (5)	0.06 ± 0.03 (5)	0.14 ± 0.09 (5)
HSV-2	0.42 ± 0.43 (3)	0.09 ± 0.06 (3)	0.23 ± 0.19 (3)
HSV-1 TK mutant			
DM21	0.25 ± 0.09 (6)	>100 (5)	>100 (5)
RSC-11	0.98 ± 0.94 (3)	>100 (3)	>100 (3)
S1-C	0.87 ± 0.99 (3)	>100 (3)	>100 (3)
HSV-2 ACV-resistant clinical isolate			
P729	0.27 ± 0.21 (3)	>100 (3)	>100 (3)
Q512	0.22 ± 0.16 (3)	>100 (3)	>100 (3)

IC_{50}, inhibitory concentration of drug required to reduce viral replication by 50%. Data are expressed as mean ± SD. The number of replicate experiments is indicated in parentheses.

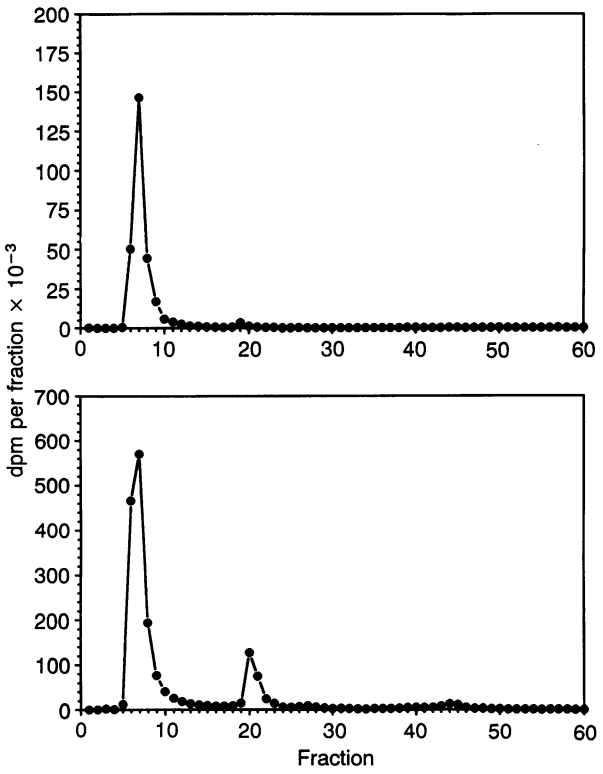

FIG. 4. Metabolism of [8-^{3}H]ACV and [8-^{3}H]ACVDP-DMG by WI38 human lung fibroblasts, *in vitro*. WI38 human lung fibroblasts (150 cm^2) were incubated for 24 hr with either 6.7 μM [8-^{3}H]ACV (24 Ci/mmol) (*Lower*) or 6.7 μM [8-^{3}H]ACVDP-DMG (24 Ci/mmol) (*Upper*), and aliquots (1 ml) of the 2.9-ml TCA-soluble supernatants were subjected to Partisil SAX HPLC. Data represent total dpm per fraction.

ACV-MP added externally was unable to restore antiviral activity in DM21 mutant-infected cells (Fig. 3, Table 1), suggesting that ACV-MP does not enter the cell. In addition, preliminary experiments show that ACV-MP is rapidly converted to ACV by phosphatases present in fetal bovine serum in the tissue culture medium, accounting for the activity of ACV-MP in wild-type HSV-1 (Fig. 2).

By what metabolic pathways did ACVDP-DMG gain its unusual biological activity against mutant DM21, which lacks TK? The prodrug can utilize several alternative metabolic pathways to generate ACV-MP, whereas ACV can only be phosphorylated by TK. ACVDP-diacylglycerol may substitute for CDP-diacylglycerol in biosynthetic reactions that form the acidic phospholipids, such as phosphatidylglycerol and phosphatidylinositol, while giving rise to the nucleoside monophosphate (28). We have shown previously that the diphosphate diacylglycerols of AZT, dideoxycytidine (ddC) and 3'-deoxythymidine (3dT), catalyze the formation of acidic phospholipids *in vitro* with microsomes and mitochondria as the enzyme source, although the rates were much slower than with CDP-diacylglycerol, the natural substrate (28). We repeated these studies with ACVDP-diacylglycerol and found that this compound was relatively active as a substrate for the biosynthesis of phosphatidylglycerol but did not serve as a substrate for phosphatidylinositol formation. ACVDP-DMG was more active as a substrate for phosphatidylglycerol synthesis than ddCDP-diacylglycerol, 3'-deoxythymidine diphosphate dimyristoylglycerol (3dTDP-DMG), and AZTDP-diacylglycerol, with an apparent V_{max} of 2.6 pmol/min per mg versus 0.7, 0.1, and <0.05, respectively, with the other liponucleotides (28). ACV-MP is the other product liberated in phosphatidylglycerol formation (28).

Table 2. Quantification of the TCA-soluble metabolites of [8-^{3}H]ACV and [8-^{3}H]ACVDP-DMG in cultured WI38 human lung fibroblasts, *in vitro*

	ACV		ACVDP-DMG		
Compound	Area %	Total pmol	Area %	Total pmol	Fold increase*
ACV	98.0	751.0	84.2	4600	6.1
ACV-MP	2.0	15.4	13.3	726	47.1
ACV-DP	0	0	2.5	136	∞

The total radioactivity represented by each peak in Fig. 4 was determined and the results are expressed as the area %. The area % values are multiplied by the total TCA-soluble dpm of ACV or ACVDP-DMG and divided by the specific activity of [8-^{3}H]ACV or [8-^{3}H]ACVDP-DMG to give total pmol of each metabolite per confluent T75 flask of WI38 cells.

*Refers to the increase in each metabolite observed with [8-^{3}H]-ACVDP-DMG relative to that observed with [8-^{3}H]ACV. Total phosphorylated [8-^{3}H]ACV metabolites (ACV-MP plus ACV-DP) with [8-^{3}H]ACVDP-DMG were increased 56-fold relative to [8-^{3}H]ACV.

In previous studies we demonstrated that a mitochondrial pyrophosphatase cleaved nucleoside diphosphate diglycerides to nucleoside monophosphate and phosphatidic acid; AZTDP, 3dTDP, and ddCDP diacylglycerols were converted to the respective nucleoside monophosphates and phosphatidic acids (29). We incubated ACVDP-DMG with mitochondrial protein at pH 5 or 7.5 at 37°C and analyzed the reaction products by HPLC at 0, 0.5, 1, 2, 4, and 8 hr as reported previously (29). ACVDP-DMG was progressively converted to ACV-MP (retention time, 18 min) and ACV (retention time, 4.0 min) by liver mitochondria, *in vitro* (data not shown). This provides evidence for pyrophosphatase cleavage as another mechanism for conversion of ACVDP-DMG directly to ACV-MP by a cellular enzyme.

DISCUSSION

Previous attempts to restore ACV activity against ACV-resistant HSV mutants using lipid prodrugs of ACV have not been successful. Phosphatidyl-ACV (*sn*-1,2-dipalmitoylglycero-3-phosphoacyclovir) was synthesized by Welch *et al.* (30) and shown to have only 10% of the antiviral activity of free ACV against wild-type HSV-1 and HSV-2. Furthermore, phosphatidyl-ACV was inactive against ACV-resistant TK-negative mutants (30), in contrast to our present findings with ACVDP-DMG. A pyrophosphate linkage is necessary in the phospholipid prodrug to generate antiviral activity in cells infected with mutant herpes viruses deficient for TK.

One would predict that ACVDP-DMG might be more toxic than ACV because of intracellular conversion to ACV-MP and to the di- and triphosphates. However, ACVDP-DMG did not cause substantial decreases in cell number or viability in WI38 cells incubated with 1 mM ACVDP-DMG. Therefore, the *in vitro* selectivity index appeared adequate in this cell line (>4000). The toxicity of the lipid prodrug *in vivo* has not yet been examined.

The fact that ACVDP-DMG is effective in cells infected with TK-deficient and TK-altered mutant HSV-1 is especially interesting in light of our recent finding that 3dTDP-DMG is 18–50 times more active in HIV-infected cells than its parent nucleoside, 3dT (31). This also appears to be due to direct cellular conversion of 3dTDP-DMG to 3dT monophosphate because 3dTDP-DMG retained activity even in HIV-infected mutant cells that lacked TK. 3dT appears to be poorly phosphorylated, which accounts for its low antiviral activity in HIV-infected normal cells. Similar results were obtained in HIV-infected TK-negative CEM cells where AZTDP-DMG exhibited antiviral activity; however, the free nucleosides

3dT and AZT had no antiviral activity in the TK-negative CEM cells because they cannot be phosphorylated (31).

In summary, ACVDP-DMG is effective *in vitro* against three different ACV-resistant TK mutants of HSV-1 and two ACV-resistant clinical isolates of HSV-2 obtained from AIDS patients. Its activity appeared to be attributable to the unique cellular metabolism of the lipid prodrug, which gives rise to ACV-MP in the target cell. Prodrugs like ACVDP-DMG may be useful in treating ACV-resistant HSV mutants that are deficient or defective in TK activity.

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1. Elion, G. B., Furman, P. A., Fyfe, J. A., DeMiranda, P., Beauchamp, L. & Schaeffer, H. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5617–5720.
2. Schaeffer, H. J., Beauchamp, L., DeMiranda, P., Elion, G. G., Bauer, D. J. & Collins, P. (1978) *Nature (London)* **272,** 583–585.
3. Fyfe, J. A., Keller, P. M., Furman, P. A., Miller, R. L. & Elion, G. B. (1978) *J. Biol. Chem.* **253,** 8721–8727.
4. Miller, W. H. & Miller, R. L. (1980) *J. Biol. Chem.* **255,** 7204–7207.
5. St. Clair, M. H., Furman, P. A., Lubbers, C. M. & Elion, G. B. (1980) *Antimicrob. Agents Chemother.* **18,** 741–745.
6. Bryson, Y. J., Dillon, M., Lovett, M., Aunna, G., Taylor, S., Cherry, J. D., Johnson, B. L., Wiesmeier, E., Growdon, W., Creagh-Kirk, T. & Keeny, R. (1983) *N. Engl. J. Med.* **308,** 916–921.
7. Reichman, R. C., Badger, G. J., Mertz, G. J., Corey, L., Richman, D. D., Connor, J. D., Redfield, D. C., Savoia, M. C., Oxman, M. N., Bryson, Y., Tyrrell, D. L., Portnoy, J., Creigh-Kirk, T., Keeney, R. E., Ashikaga, T. & Dolin, R. (1984) *J. Am. Med. Assoc.* **251,** 2103–2107.
8. Straus, S. E., Seidlin, M., Takiff, H., Jacobs, D., Bowen, D. & Smith, H. A. (1984) *Ann. Intern. Med.* **100,** 522–524.
9. Meyers, J. D., Reed, E. C., Shepp, D. H., Thornquist, M., Dandliker, P. S., Vicary, C.-A., Fluornoy, N., Kirk, L. E., Kersey, J. H., Thomas, E. D. & Balfour, H. H. (1988) *N. Engl. J. Med.* **318,** 70–75.
10. Huff, J. C., Bean, B., Balfour, H. H., Laskin, O. L., Connor, J. D., Corey, L., Bryson, Y. J. & McGuirt, P. (1988) *Am. J. Med.* **85,** 84–89.
11. Hill, E. L., Hunter, G. A. & Ellis, M. N. (1991) *Antimicrob. Agents Chemother.* **35,** 2322–2328.
12. Collins, P., Larder, B. A., Oliver, N. M., Kemp, S., Smith, I. W. & Darby, G. (1989) *J. Gen. Virol.* **70,** 375–382.
13. Englund, J. A., Zimmerman, M. T., Swierkosz, E. M., Goodman, J. L., Scholl, D. R. & Balfour, H. H. (1990) *Ann. Intern. Med.* **112,** 416–422.
14. Barry, D. W., Nusinoff-Lehrman, S., Ellis, M. N., Biron, K. & Furman, P. A. (1983) *Scand. J. Infect. Dis. Suppl.* **47,** 155–164.
15. Erlich, K. S., Mills, J., Chatis, P., Mertz, G. J., Busch, D. F., Follansbee, S. E., Grant, R. M. & Crumpacker, C. S. (1989) *N. Engl. J. Med.* **320,** 293–296.
16. Ljungman, P., Ellis, M. N., Hackman, R. C., Shepp, D. H. & Meyers, J. D. (1990) *J. Infect. Dis.* **162,** 244–248.
17. Norris, S. A., Kessler, H. A. & Fife, K. H. (1988) *J. Infect. Dis.* **157,** 209–210.
18. Gateley, A., Gander, R. M., Johnson, P. C., Kit, S., Otsuka, H. & Kohl, S. (1990) *J. Infect. Dis.* **161,** 711–715.
19. Marks, G. L., Nolan, P. E., Erlich, K. S. & Ellis, M. N. (1989) *Rev. Infect. Dis.* **11,** 474–476.
20. Hostetler, K. Y., Stuhmiller, L. M., Lenting, H. B. M., van den Bosch, H. & Richman, D. D. (1990) *J. Biol. Chem.* **265,** 6112–6117.
21. Van Wijk, G. M. T., Hostetler, K. Y. & van den Bosch, H. (1992) *J. Lipid Res.* **33,** 1211–1219.
22. Redfield, D. C., Richman, D. D., Albanil, S., Oxman, M. N. & Wahl, G. M. (1983) *Diagn. Microbiol. Infect. Dis.* **1,** 117–128.
23. Safrin, S., Crumpacker, C., Chatis, P., Davis, R., Hafner, R., Rush, J., Kessler, H. A., Landry, B., Mills, J. and other members of the AIDS Clinical Trials Group. (1991) *N. Engl. J. Med.* **325,** 551–555.
24. Efstathiou, S., Kemp, S., Darby, G. & Minson, A. C. (1989) *J. Gen. Virol.* **70,** 869–879.
25. Dangl, J. L., Parks, D. R., Oi, V. T. & Herzenberg, L. A. (1982) *Cytometry* **2,** 395–401.
26. Hostetler, K. Y., Carson, D. A. & Richman, D. D. (1991) *J. Biol. Chem.* **266,** 11714–11717.
27. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem.* **31,** 911–914.
28. Van Wijk, G. M. T., Hostetler, K. Y., Schlame, M. & van den Bosch, H. (1991) *Biochim. Biophys. Acta* **1086,** 99–105.
29. Van Wijk, G. M. T., Hostetler, K. Y. & van den Bosch, H. (1991) *Biochim. Biophys. Acta* **1084,** 307–310.
30. Welch, C. J., Larsson, A., Ericson, A. C., Oberg, B., Datema, R. & Chattopadhyaya, J. (1985) *Acta Chem. Scand. B* **39,** 47–54.
31. Hostetler, K. Y., Richman, D. D., Carson, D. A., Stuhmiller, L. M., van Wijk, G. M. T. & van den Bosch, H. (1992) *Antimicrob. Agents Chemother.* **36,** 2025–2029.