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Alkoxy propane prodrugs of foscarnet: effect of alkyl chain length on in vitro antiviral activity in cells infected with HIV-1, HSV-1 and HCMV

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Abstract

The identification of more effective and less toxic foscarnet (PFA) analogs for antiviral therapy would be useful. We recently synthesized 1-*O*-octadecyl-*sn*-glycero-3-phosphonoformic acid (ODG-PFA) and noted a 93-fold increase in its anti-HCMV activity relative to PFA. In addition, the antiviral activity of ODG-PFA in herpes simplex virus type-1 (HSV-1) and human immunodeficiency virus type-1 (HIV-1) infected cells was increased 40-fold relative to PFA (Hostetler et al., 1996. Antiviral Res. 31, 59). To evaluate structure–activity relationships further, we synthesized alkoxypropyl esters of foscarnet with varying alkyl chain lengths and degrees of saturation. These compounds were tested in vitro for activity and selectivity in comparison with PFA and ODG-PFA in cells infected with HCMV, HSV-1 or HIV-1. Antiviral activity was strongly dependent on chain length with alkyl ethers 14–18 carbon atoms long exhibiting the greatest antiviral activity against HCMV and HSV-1. In HIV-infected HT4-6C cells, optimal activity was observed at 18–22 carbon chain lengths. The antiviral activities of 1-octadecyloxypropane-3-PFA and 1-docosyloxypropane-3-PFA were 135- and 338-fold greater than that of PFA in HT4-6C cells infected with HIV-1. This also represents a 2.6–6-fold improvement in antiviral activity over ODG-PFA, the previously reported analog.

Keywords: Alkoxy propane; Prodrugs; Foscarnet; HIV-1; HSV-1; HCMV

1. Introduction

Foscarnet (phosphonoformic acid, PFA) is a pyrophosphate analog that inhibits various viral

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DNA and RNA polymerases (Öberg, 1983). PFA inhibits the replication of herpes group viruses (Öberg, 1983) and HIV (Sandstrom et al., 1985) and is currently approved for clinical use for the treatment of HCMV retinitis in AIDS and for acyclovir-resistant HSV. However, foscarnet is not highly active in vivo and must be administered by intravenous infusion, because of its low oral bioavailability. Foscarnet toxicity is significant with kidney damage and hypocalcemia as prominent side effects (Chrisp and Clissold, 1991). In AIDS patients with CMV retinitis, treatment with PFA has been suggested to prolong life versus historical controls either untreated or treated with ganciclovir (Polis et al., 1993). PFA treatment of AIDS patients with CMV retinitis has been shown to induce a series of mutations in HIV reverse transcriptase (RT) which confer 2–14-fold PFA resistance; importantly, the PFA resistant virus was hypersensitive to zidovudine and non-nucleoside RT-inhibitors (Mellors et al., 1995). The PFA resistance mutations detected at codons 88, 89, 91 or 161 of the HIV reverse transcriptase were found to reverse phenotypic zidovudine (AZT) resistance. Moreover, the zidovudine resistance mutations increased the activity of PFA in vitro (Tachedjian et al., 1996). These authors suggested that zidovudine and PFA resistance might be mutually exclusive. Clearly, it would be useful to have more potent and less toxic forms of PFA for human use.

As part of our ongoing program to identify lipid prodrugs of foscarnet with increased antiviral activity and enhanced oral bioavailability, we synthesized 1-*O*-octadecyl-*sn*-glycero-3-phosphonoformic acid (ODG-PFA). Compared with PFA, this compound was 93-fold more active against HCMV; the antiviral activity of ODG-PFA in HSV-1 and HIV-1 infected cells was increased more than 40-fold relative to foscarnet (Hostetler et al., 1996). We now report the synthesis and biological evaluation of a series of alkoxypropane conjugates of PFA, which are structurally related to ODG-PFA, except that they lack the *sn*-2-hydroxyl of glycerol. To evaluate the optimal structure for the lipid moiety, we synthesized a series of PFA conjugates varying the alkyl chain length and saturation of the 1-*O*-alkoxy group. PFA was esterified via its phosphonate to the 3-alkoxy-1-propanols. These compounds were tested for antiviral activity and selectivity in MRC-5 human lung fibroblasts infected with HCMV or HSV-1 or in HT4-6C cells infected with HIV-1.

2. Methods

2.1. Chemistry

NMR spectra were obtained on a 300 MHz General Electric QE-300 spectrometer, with TMS as internal standard (s: singlet, d: doublet, t: triplet, q: quartlet, m: multiplet, bs: broad singlet). Flash chromatography was performed with silica gel EM Keiselgel 60 (230–400 mesh). Thin layer chromatography was carried out with Analtech Silica gel-GF (250 micron) plates. The products were visualized with UV light, phosphorus spray and charring. Combustion analyses were performed by Oneida Research Services, Whitesboro, NY.

2.2. General procedure for the synthesis of 3-alkoxy-1-propanols

*2.2.1. Example: 3-octadecyloxy-1-propanol (**1b**)*

To a solution of 1,3-propanediol (20 ml, 300 mmol) in *N*,*N*-dimethylformamide (500 ml), maintained under an atmosphere of nitrogen, was added hexane-washed sodium hydride (6.0 g, 60% suspension in paraffin oil), and the resulting mixture stirred for 15 min. Octadecyl methanesulfonate (20 g, 57 mmol) was added in one portion, and the reaction mixture was stirred at room temperature overnight.

The mixture was poured into ice-water (1000 ml), with stirring, upon which a white solid separated. The solid was filtered off, dried and recrystallized from hexane to yield 14.5 g (77%) of 3-(octadecyloxy)-1-propanol (**1b**) as an amorphous solid: ^{1}H NMR ($CDCl_3$): δ 0.9 (t, 3H), 1.23 (bs,30 H), 1.57 (m, 2H), 1.83 (m, 2H), 3.43 (t, 2H), 3.62 (t, 2H), 3.77 (q, 2H).

*2.2.2. 3-(docosyloxy)-1-propanol (**1a**)*

3-(docosyloxy)-1-propanol (**1a**) yield: (88.0%); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H), 1.25 (bs, 38 H), 1.58 (m, 2H), 1.83 (m, 2H), 3.46 (m, 2H), 3.63 (m, 2H), 3.78 (m, 2H).

*2.2.3. 3-(hexadecyloxy)-1-propanol (**1c**)*

3-(hexadecyloxy)-1-propanol (**1c**) yield (73.0%); ^{1}H NMR ($CDCl_3$): δ 0.84 (t, 3H), 1.24 (bs, 26 H), 1.59 (m, 2H), 1.86 (m, 2H), 3.40 (t, 2H), 3.61 (t, 2H), 3.82 (t, 2H).

*2.2.4. 3-(tetradecyloxy)-1-propanol (**1d**)*

3-(tetradecyloxy)-1-propanol (**1d**) yield (70.0%); ^{1}H NMR ($CDCl_3$): δ 0.88 (t,3H), 1.30 (bs, 22 H), 1.57 (m, 2H), 1.83 (m, 2H), 3.43 (t, 2H), 3.62 (t, 2H), 3.78 (t, 2H).

*2.2.5. 3-(dodecyloxy)-1-propanol (**1e**)*

3-(dodecyloxy)-1-propanol (**1e**) yield (90.1%); ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H), 1.29 (bs, 18 H), 1.58 (m, 2H), 1.83 (m, 2H), 3.42 (t, 2H), 3.62 (t, 2H), 3.79 (t, 2H).

*2.2.6. 3-(octyloxy)-1-propanol (**1f**)*

3-(octyloxy)-1-propanol (**1f**) yield (quantitative); ^{1}H NMR ($CDCl_3$): δ 0.91 (t, 3H), 1.32 (bs, 10 H), 1.59 (m, 2H), 1.77 (m, 2H), 3.50 (m, 2H), 3.64 (t, 2H), 3.82 (t, 2H).

*2.2.7. 3-(oleyloxy)-1-propanol (**1g**)*

3-(oleyloxy)-1-propanol (**1g**) yield: (55.0%); ^{1}H NMR ($CDCl_3$); δ 0.92 (t, 3H), 1.29 (bs, 16H), 1.58 (m, 2H), 1.83 (m, 2H), 2.0 (m, 4H), 3.42 (t, 2H), 3.63 (t, 2H), 3.79 (t, 3H), 5.38 (t, 2H).

2.3. General procedure for the synthesis of 1-(alkoxy)-propane-3-phosphonoformate ethyl ester

*2.3.1. Example: 1-(octadecyloxy)-propane-3-phosphonoformate ethyl ester (**2b**)*

To a solution of 1-*O*-octadecyl-1,3-propanediol (1.31 g, 4 mmol) in pyridine (50 ml) was added ethyl phosphonoformate monopyridinium (1.19 g, 5 mmol). The resulting suspension was cooled to 0°C in an ice-bath and a solution of *N*,*N*-dicyclohexylcarbodiimide (2.29 g, 12 mmol) in dichloromethane (25 ml) was added dropwise with stirring. The resulting mixture was stirred at 0°C for 3 h and at room temperature overnight. The mixture was filtered, and the precipitate was washed with dichloromethane (2×20 ml). The filtrate and washings were combined, and concentrated to dryness in vacuo. The residue was adsorbed on to silica gel, and flash chromatographed over silica gel with an increasing gradient of methanol in dichloromethane (0–10%) as eluent. The appropriate fractions were pooled and evaporated under reduced pressure to yield target compound **2b** (1.15 g, 62%) as an amorphous solid: ^{1}H NMR ($CDCl_3$–CD_3OD) δ 0.80 (t, 3H), 1.20 (overlapping t and bs, 33H), 1.52 (m 2H), 1.85 (m, 2H), 3.35 (t, 2H), 3.48 (t,2H), 4.0 (q, 2H), 4.22 (q, 2H).

*2.3.2. 1-(docosyloxy)-propane-3-phosphonoformate ethyl ester (**2a**)*

1-(docosyloxy)-propane-3-phosphonoformate ethyl ester (**2a**): yield (63.0%); ^{1}H NMR ($CDCl_3$–CD_3OD) δ 0.92 (t, 3H), 1.50 (overlapping t and bs, 41 H), 1.56 (m, 2H), 1.91 (t, 2H), 3.38 (m, 2H), 3.41 (t, 2H), 3.54 (t, 2H), 4.25 (q, 2H).

*2.3.3. 1-(hexadecyloxy)-propane-3-phosphonoformate ethyl ester (**2c**)*

1-(hexadecyloxy)-propane-3-phosphonoformate ethyl ester (**2c**): yield (70.0%). This compound was used directly for the next step.

*2.3.4. 1-(tetradecyloxy)-propane-3-phosphonoformate methyl ester (**2d**)*

1-(tetradecyloxy)-propane-3-phosphonoformate methyl ester (**2d**): yield (48.0%). This compound was synthesized as the methyl ester using a procedure identical to the ethyl ester, with methyl phosphonoformate. ^{1}H NMR ($CDC1_3$:CD_3OD): δ 0.92 (t, 3H), 1.25 (bs, 22 H), 1.55 (m, 2H), 1.90 (bs, 2H), 3.40 (t, 2H), 3.76 (s, 3H), 4.05 (m, 2H).

*2.3.5. 1-(dodecyloxy)-propane-3-phosphonoformate ethyl ester (**2e**)*

1-(dodecyloxy)-propane-3-phosphonoformate ethyl ester (**2e**): yield (36.0%); ^{1}H NMR ($CDCl_3$): δ 0.83 (t, 3H), 1.25 (bs, 21 H), 2.04 (m, 2H), 1.83 (bs, 2H), 3.29 (t, 2H), 3.38 (bs, 2H), 4.08 (bs, 2H), 4.25 (bs, 2H).

*2.3.6. 1-(octyloxy)-propane-3-phosphonoformate ethyl ester (**2f**)*

1-(octyloxy)-propane-3-phosphonoformate ethyl ester (**2f**): yield 31%; ^{1}H NMR ($CDCl_3$–CD_3OD): δ 0.87 (t, 3H), 1.29 (bs, 13H), 1.50 (m, 2H), 1.84 (m, 2H), 3.33 (t, 2H), 3.46 (t, 2H), 4.04 (q, 2H), 4.21 (q, 2H).

*2.3.7. 1-(oleyloxy)-propane-3-phosphonoformate ethyl ester (**2g**)*

1-(oleyloxy)-propane-3-phosphonoformate ethyl ester (**2g**): yield: (28.2%); ^{1}H NMR ($CDCl_3$): δ 0.83 (t, 3H), 1.29 (bs, 19 H), 1.50 (m, 2H), 1.84 (bs, 2H), 2.0 (q, 4H), 3.29 (t, 2H), 3.46 (bs, 2H), 4.0 (bs, 2H), 4.25 (bs, 2H), 5.29 (t, 2H).

2.4. General procedure for the synthesis of 1-(alkoxy)-propane-3-phosphonoformate (disodium salt)

*2.4.1. Example: 1-(octadecyloxy)-propane-3-phosphonoformate (disodium salt) (**3b**)*

To a suspension of 1-(octadecyloxy)-propane-diol-3-phosphonoformate ethyl ester (0.8 g, 1.7 mmol) in absolute ethanol (40 ml) was added 1 M aqueous sodium hydroxide, and the mixture was stirred at room temperature for 1 h. The resulting suspension was filtered, and the precipitate was washed with ethanol (3 × 25 ml). The precipitate was dried. Yield 0.64 g (76.2%) of **3b** as an amorphous solid; ^{1}H NMR ($CDCl_3$:CD_3OD: D_2O::2:3:1:): 0.93 (m, 3H), 1.38 (bs, 30H), 1.57 (bs, 2H), 1.93 (m, 2H), 3.43 (t, 2H), 3.59 (t, 2H), 4.0 (t, 2H). Analysis calculated for $C_{22}H_{43}O_6PNa_2$. 0.9 H_2O: %C 53.23, %H 9.09. Found: %C 53.46, H 8.62.

*2.4.2. 1-(docosyloxyl-propane-3-phosphonoformate (disodium salt) (**3a**)*

1-(docosyloxyl-propane-3-phosphonoformate (disodium salt) (**3a**): yield (83.1%); ^{1}H NMR ($CDCl_3$:CD_3OD: D_2O::2:3:1:): δ 0.88 (bs, 3H), 1.38 (bs, 38 H), 1.56 (m, 2H), 1.95 (m, 2H), 3.45 (m, 2H), 3.59 (m, 2H), 3.97 (m, 2H). Analysis calculated for $C_{26}H_{51}O_6P$. 0.7 H_2O: %C 56.86, %9.62. Found: %C 56.97, %H 9.37.

*2.4.3. 1-(hexadecyloxy)-propane-3-phosphosphono-formate (disodium salt) (**3c**)*

1-(hexadecyloxy)-propane-3-phosphosphono-formate (disodium salt) (**3c**): yield (45.7%); ^{1}H NMR ($CDCl_3$:CD_3OD: D_2O::2:3:1): 0.95 (m, 3H), 1.25 (bs, 26H), 1.56 (bs, 2H), 1.95 (m, 2H), 3.45 (m, 2H), 3.60 (m, 2H), 3.95 (m, 2H). Analysis calculated for $C_{20}H_{39}O_6PNa_2$. 0.14 H_2O: %C 52.80, %H 8.70. Found: %C 52.56, %H 8.49.

*2.4.4. 1-(tetradecyloxy)-propane-3-phosphonoformate (disodium salt) (**3d**)*

1-(tetradecyloxy)-propane-3-phosphonoformate (disodium salt) (**3d**): yield (95.0%); ^{1}H NMR ($CDCl_3$:CD_3OD: D_20::2:3:1): 0.90 (m, 3H), 1.28 (bs, 26H), 1.57 (bs, 2H), 1.93 (m, 2H), 3.45 (m, 2H), 3.58 (m, 2H), 4.01 (m, 2H). Analysis calculated for $C_{18}H_{35}O_6PNa_2$. 0.25 H_2O: %C 50.40, %H 8.34. Found: %C 50.01; %H 7.85.

*2.4.5. 1-(dodecyloxy)-propane-3-phosphonoformate (disodium salt) (**3e**)*

1-(dodecyloxy)-propane-3-phosphonoformate (disodium salt) (**3e**): yield (57.0%); ^{1}H NMR ($CDCl_3$:CD_3OD: D_2O::2:3:1): 0.91 (m, 3H), 1.27 (bs, 18H), 1.57 (m, 2H), 1.93 (m, 2H), 3.43 (m, 2H), 3.55 (m, 2H), 3.98 (q, 2H). Analysis calculated for $C_{16}H_{31}O_6PNa_2$. 1.5 H_2O: %C 45.39, %H 8.09. Found: %C 45.02, %H 7.15.

*2.4.6. 1-(octyloxy)-propane-3-phosphonoformate (disodium salt) (**3f**)*

1-(octyloxy)-propane-3-phosphonoformate (disodium salt) (**3f**): yield (67.0%); ^{1}H NMR

($CDCl_3$:CD_3OD: D_2O::2:3:1): δ 0.94 (m, 2H), 1.25 (bs, 10H), 1.56 (m, 2H), 1.94 (M, 2H), 3.44 (M, 2H), 3.50 (m, 2H), 3.93 (m, 2H). Analysis calculated for $C_{12}H_{23}O_6PNa_2$. 1.5 H_2O: %C 39.24, %H 7.14. Found: %C 39.14, %H 6.42.

2.4.7. 1-(oleyloxy)-propane-3-phosphonoformate (disodium salt) (3g)

1-(oleyloxy)-propane-3-phosphonoformate (disodium salt) (**3g**): yield (77.8%); ^1H NMR ($CDCl_3$:CD_3OD: D_2O::2:3:1) δ 0.95 (bs, 3H), 1.38 (bs, 16H), 1.60 (m, 2H), 1.95 (m, 2H), 2.0 (m, 4H), 3.45 (m, 2H), 3.60 (m, 2H), 3.95 (m, 3H), 5.35 (bs, 2H). Analysis calculated for $C_{22}H_{41}O_6PNa_2$. 1.3 H_2O: %C 52.64, %H 8.76. Found: %C 52.62, %H 8.55.

2.5. Antiviral testing

2.5.1. HCMV and HSV-1

Liposomes containing dioleoylphosphatidylcholine, dioleoylphosphatidylglycerol, cholesterol and drug at a molar ratio of 50/10/30/10 respectively, were prepared as previously described (Hostetler et al., 1996) to obtain a final drug concentration of 5 mM (stock). Blank liposomes without drug were similarly prepared and added to provide 1 mM liposomal lipid as a control. Subconfluent MRC-5 (human fibroblast) cells were pretreated for 24 h with the indicated concentrations of drug in MEM medium containing 2% fetal bovine serum and antibiotics. The medium was removed, HCMV virus (AD-169 strain) added at a dilution that resulted in a 3–4+ CPE in the no drug wells in 5 days, or an increase in HCMV DNA 31 times the control blank value, and incubated at 37°C for 1 h. The medium was removed and replaced with drug dilutions and incubated for five days (2–3 cycles). The HSV-1 assay was done the same way but without a drug pretreatment. The input HSV-1 virus was sufficient to provide 3–4+ CPE in 24 h and an HSV-1 DNA level 40 times the blank value for uninfected cells. It was then incubated about 24 h (single cycle). The HCMV or HSV-1 DNA present was quantified by nucleic acid hybridization using a CMV or HSV antiviral susceptibility test kit from Diagnostic Hybrids (Athens, OH). The medium was removed and the cells were lysed and adsorbed onto Hybriwix™ filters. The filters were hybridized overnight at 60°C, then washed for 30 min at 73°C and counted in a gamma counter.

2.5.2. HIV

Liposomes containing dioleoylphosphatidylcholine, dioleoylphosphatidylglycerol, cholesterol and drug at a molar ratio of 50/10/30/10, respectively, or blank control liposomes were prepared to obtain a final drug concentration of 5 mM (stock). To HT4-6C (CD4 positive HeLa) cells at 25% confluency, a virus dilution was added that resulted in 100–200 plaques (syncytial foci) in the control wells in 3 days and incubated for 2 h at 37°C. Blank liposomes or various dilutions of the drug were then added in DMEM medium containing 4% FBS and antibiotics at the indicated concentrations. The cells were then incubated at 37°C for 3 days. The plates were fixed, stained with crystal violet, and the plaques were then counted as previously described (Larder et al., 1990).

2.6. Cytotoxicity testing

Subconfluent MRC-5 and HT4-6C cells were exposed to drugs for 5 days and evaluated by a visual grading system as previously described (Hostetler et al., 1996). Rapidly dividing human T-lymphoblast cells (CEM cells) were exposed to drug and the number of viable cells were determined by flow cytometry after staining with propidium iodide as previously described (Dangl et al., 1982). The cytotoxic concentration of drug which reduced viable cell number by 50% (TC_{50}) was determined (Table 1).

3. Results

3.1. Chemistry

The synthesis of compounds **3a–g** is outlined in Fig. 1. There are some reports of the synthesis of 3-alkyloxy-1-propanols in the literature (Baumann et al., 1967; Tsushima et al., 1982; Pascher

Table 1
Cytotoxicity of alkoxypropyl conjugates of foscarnet in MRC-5, HT4-6C and CEM cells

Compound	Chain length:double bonds	TC_{50}, μM		
		MRC-5	HT4-6C cells	CEM
1-octyloxypropane-3-PFA (**3f**)	8:0	1000	320	457
1-dodecyloxypropane-3-PFA (**3e**)	12:0	320	210	154
1-tetradecyloxypropane-3-PFA (**3d**)	14:0	1000	320	220
1-hexadecyloxpropasen-3-PFA (**3c**)	16:0	320	100	233
1-octadecyloxypropane-3-PFA (**3b**)	18:0	660	100	169
1-docosyloxypropane-3-PFA (**3a**)	22:0	210	100	410
1-oleyloxypropane-3-PFA (**3g**)	18:1	32	32	69
1-*O*-octadecyl-*sn*-glycero-3-PFA	18:0	>1000	320	347
PFA	—	>1000	>1000	800

TC_{50} toxic concentration of drug which reduces viable cell number by 50%; PFA, phosphonoformate.

et al., 1984; Kosar-Hashemi and Armarego, 1993; Yoshida et al., 1995). We used a procedure similar to the one reported by Brachwitz and co-workers (Brachwitz et al., 1990) to synthesize 3-alkoxy-1-propanols (**1a–g**) by alkylation of an excess of 1,3-propanediol with the appropriate alkylmethanesulfonate in the presence of sodium hydride in DMF. Compounds **1a–e** were isolated by crystallization, while **1f–g** were purified by chromatography. Compounds **1a–g** were coupled to ethyl phosphonoformate with *N*,*N*-dicyclohexylcarbodiimide in a mixture of dichloromethane and pyridine. The products (**2a–g**) were isolated by flash chromatography over silica gel and subsequently saponified with aqueous sodium hydroxide in ethanol to yield the target compounds (**3a–g**) as the disodium salts. The salt was purified by washing with ethanol to yield analytically pure **3a–g** as amorphous solids in yields ranging from 46–95%.

3.2. Antiviral testing

In MRC-5 cells infected with HCMV, the 50% effective concentration (EC_{50}) of 1-octadecyloxypropane-3-PFA (**3b**) was 1.0 μM versus 0.6 μM for ODG-PFA (Fig. 2 and Table 2). This represents a substantial increase in antiviral activity over free foscarnet which has an EC_{50} of 40 μM. Similar results were obtained in HSV-1 infected cells (Table 2). When the alkyl ether chain length was reduced to eight carbons (**3f**), there was a marked reduction in antiviral activity against HCMV and HSV-1, (EC_{50} 76–85 μM). In HCMV-infected cells, the optimal chain length was 14–18 carbons with EC_{50} values of 0.9–1.2 μM. In HSV-1 infected cells, the optimal alkyl chain length was 16 or 18 carbons, EC_{50} 3.1 and 1.8 μM, respectively (Fig. 2). The 50% toxic concentration (TC_{50}) of the 14, 16, and 18 carbon alkoxypropane analogs in MRC-5 cells ranged from 320 to 1000 μM (Table 1). Increasing the alkyl chain to 22 carbons resulted in a 3-fold reduction in antiviral activity against HCMV and HSV-1 and a moderate increase in toxicity to MRC-5 cells (TC_{50} 210 μM). Introduction of a double bond into the 18 carbon saturated alkyl chain (**3g**) resulted in a slight increase in antiviral activity but cytotoxicity increased dramatically (Table 1). The alkoyxpropane analogs with 18 carbon alkyl chains (**3b**) had antiviral activity similar to that of the 18-carbon glycerol derivative, ODG-PFA in cells infected with HCMV or HSV-1.

In HIV-1 infected HT4-6C cells, a slightly different situation was observed. The alkoxypropane analog having the greatest antiviral activity was the 22 carbon alkyl chain, 1-docosyloxypropane-3-PFA (**3a**) with an EC_{50} of 0.4 μM versus 135 μM for PFA, representing a 338-fold increase in antiviral activity (Fig. 2, Table 2). The 18 carbon compound, 1-octadecyloxypropane-3-PFA (**3b**), was also highly active with an EC_{50} value of 1 μM, a 135-fold increase over PFA. These two

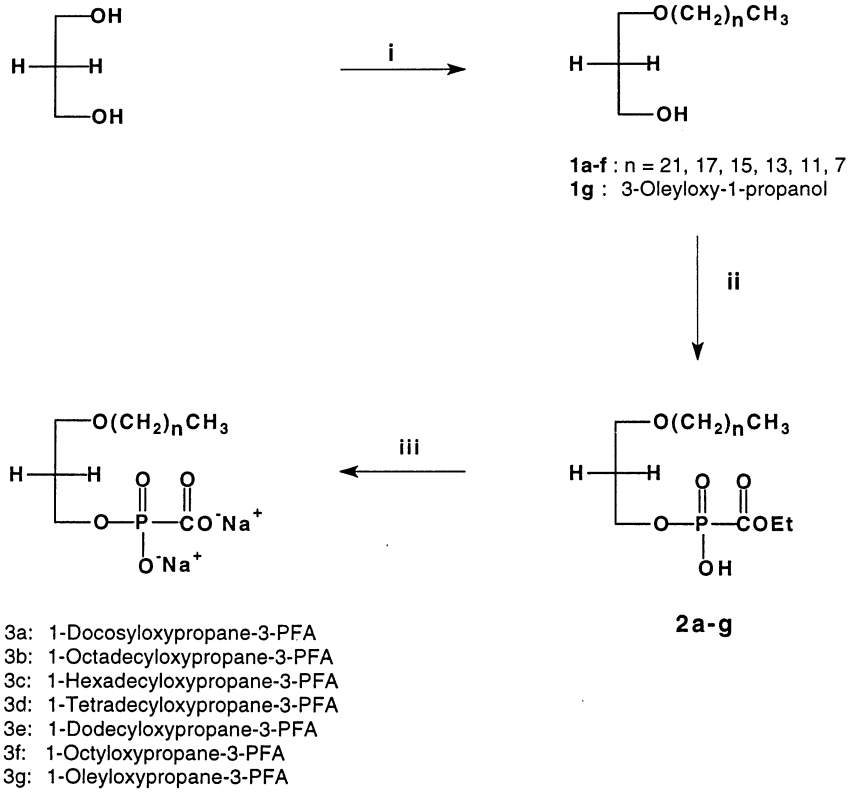

Fig. 1. Synthetic scheme for alkoxypropane foscarnet conjugates.

compounds were 2–6 times more active than previously reported for ODG-PFA (Hostetler et al., 1996). Introduction of a double bond into 1-octadecyloxy-propane-3-PFA (**3b**) resulted in an analog with similar activity to the saturated compound; however, cytotoxicity was greater with 1-oleyloxypropane-3-PFA (**3g**), TC_{50} 32 versus 100 μM in HT4-6C cells (Table 1).

3.3. Cytotoxicity

The toxicity of alkoxypropyl-foscarnet analogs was also chain length-dependent (Table 1) but much less so than antiviral activity (Table 2). HT4-6C cells (HeLa cells containing the CD4+ receptor) were more sensitive to these compounds than MRC-5 human lung fibroblasts or rapidly-dividing human T-lymphoblastic leukemia cells (CEM). The highest toxicities were noted at 16–22 carbons while 8–14 carbon analogs were generally less toxic. The degree of change in antiviral activity as a function of chain length (least active to most active) was 47–110-fold. However, the cytotoxicity range from least toxic to most toxic was only 2.7–4.8-fold.

4. Discussion

This study shows that the alkyl chain length exhibits a fairly narrow window with optimal antiviral activity observed at 16 and 18 carbon

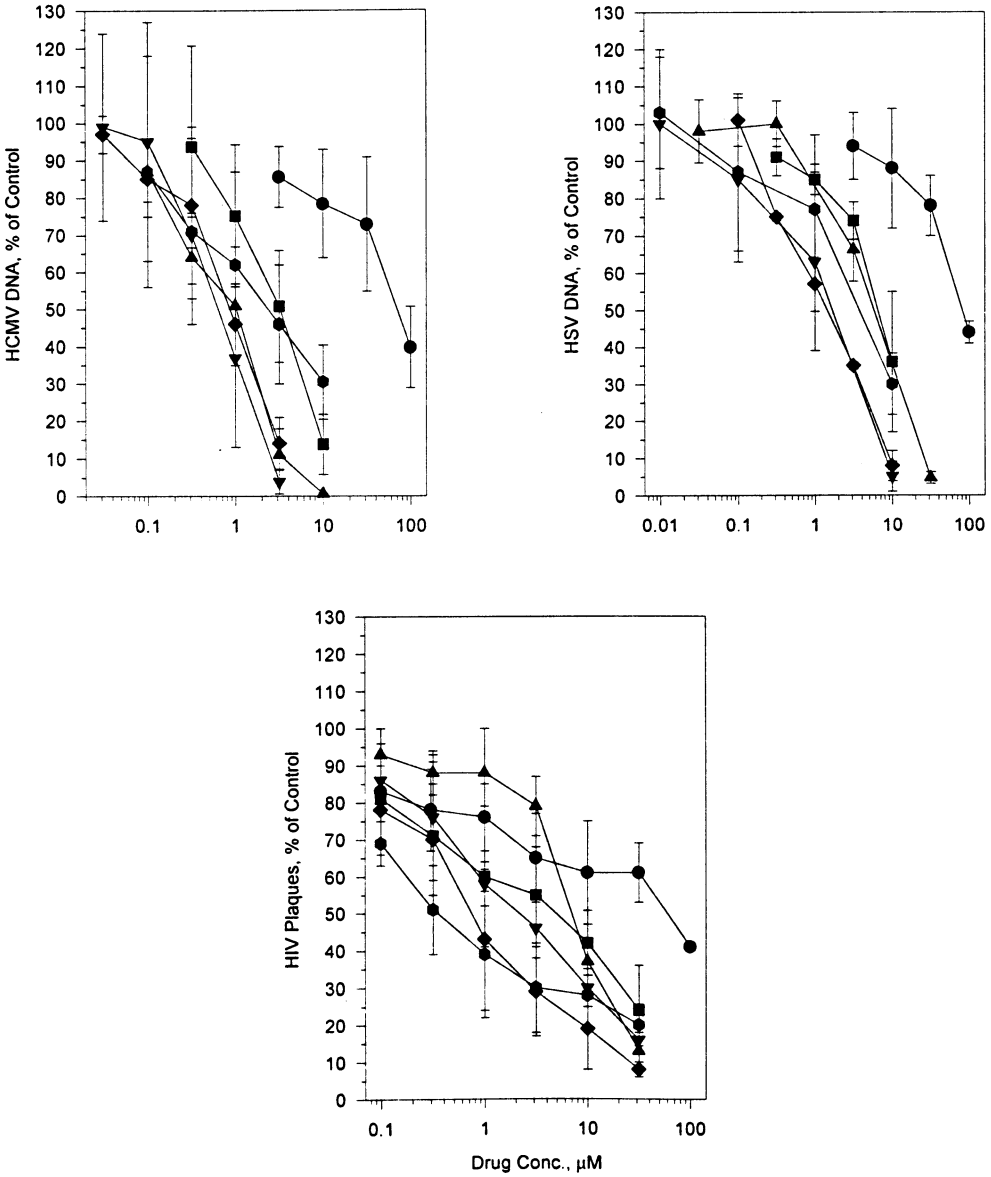

Fig. 2. Effect of alkoxypropane foscarnet conjugates on replication of HCMV, HSV-1 and HIV-1, in vitro. Upper left panel: HCMV; right panel: HSV-1 and Lower panel, HIV-1. Symbols: circle, 1-octyloxypropane-3-PFA; square, 1-dodecyloxypropane-3-PFA; triangle, 1-tetradecyloxypropane-3-PFA; inverted triangle, 1-hexadecyloxypropane-3-PFA; diamond, 1-octadecyloxypropane-3-PFA; and hexagon, 1-docosyloxypropane-3-PFA.

chains. The EC_{50} of the alkoxypropane conjugates of PFA was plotted as a function of chain length. Prodrugs of PFA with chain length less than 16 progressively lose antiviral activity in HCMV, HSV-1 or HIV-1 infected cells (Fig. 3). The activity of the respective optimal chain length analog was 84-, 47- and 100-fold greater than the eight carbon analog, 1-(octyloxy)-propane-3-PFA (**3f**), in HCMV-, HSV-1 or HIV-1 infected cells, respectively. The lower antiviral activity of eight

Table 2
Effect of chain length on the antiviral activity of alkoxy propane foscarnet conjugates in HCMV and HSV-1 infected MRC-5 cells

Compound	Chain length:double bonds	EC_{50}, μM		
		HCMV	HSV-1	HIV-1
1-octyloxypropane-3-PFA (**3f**)	8:0	76 ± 27 (3)	85 ± 3.6 (3)	44 ± 30 (3)
1-dodecyloxypropane-3-PFA (**3e**)	12:0	3.5 ± 2.1 (3)	8.9 ± 3.9 (3)	8.3 ± 1.2 (3)
1-tetradecyloxypropane-3-PFA (**3d**)	14:0	1.2 ± 0.7 (3)	10.9 ± 3.7 (3)	7.2 ± 1.8 (3)
1-hexadecyloxpropane-3-PFA (**3c**)	16:0	0.9 ± 0.6 (3)	3.1 ± 2.2 (3)	2.5 ± 1.4 (4)
1-octadecyloxypropane-3-PFA (**3b**)	18:0	1.0 ± 0.4 (3)	1.8 ± 0.7 (3)	1.0 ± 0.7 (4)
1-docosyloxypropane-3-PFA (**3a**)	22:0	2.9 ± 3.7 (3)	5.8 ± 2.3 (3)	0.4 ± 0.2 (3)
1-oleyloxypropane-3-PFA (**3g**)	18:1	0.8 ± 0.4 (3)	0.9 (2)	1.5 ± 1.2 (5)
1-*O*-octadecyl-*sn*-glycero-3-PFA	18:0	0.7 ± 0.2 (7)	0.6 ± 0.2 (5)	2.6 ± 1.4 (7)
PFA	—	40 ± 16 (6)	41 ± 16 (5)	135 ± 25 (8)
Control Liposomes	—	>1000	>1000	>1000

EC_{50}, the μM concentration of drug which is effective in reducing viral DNA by 50%; PFA, phosphonoformate.

carbon chains may be due to greater water solubility and the relative lack of ability to insert into the target cell plasma membrane lipid bilayer. In HCMV- and HSV-1 infected cells, the 22 carbon alkoxypropyl-PFA prodrug was 3-fold less active than the 16 or 18 carbon analog (Fig. 3 and Table 2). However, 1- (docosyloxy)-propane-3-PFA (**3a**) was 2.5–6-fold more active than the 16 and 18 carbon analogs in HIV-1 infected HT4-6C cells. The reasons for this difference are not clear but may represent metabolic differences in the MRC-5 and HT4-6C cell lines.

Early studies with PFA encapsulated in liposomes indicated 38–40-fold increases in antiviral activity versus free PFA in cells infected with HSV-2 (Szoka and Chu, 1988) and HCMV (Bakker-Woudenberg et al., 1991). However, subsequent studies in HCMV (Gümbel et al., 1994) and HIV-1 (Dusserre et al., 1995) showed either no increase or a 1–2-fold increase in antiviral activity in vitro. For convenience, we have routinely measured the antiviral activity of the lipophilic PFA analogs in a liposomal formulation. To evaluate the effect of formulation variables on antiviral activity, we compared the anti-HCMV activity of ODG-PFA in liposomes with the activity of micellar or albumin-bound ODG-PFA. Liposomal ODG-PFA had an EC_{50} of 0.7 μM versus an EC_{50} of 0.6 μM for micellar ODG-PFA and 0.4 μM for albumin-bound ODG-PFA. We conclude that the liposomal formulation is slightly less active than micellar or albumin-bound ODG-PFA and that the liposome does not play a key role in the observed increase in antiviral activity.

Previous studies with 1-*O*-octadecyl-*sn*-glycero-3-[^{14}C]PFA have shown that the cellular uptake of the lipid prodrug is much greater than that of [^{14}C]PFA. ODG-[^{14}C]PFA is hydrolyzed in the cell to [^{14}C]PFA by a phosphodiesterase of the phospholipase C type (Hostetler et al., 1996). The increased antiviral activity of the propanediol analogs is also presumed to be due to enhanced cellular uptake and its cellular conversion to PFA. Our earlier studies identified 1-*O*-octadecyl-*sn*-glycero-3-PFA as a highly active antiviral, 40–100 times more active than PFA in HCMV, HSV or HIV infected cells (Hostetler et al., 1996). The present experiments indicate that the *sn*-2 hydroxyl of glycerol is not required for enhancement of antiviral activity and suggest that the 1-(alkyloxy)-propane-3-PFAs may be useful for treating HIV or CMV infections.

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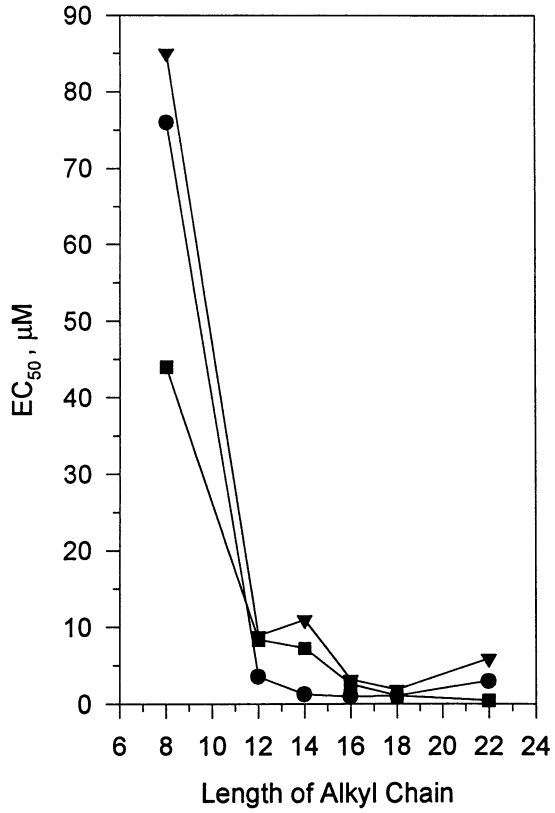

Fig. 3. Effect of alkyl chain length on the antiviral activity (EC_{50}) of alkoxypropane foscarnet analogs, in vitro. Symbols: circle, HCMV; square, HSV-1 and triangle, HIV-1.

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