

Hexadecyloxypropyl Tenofvir Associates Directly with HIV and Subsequently Inhibits Viral Replication in Untreated Cells

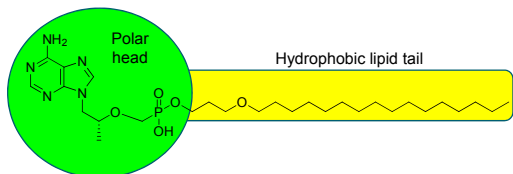
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INTRODUCTION

CMX157 is a lipid (1-(0-hexadecyloxypropyl), HDP) conjugate of tenofvir (TFV). Unlike tenofvir disoproxil fumarate (Viread®) and most prodrugs, CMX157 is not efficiently cleaved in plasma. This should both increase the levels of active TFV-PP in target cells for HIV and decrease the levels of free TFV in blood. Compared to TFV, CMX157 is >300 times more potent against wild-type and NRTI-resistant HIV *in vitro*; it effectively penetrates isolated human PBMCs, producing >30-fold higher intracellular levels of the active anabolite, TFV-diphosphate (TFV-PP).

Since CMX157 has a lipid side chain and HIV has a lipid bilayer, it was postulated that CMX157 associates directly with virions. To test this hypothesis, purified HIV was incubated with CMX157 or TFV followed by quantification of virus-associated drug and determination of the tissue culture infectious dose (TCID₅₀).

The goal of lipid conjugation is to increase oral bioavailability, increase potency and target delivery to tissues. The novel molecules are designed to resemble natural lipids that are readily absorbed from the small intestine and distributed to tissues via lymph and/or plasma. Once in the cell the drug is cleaved from the lipid carrier by hydrolytic action of phospholipases.



METHODS

Concentrated HIV-1_{IIIIB} was incubated with 500 nM CMX157 or TFV for 2 hours, pelleted to remove unbound compound and lysed with 70% methanol. Supernatants were analyzed in triplicate using LC/MS/MS. Analyses were separated by gradient, reverse phase, ion-pairing chromatography and detected by positive ion electrospray; separate viral aliquots were used to determine TCID₅₀ by XTT, RT and p24 assays in CEM-SS cells. To assess the effect of exposure time, concentrated HIV-1_{IIIIB} was incubated with 500nM CMX157 for 1, 15, 30, 60, and 120 minutes prior to TCID₅₀. To determine the effect of drug dose, TCID₅₀ was determined following a 15 minute incubation of virus with a range of concentrations of CMX157 ranging from 0.039 to 125nM. HDP-acyclovir was evaluated in parallel as a control.

BACKGROUND

IC₅₀s for CMX157 ranged from 0.66 nM for L74V/M184V to 57 nM for A62V/T69SVG/N75I/215I in the PhenoSense™ assay; corresponding IC₅₀s for tenofvir were 227 nM and 16,959 nM. CMX157 IC₅₀s for M41L/L210W/ T215Y averaged 6.3 nM without M184V and 2.2 nM with M184V (2,240 and 770 nM for tenofvir respectively).

Reasons for Increased In Vitro Efficacy?

- More active anabolite (TFV-PP) inside relevant cells:
- 1) Direct penetration of CMX157 into cells
 - 2) HIV mediated transport of CMX157 into cells

Levels of active anabolite in human PBMCs:

Following 24 hour incubations with equimolar TFV or CMX157, human PBMCs exposed to CMX157 had much higher intracellular levels of active drug (TFV-PP). For example, 10nM CMX157 yielded higher levels of TFV-PP (70 fmol/million cells) than 1000nM TFV (50 fmol/million cells) which approximates the human Cmax obtained with standard dosing of Viread®.

Does CMX157 bind directly to HIV virions?

- Incubate purified HIV with CMX157 or TFV
- Vary time of incubation and concentration
- Pellet HIV and remove unbound drug
- Quantify drug associated with viral pellet
- Determine effect of HIV-associated drug on infection of untreated cells (TCID₅₀)

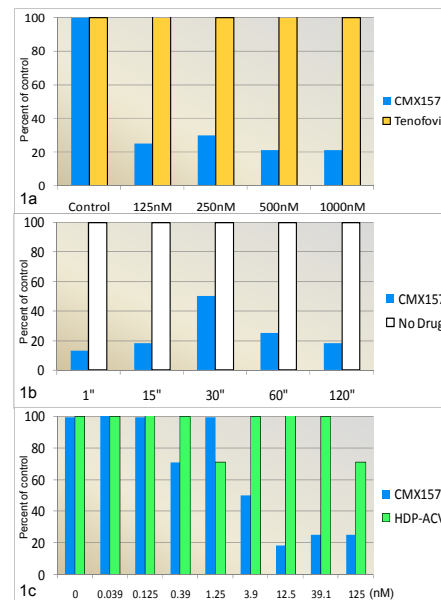
RESULTS

Table 1: Concentration of CMX157 and TFV in HIV_{IIIIB} lysates

Sample	CMX157 (pmol)	TFV (pmol)
HIV + 500 nM CMX157	5406	0.3
HIV + 500 nM TFV	ND	17
HIV + DMSO	ND	ND

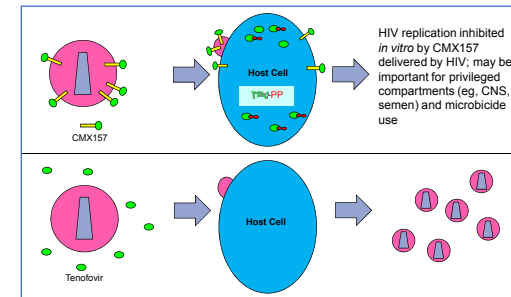
Analysis of purified HIV pellets following incubation with 500 nM drug showed ≈30,000 molecules of CMX157 were associated with each virion versus ≈100 molecules/virion for TFV.

Figure 1: TCID₅₀ effects of pre-incubating HIV_{IIIIB} with CMX157, TFV or lipid control (HDP-Acyclovir)



As shown in Fig 1a, TFV did not reduce the infectivity of pre-incubated virus on untreated cells while CMX157 reduced TCID₅₀ to about 25% of control with no apparent dose effect between 125 and 1000nM. As shown in Fig 1b, 500nM CMX157 had a similar effect when virus was exposed for a very short time period (1 minute) or 2 hours. Fig 1c reveals a dose effect where concentrations of CMX157 down to 3.9 nM resulted in 3-4 fold decreases in TCID₅₀ while the lipid control bearing the same alkyl modification as CMX157, HDP-acyclovir, showed no consistent effect. Data shown are for p24; similar data were obtained using XTT or RT endpoints. Incubation of CMX157 with media followed by pelleting, supernatant removal and addition of virus had no effect on TCID₅₀.

Figure 2: Proposed mechanism of CMX157 "hitchhiking" on HIV



DISCUSSION/CONCLUSIONS

Overall, these results indicate CMX157 associates directly with HIV and that this association enhances its antiviral activity *in vitro*. The most likely mechanism is direct targeting of CMX157 to the cell being infected; this has implications for potential treatment and prevention of HIV infection. Once inside the cell, CMX157 would be converted to TFV-PP which would then inhibit HIV replication via chain termination. CMX157 could have advantages over TFV via this mechanism of cell targeting as any HIV virion exposed to drug would carry CMX157 into any compartment or cell type it subsequently enters.

- CMX157 associated directly with HIV at high levels (≈30,000 molecules/virion); TFV did not (≈100 molecules/virion).
- Pre-exposure to CMX157 consistently reduced HIV infection of untreated cells ~4 fold at ≥40 nM; TFV had no effect at 1000 nM.
- The association of CMX157 with HIV appears to occur very quickly (1minute).
- CMX157 may have efficacy in anatomical or cellular compartments not treated by TFV.
- CMX157 is an attractive candidate microbicide for HIV.