Improved Intracellular Concentrations of Cidofovir Diphosphate Using an Oral Prodrug Approach

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Objective

To determine the intracellular concentration of cidofovir diphosphate in USC-505 and cidofovir treated HFF-1 cells *in vitro*.

Introduction

Cidofovir (CDV) is a nucleoside phosphonate analog, which has broad spectrum antiviral activity but lacks oral bioavailability. To overcome this problem we have developed an oral prodrug of cidofovir (USC-505). This prodrug, when administered orally, is readily absorbed, taken up by cells and converted to the pharmacologically active antiviral cidofovir diphosphate (CDV-PP), which inhibits the viral replication (Figure 1). The goal of this study was to determine the relative increase of CDV-PP in human foreskin fibroblasts (HFF-1) cells exposed to USC-505 versus cidofovir.

Methods

Cells
Human foreskin fibroblasts (HFF-1) cells were obtained from ATCC and grown in the recommended DMEM medium in the presence of 15% FBS. During treatment with CDV and USC-505, the FBS concentration was reduced to 2% to reflect the FBS concentration used in the *in vitro* antiviral assays.

Treatment of cells with USC-505 and CDV
Cells were seeded in triplicate sets of T75 flasks at 6E+06 cells/flask. The cells were incubated for 24 hours in 37˚C with 5% CO_2. Drugs (CDV or USC-505) were added to the final concentration of 1µM in 2% FBS containing DMEM. Vehicle control of DMSO was added at 0.6% concentration for cell count determination. The flasks were incubated for 72 hours in 37˚C with 5% CO_2.

Observation and sample collection
The flasks were observed under phase contrast microscope every 24 hours for any changes in cell morphology (e.g. vacuolation/granulation or floating dead cells in media).

The vehicle control flasks were trypsinized with 0.05% trypsin for 2 minutes at 37˚C to dislodge the cells. Cells were washed with D-PBS by centrifuging at 2000 rpm for 5 minutes. The cell pellet was resuspended in 5mL of D-PBS and live cells (trypan blue exclusion) and counted in a Neubauer chamber.

The media from the drug treated T75 flasks were collected in sterile 50mL conical tubes and cells were rinsed twice with 40mL of chilled DPBS. The cells were lysed in 1mL of chilled 50% Acetonitrile and cells were scraped off using a cell scraper. The cell lysate was collected in vials and vortexed and frozen immediately on -80˚C (Figure 2).

Sample analysis
The samples were thawed, centrifuged and the supernatant was analyzed using a qualified LC-MS/MS for CDV, CDV-PP and prodrug.

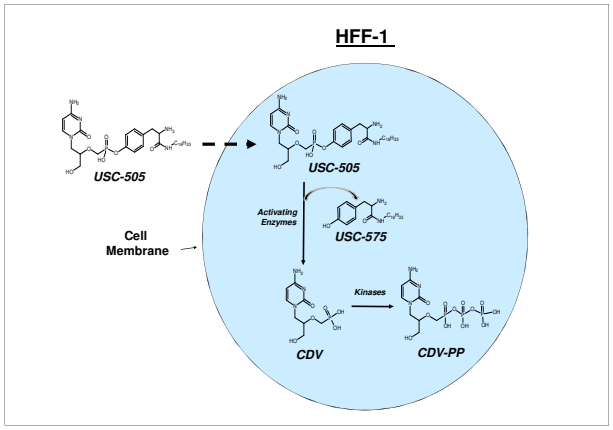

Figure 1: Proposed uptake and conversion of USC-505 to CDV-PP

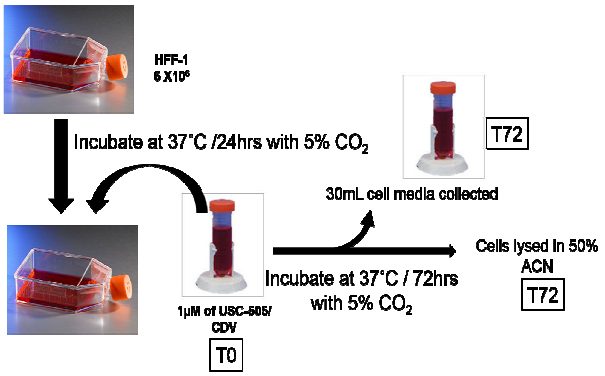

Figure 2: Schematic representation of experimental protocol for intracellular prodrug conversion and accumulation of CDV-PP in HFF-1 cells.

Results

	T72h	
Compound	**Media (nmol)**	**Intracellular (nmol)**
USC-505	44.4	3.9
USC-505 Degradation	8.5*	ND
CDV-PP	0	0.98
CDV	ND	0.22
Total nmol	58	
* Inferred from stability study		

Table 1. CDV and CDV-PP levels in HFF-1 cells treated with 1µM USC-505 for 72 hours.

	Mass Balance (%)	
Compound	**Media (% recovered)**	**Cell Lysate (%recovered)**
USC-505	75.95%	6.74%
USC-505 Degradation	14.70%	ND
CDV-PP	0.00%	1.68%
CDV	ND	0.92%
Total Micromoles	0.058	

Table 2. Mass balance of USC-505 in HFF-1 cells.

Viral Family	Virus	Activity (EC50, µM)		Enhanced Activity (CDV/USC-505)	Cell Line
		USC-505	CDV		
Herpes	CMV	0.001	0.625	625	HFF
	HSV-2	0.12	26.7	223	HFF
	VZV	0.01	60	6000	HFF
Adenovirus	AdV5	0.03	3.7	123	HFF
Pox	Cowpox	0.25	23	92	HFF
	Vaccinia	8.7	22.4	3	HFF

Table 3. Increased *in vitro* antiviral activity of USC-505 in comparison to CDV.

Summary

- HFF-1 cells were chosen and used to study the intracellular CDV-PP accumulation since all the *in vitro* antiviral assays were performed in this cell line.
- Neither CDV nor CDV-PP was not detected in CDV treated HFF-1 cells. USC-505 treated HFF-1 cells showed appreciable uptake and conversion to CDV and CDV-PP compared to parent CDV treated cells (Table 1 & 2).
- No toxic changes of vacuolation or dead cells were seen with either CDV/ USC-505 treatment.
- Increased intracellular concentrations of CDV-PP indirectly corresponds to increase in potency of USC-505 over CDV in antiviral assays *in vitro* (Table 3).

Conclusions

- USC 505, an oral prodrug of CDV has shown increased intracellular accumulation of CDV-PP in comparison to CDV treated HFF-1 cells.

Future studies

- Intracellular CDV-PP concentrations will be analyzed in virus infected, USC-505 and CDV treated cells.
- The activating enzymes associated with the intracellular conversion of USC-505 to Cidofovir (the parent compound) will be determined. In vitro enzyme assays will be applied to assess USC-505 substrate activity against various enzymes (e.g. phosphatases, lipases, etc.).
- Dynamic concentration relationships between absorbed drug and metabolites will be studied in a time course experiment.

References

- Krylov, I. et al. Mol Pharm 2013, 10(2):512-522.
- Lanier R.et al. Brincidofovir (BCV) delivers high intracellular concentrations of cidofovir diphosphate. 27th ICAR, Chimerix (2014).
- Lanier R. et al. Viruses, *2*, 2740 (2010).

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