Phosphoramidate prodrugs of uridine analogs, including sofosbuvir, are metabolized to both uridine and cytidine triphosphates in Huh7 cells and primary human hepatocytes

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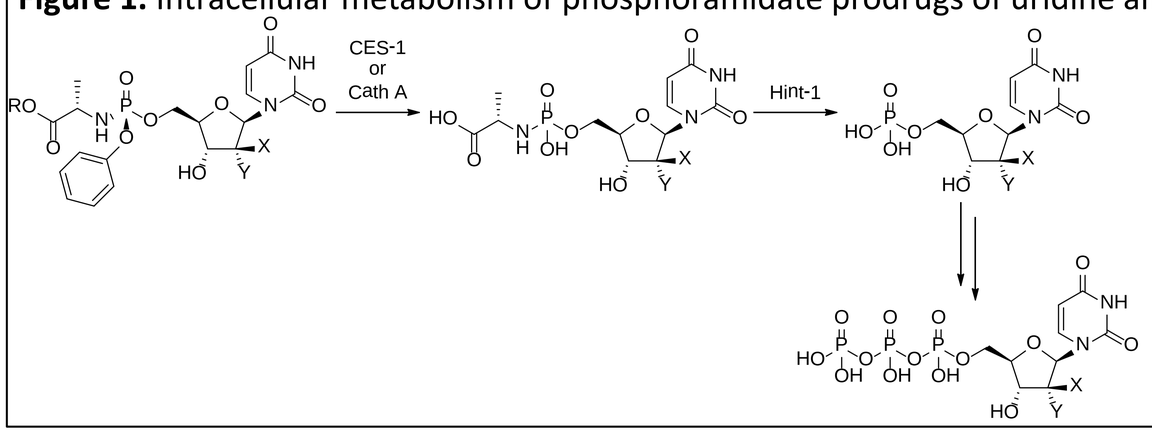
Background and Introduction

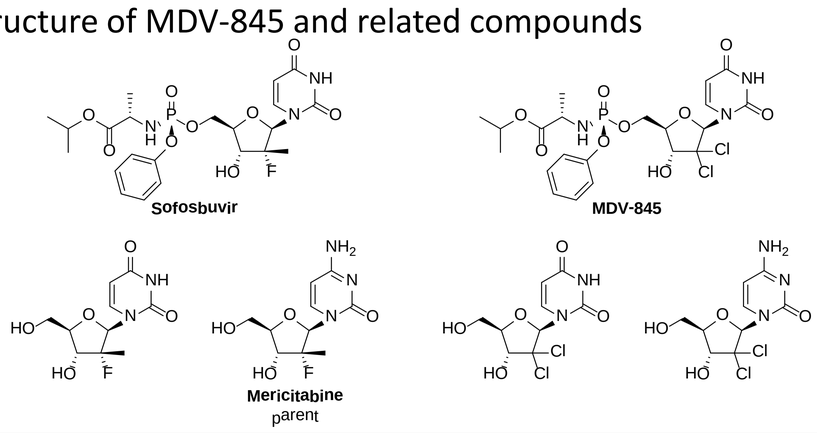
Sofosbuvir is a prodrug of a uridine nucleoside analog that is converted intracellularly to its active anti-HCV metabolite, the uridine triphosphate. While cytdine analogs such as mericitabine have been shown to be metabolized to both cytidine and uridine triphosphates , the intracellular metabolism of phosphoramidate prodrugs of uridine analogs has only been reported to yield uridine triphosphates[1,2] as shown in Figure 1 .

Figure 1. Intracellular metabolism of phosphoramidate prodrugs of uridine analogs



As part of a program to discover new nucleotide inhibitors of HCV replication we synthesized a number of phosphoramidate prodrugs of novel uridine analogs, and assessed their antiviral properties. MDV-845 (Figure 2) was identified as a novel inhibitor of HCV RNA replication *in vitro*. Its mechanism of action, intracellular metabolism and PK properties were profiled and compared with those of sofosbuvir.

Figure 2. Structure of MDV-845 and related compounds



Results

MDV-845 is a potent inhibitor of HCV RNA replication , with activity against HCV genotypes 1-6 (Table 1) and improved anti-HCV activity relative to sofosbuvir. It is not cytotoxic to Huh7 cells, with a CC_{50} value of >100μM (n=28) in 6 day XTT cytotoxicity assay. The UTP derived from MDV-845 inhibits HCV GT1b polymerase (Table 2), and has chain terminating properties similar to those of sofosbuvir UTP (data not shown).

Assay	EC_{50} (nM)		Assay	EC_{50} (nM)	
	Sofosbuvir	MDV-845		Sofosbuvir	MDV-845
HCV GT1b (stable)	98 *(89-110; n=130)*	57 *(50-64; n=40)*	HCV GT3a*	130 *(110-160; n=8)*	67 *(57-79; n=8)*
HCV GT1b (transient)	82 *(59-110; n=32)*	52 *(43-61; n=16)*	HCV GT4a*	200 *(130-300; n=10)*	65 *(55-76; n=8)*
HCV GT1a*	130 *(96-190; n=18)*	68 *(60-77; n=18)*	HCV GT5a*	120 *(0.085-0.16; n=6)*	51 *(37-70; n=7)*
HCV GT2a replicon	48 *(31-75; n=2)*	18 *(6.6-52; n=2)*	HCV GT6a*	170 *(120-250; n=5)*	68 *(55-84; n=6)*
HCV GT2a virus	54 *(46-0.63; n=4)*	12 *(3.7-42; n=3)*			

Table 1. Inhibition of HCV replication by MDV-845 EC_{50} values (geometric means with 95% confidence limits) determined in Huh7 cells containing luciferase reporter-encoding HCV subgenomic replicons (or GT2a JFH-1 HCV virus) following 72h of compound treatment. *Chimeric replicons: HCV GT1b backbone with NS5B ORFs from specified GTs inserted.

HCV GT1b NS5B polymerase K_i (μM)			
Sofosbuvir-UTP	Mericitabine-TP	MDV-845-UTP	MDV-845-CTP
0.44 *(0.37-0.52; n=28)*	0.12 *(n=1)*	1.90 *(1.50-2.50; n=6)*	0.20 *(0.092-0.42; n=3)*

Table 2. Inhibition of HCV NS5B polymerase activity by triphosphate metabolites of sofosbuvir and MDV-845. Triphosphates were evaluated for inhibition of GT1b HCV NS5B polymerase. K_i values are presented as geometric means with 95% confidence limits.

References:

1. MJ Sofia *et al*., J. Med. Chem. (2010), **53**, 7202–7218;
2. 2. FDA review documentation (accessed May 29th 2016): http://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204671Orig1s000PharmR.pdf

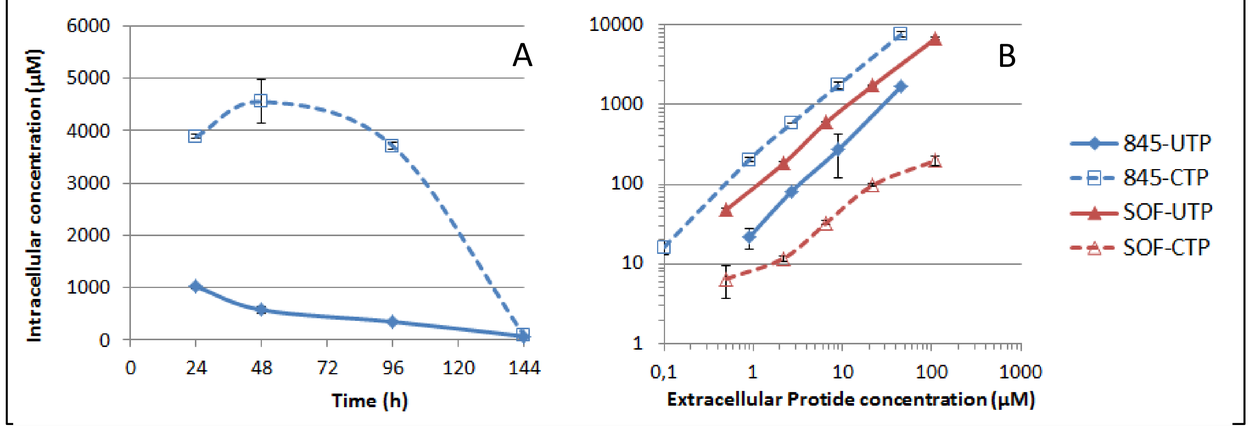
Conclusions

- **Phosphoramidate prodrugs of uridine nucleoside analogs yield both cytidine and uridine triphosphates in Huh7 cells and primary human hepatocytes; the high levels of MDV-845 CTP formed in Huh7 cells account for the superior potency of MDV-845 relative to sofosbuvir in replicon assays**
- **Exposures to both cytidine and uridine metabolites need to be assessed in safety and pharmacology studies on drug candidates from this class**
- **Differences in the intracellular metabolism of such prodrugs between Huh7 cells, in which pharmacological activity is assessed, and primary cells may confound human dose predictions based on *in vitro* antiviral activity**

Results (continued)

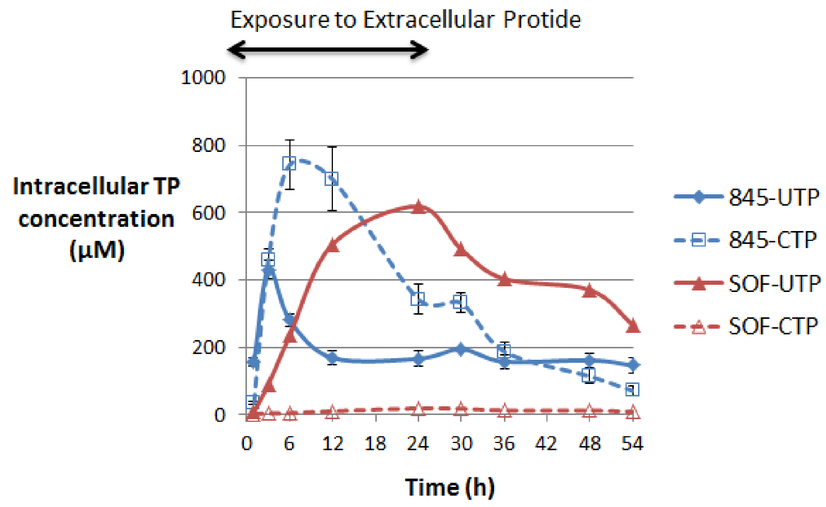
Based on the superior antiviral activity of MDV-845 and similar potency of MDV-845 UTP against HCV NS5B polymerase, it was expected that MDV-845 would generate high levels of its UTP in Huh7 cells. However initial intracellular metabolism studies in Huh7 cells comparing intracellular UTP levels derived from equivalent extracellular concentrations of either sofosbuvir or MDV-845 showed that MDV-845 UTP levels were lower than those of sofosbuvir UTP. This prompted a more detailed investigation of the intracellular metabolism of both MDV-845 and sofosbuvir in Huh7 cells, and led to identification of sofosbuvir CTP (mericitabine triphosphate) and MDV-845 CTP as intracellular metabolites of sofosbuvir and MDV-845 respectively (Figure 3).

Figure 3. Intracellular metabolism of MDV-845 and sofosbuvir in Huh7 cells. (A) UTP and CTP metabolite time course in Huh7 cells after extracellular incubation with MDV-845 (50 μM); (B) Concentration dependence of UTP and CTP levels in Huh7 cells after extracellular incubation with MDV-845 or sofosbuvir for 24h.



Intracellular metabolism studies with MDV-845 and sofosbuvir in primary human hepatocytes showed that CTP:UTP metabolite ratios were substantially lower in these cells compared to Huh7 cells (Figure 4). Pharmacologically relevant CTP levels were detectable in dog liver after oral dosing of MDV-845 (Table 3) as well as after exposure to dog hepatocytes *in vitro* (data not shown). It is therefore expected that cytidine metabolites of these prodrugs will be formed in humans after dosing of such prodrugs.

Figure 4. Time course of UTP and CTP metabolites in primary human hepatocytes after incubation with extracellular MDV-845 or sofosbuvir (10μM).



Liver Metabolite	MDV-845 (n=3)	Sofosbuvir[2]
Protide (μM)	0.03 ± 0.02	1.1
Alanine metabolite (μM)	1.7 ± 1.0	45
U-nucleoside (μM)	12 ± 5.1	10
C-nucleoside (μM)	3.7 ± 1.0	Not reported
UMP (μM)	15 ± 7.8	Not reported
UTP (μM)	3.5 ± 1.3	20
CTP (μM)	0.93 ± 0.13	Not reported
CTP/UTP ratio	0.31 ± 0.16	Not reported

Table 3. Liver metabolite levels after oral administration of protides to dogs. Dogs were dosed with 50mg/kg qd p.o. for 4 days. Livers were at harvested 4h post dose on Day 4.