Potent Broad Spectrum Anti-DNA Viral Activity for HPMPA and HPMPC Tyrosinamide Prodrugs

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Abstract

The broad spectrum antiviral activity of acyclic nucleoside phosphonates (ANPs) makes this prominent class of therapeutics useful agents in the treatment of many DNA virus infections. The prototypic molecules cidofovir ((S)-HPMPC, Vistide®) and ((S)-HPMPA) exhibit antiviral activity against a wide variety of DNA viruses, including the herpesviruses, poxviruses, polyomaviruses and papillomaviruses. A major hindrance in meeting their therapeutic potential is the presence of an ionizable phosphonic acid group, which results in poor cell membrane permeability and low oral bioavailability at physiological pH. We previously reported the synthesis and preliminary studies of a series of lipophilic tyrosinamide prodrugs of (S)-HPMPA and (S)-HPMPC. Here we present structure activity relationships of alkyl group size in a set of 8 viruses including representatives of each of the four virus families described above. In all viruses, increased length of the alkyl moiety correlated well with increased potency with maximal activity observed with alkyl chains of at least 14 carbon atoms in length. In the HPMPA series, up to 30-fold improvements were observed in the therapeutic indices for some viruses and were virus specific. The most marked improvements in antiviral activity were observed against vaccinia virus, varicella-zoster virus, adenovirus and human papillomavirus and only modest changes occurred against herpes simplex virus. This strategy has the potential to greatly improve the activity ANP and suggests viral infections that might be best targeted with this approach.

Methods

In vitro antiviral assays were performed by methods similar to that described previously[1]. Briefly, Human foreskin fibroblast (HFF) cells were prepared and expanded through serial passages in standard growth medium of MEM with Earl's salts supplemented with 10% FBS and antibiotics. Low passage (3-10) HFF cells were seeded into tissue culture plates in MEM containing 10% FBS. Media containing serial dilutions of the experimental drug was added in triplicate wells. Media alone was added to both cell and virus control wells. Virus suspension was added to each well, excluding cell control wells which received MEM. Plates were incubated at 37°C in a CO_2 incubator for three days to fourteen days depending the virus strain. After the incubation, cytopathic effect (CPE) was determined to assess antiviral activity. EC_{50} and CC_{50} values were determined by fitting dose response curves of drug treated and untreated cells in the presence and absence of virus.

For the pharmacokinetic and distribution studies, ^{3}H-prodrugs of HPMPA were prepared by tritium exchange. All label resided on the HPMPA core. Mice were dosed either with HPMPA (IV) or the prodrugs C8, C12 and C16 (PO). Blood samples were collected at regular intervals and analyzed by liquid scintillation counting. For the distribution studies, mice were sacrificed at 12, 24, and 72 hours post dose and tissues were harvested, homogenized and analyzed for total radioactivity by liquid scintillation counting.

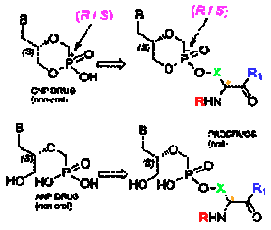

Fig 1. Modification of ANP drugs to increase oral bioavailability and potency. The phosphonic acid group is esterified by the OH side-chain X of an amino acid (Ser, Thr or Tyr) that has a free (R = H) or modified (R = Val) α-amino group and a modified carboxyl group (R_1 = OR_2 or NHR_2, where R_2 = C4-C18 alkyl).

Results

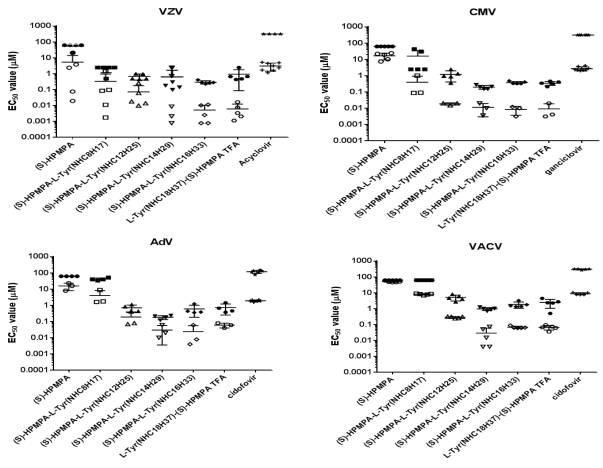

Fig 2. Potency (EC_{50}, open symbols) and Toxicity (CC_{50}, filled symbols) of HPMPA Prodrugs against Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), Adenovirus (AdV) and Vaccina (VACV)

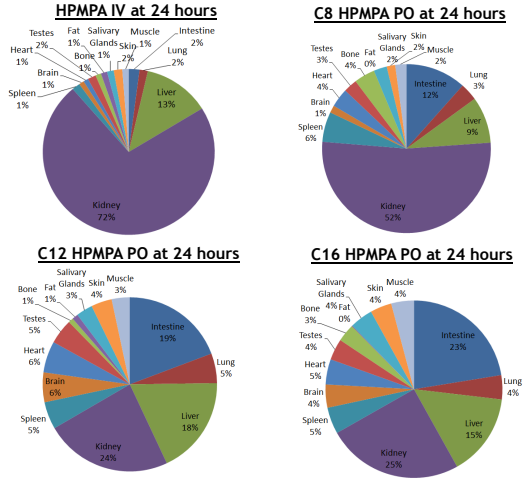

Fig 3. Tissue distribution of HPMPA prodrugs

Table 1. Potency (EC_{50}, μM) and toxicity (CC_{50}, μM) and selectivity index (SI)

		(S)-HPMPC	(S)-HPMPC-L-Tyr($NHC_{16}H_{33}$)	(S)-HPMPA	(S)-HPMPA-L-Tyr($NHC_{16}H_{33}$)
HSV	EC_{50}	32.0 ± 16.0	0.2 ± 0.11	59.2 ± 1.96	0.46 ± 0.05
	CC_{50}	60.0 ± 0.00	1.32 ± 0.24	60 ± 0	0.96 ± 0.25
	SI	2	7	1	2
VZV	EC_{50}	60.0 ± 0.00	0.01 ± 0	47.5 ± 25.1	0.05 ± 0.07
	CC_{50}	60.0 ± 0.00	0.3 ± 0.05	60.0 ± 0.00	0.32 ± 0.05
	SI	1	30	1	6
CMV	EC_{50}	0.625 ± 0.358	0.001 ± 0	19.0 ± 6.21	0.011 ± 0.001
	CC_{50}	60.0 ± 0.00	0.34 ± 0.07	60 ± 0	0.38 ± 0.07
	SI	96	340	3	35
VACV	EC_{50}	22 ± 11	0.153 ± 0.107	50.5 ± 5.66	0.068 ± 0.011
	CC_{50}	60 ± 0	1.81 ± 0.39	60 ± 0	1.83 ± 0.78
	SI	3	12	1	27
AdV	EC_{50}	3.75 ± 2.48	0.035 ± 0.007	11.9 ± 5.50	0.032 ± 0.04
	CC_{50}	60.0 ± 0.00	0.305 ± 0.064	60 ± 0	0.39 ± 0.057
	SI	16	9	5	12

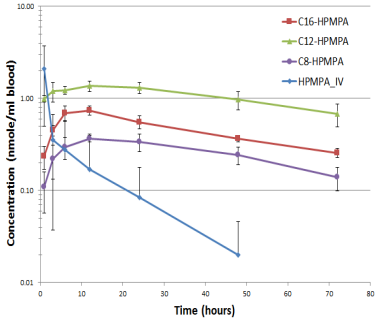

Fig 4. Pharmacokinetic profile of HPMPA prodrugs

Conclusions

- As shown in Fig 2, all prodrugs of HPMPA were more potent than the parent HPMPA against VZV, CMV, AdV and VACV. Structure-activity relationships suggest that longer alkyl chain length leads to higher potency, likely due to higher lipophilicity enabling better cell penetration.
- Drug distribution of HPMPA vs. prodrugs shows remarkable differences in kidney uptake and differential organ distribution as a function of alkyl chain length. While HPMPA after IV administration shows high kidney uptake (>70%) 24 hours post-dose, the C8 analog showed ~50% kidney uptake, while the C12 and C16 analogs were kidney-sparing (≤25%), suggesting lower nephrotoxicity liability for these analogs (Fig 3).
- Fig 4 shows the pharmacokinetic profile of C8, C12 and C16 HPMPA prodrugs. All three compounds resulted in good oral exposure with terminal half-lives much longer (~50 hours) than IV HPMPA.
- HPMPA and HPMPC C16 analogs show broad-spectrum activity against HSV, VZV, CMV, VACV, and AdV. with selectivity indices acceptable for further development (Table 1).

References

Zakharova VM et al.: Tyrosine-based 1-(S)-[3-hydroxy-2-(phosphonomethoxy) propyl] cytosine and -adenine ((S)-HPMPC and (S)-HPMPA) prodrugs: synthesis, stability, antiviral activity, and in vivo transport studies. J Med Chem 2011;54:5680-93.

Acknowledgements

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