Pharmacokinetics of Potent HPMPA and HPMPC Tyrosinamide Prodrugs with Broad Spectrum Anti-DNA Viral Activity

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Purpose

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 herpes viruses, poxviruses, polyomaviruses and papillomaviruses. A major hindrance in meeting their therapeutic potential is low oral bioavailability and dose limiting kidney toxicity. The study was conducted to demonstrate that these undesirable effects could be mitigated by synthesizing tyrosinamide prodrugs while improving potency against viral targets.

Methods

In vitro antiviral assays were performed as follows: 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 37oC in a CO2 incubator for three days to fourteen days depending the virus strain. After the incubation, cytopathic effect (CPE) was determined to assess antiviral activity. EC50 and CC50 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, 3H-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. In addition, an LC/MS/MS method was developed that was able to quantitate both the prototypic molecules and the prodrugs in plasma.

Results

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 (VACV), varicella-zoster virus (VZV), adenovirus (AdV) and human papillomavirus (HPV) and only modest changes occurred against herpes simplex virus (HSV).

Distribution studies demonstrate that kidney exposure was greatly reduced following oral administration of prodrugs (25%) as compared to parenteral administration of the parent compound (72%). Both liquid scintillation and LC/MS/MS methods indicate that the fraction absorbed following an oral dose is ~43%.

Conclusion

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. This strategy has the potential to greatly improve the activity of ANPs and suggests viral infections that might be best targeted with this approach. In addition, the prodrugs were demonstrated to limit kidney exposure and were orally available, making these good candidates for further development.