cell culture, application of FPAC to virus-infected mice resulted in dramatic decreasing of mortality (50–90% and 14–33% in the group of non-treated and treated mice, respectively, depending on the dose of the virus) and increasing of mean day of death (9.3–11.8 and 12.8–14.3 days). Virus titer in lungs of treated animals was slightly lower (4.5 against 5.3 $\log_{10}$ $TCID_{50}$/20 mg tissue in control). Rimantadine also appeared effective against influenza in mouse model decreasing mortality (10–20%) and reducing virus titer (2.5 $\log_{10}$ $TCID_{50}$/20 mg tissue). Morphological signs of virus infection in the lungs, such as bronchial epithelium damage, hemorrhagic and serous edema and perivascular and peribronchial cell infiltration were less manifested than in control anilmals.

Conclusion: Taken together, these data suggest that a novel low-toxic fullerene derivative might be prospective anti-influenza drug and should be further developed.

doi:10.1016/j.antiviral.2010.02.422

Poster Session 2: Herpes Viruses, Pox Viruses, Other Antiviral, Medicinal Chemistry and Topical Microbicides

Chairs: 4:00–6:00 pm

Pacific D-O

113

Synthesis and Antiviral Activity of 3-O-Phosphonomethyl Nucleosides

Michal Cesnek*, Piet Herdewijn

Laboratories of Medicinal Chemistry and Virology, Rega Institute for Medical Research, Minderbroedersstraat 10

Nucleoside phosphonates are widely used group of therapeutic agents with a broad spectrum of antiviral activity (De Clercq, 2000). There are two main advantages of the phosphorylated sugar moiety in comparison with non-phosphorylated nucleoside. The phosphonate group is stable against enzymatic hydrolysis (Holy, 1993) and the phosphonate nucleoside is skipping the requisite first phosphorylation step, which is an inefficient and often rate-limiting step, to reach its active metabolic form. In past few years strong antiviral activity and a good anti-HIV-1/HIV-2 selectivity were observed at compounds containing adenine and thymine derivatives of 3-O-phosphonomethyl-L-2-deoxythreose PMDTA, PMDTT, respectively (Wu et al., 2005). Based on this observation a series of 3-O-phosphonomethyl-L-2-deoxythreose analogues were prepared (Vina et al., 2007; Huang and Herdewijn, 2009). We attempt to elucidate the influence of 5′,6′-dihydroxyethyl substituent on the antiviral activity. To avoid possible steric hindrance of 3′-phosphonate during enzymatic phosphorylation reaction we chose α-D-galactofuranose as a starting material which was transformed subsequently into the appropriate 3-O-phosphonomethyl-β-D-galactose base. All compounds were evaluated in vitro for their antiviral activity.

References

De Clercq, E., 2004. J. Clin. Virol. 30, 115–133.
Holy, A., 1993. In: De clercq, E. (Ed.), Advances in Antiviral Drug Design vol. 1. Jai Press Inc., London, 179 pp.
Wu, T., Froeyen, M., Kempeneers, V., Pannecouque, C., Wang, J., Busson, R., De Clercq, E., Herdewijn, P., 2005. J. Am. Chem. Soc. 127, 5056.
Vina, D., Wu, T., Renders, M., Laflamme, G., Herdewijn, P., 2007. Tetrahedron 63, 2634.
Huang, Q., Herdewijn, P., 2009. Nucleosides Nucleotides Nucleic Acids 28, 337.

doi:10.1016/j.antiviral.2010.02.423

114

Oral Pharmacokinetics of hexadecyloxypropyl 9-(R)-[2-(Phosphono-methoxy)propyl]guanine (HDP-(R)-PMPG) in Mice using LC/MS/MS

Kathy A. Aldern[1,*], Julissa Trahan[2], James R. Beadle[1], Yongxuan Su[1], Karl Y. Hostetler[1,2]

[1] *University of California, San Diego, La Jolla, USA;* [2] *Veterans Medical Research Foundation, San Diego, USA*

Tenofovir [(R)-PMPA] is an acyclic nucleoside phosphonate (ANP) which is a potent inhibitor of HIV reverse transcription after intracellular conversion to its active metabolite, tenofovir diphosphate. The polar phosphonic acid is poorly absorbed after oral administration, but bioreversible masking of the phosphonate as the disoproxil ester (tenofovir disoproxil fumarate, Viread™) provides oral bioavailability of approximately 39%. Alternatively, esterification with alkoxyalkyl groups has proven to be an effective method for the oral delivery of several ANP drugs. For example, the hexadecyloxypropyl ester of (R)-PMPA (HDP-(R)-PMPA, CMX157) was studied in rats, demonstrating that the intact alkoxyalkyl ester is efficiently delivered to the systemic circulation after oral administration. Intracellular metabolism studies have shown that alkoxyalkyl ANPs are taken up rapidly by cells and metabolized to the active diphosphate metabolites.

To identify additional phosphonate nucleosides with potent anti-HIV activity, we recently prepared a series of alkoxyalkyl PMP-analogs and, following their in vitro evaluation against HIV, selected HDP-(R)-PMPG (EC_{50} vs. HIV = 2 nM in PBMCs) for additional study in mice. To assess the oral pharmacokinetics of HDP-(R)-PMPG, we developed an analytical method based on LC/MS/MS. A single oral dose of HDP-(R)-PMPG (10 and 30 mg/kg doses) was given to male mice and plasma collected at various time points up to 24 h. Plasma samples (50 μl) were prepared, analyzed by LC/MS/MS and quantitated using an internal standard. HDP-(R)-PMPG was rapidly absorbed and passed the intestinal epithelium intact. For both doses, the maximum plasma levels were detected at 4 h. After peaking, plasma levels declined during 24 h. The LC/MS/MS method for measuring plasma levels of alkoxyalkyl ANPs provides a useful tool for optimizing the oral delivery of acyclic nucleoside phosphonates. These findings warrant further study of HDP-(R)-PMPG for the treatment of HIV infections.

doi:10.1016/j.antiviral.2010.02.424