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1-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine [(*S*)-HPMP-5-azaC] emerged as a potent and selective inhibitor of several DNA viruses, including herpesviruses [i.e. herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2, varicella-zoster virus (VZV), human herpes virus 6 (HHV-6) and cytomegalovirus (CMV)], adenovirus type2 (Ad2) and poxviruses [i.e. vaccinia virus (VV), cowpox virus (CPV) and orf virus]. The antiviral activity of (*S*)-HPMP-5-azaC was comparable to that of the reference drug (*S*)-HPMPC (cidofovir) against HSV-1, HSV-2 and vaccinia virus, and two to seven-fold higher than cidofovir against VZV, HCMV, HHV-6 and Ad2. The cyclic derivative of (*S*)-HPMP-5-azaC (i.e. cHPMP-5-azaC) was also able to inhibit the replication of these DNA viruses. We have now evaluated the in vivo activity of HPMP-5-azaC and cHPMP-5-azaC against HSV-1, HSV-2, VV and CPV using two murine lethal challenge models. Five NMRI mice per group were infected with a lethal dose of HSV-1 or HSV-2 (intraperitoneal infection) or VV or CPV (intranasal infection) and treated subcutaneously with (*S*)-HPMP-5-azaC, cHPMPC-5-azaC, (*S*)-HPMPC or cHPMPC once daily from day 0 to day 4 post-infection at a dose of 50 mg/kg. The virus dose used in each experiment resulted in 100% mortality in the untreated animals. (*S*)-HPMP-5-azaC and cHPMP-5-azaC proved to be as effective as, respectively, (*S*)-HPMPC and cHPMPC in reducing mortality, affording 100% survival. Furthermore, a single dose of 50 mg/kg of each of the four compounds administered on day 0 was sufficient to provide full protection. Oral prodrugs were synthesized [i.e. alkyl (e.g. octadecyl), acyloxyalkyl (pivaloyloxymethyl) and alkoxyalkyl (e.g. hexadecyloxyethyl)], the latter being the most active prodrug in vitro. The in vivo activities of these prodrugs are currently under investigation. These findings warrant further development on this new class of acyclic nucleoside phosphonates.

doi:10.1016/j.antiviral.2007.01.025

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Isolation and Characterization of an Helicase-Primase Inhibitor (HPI)-Resistant HSV-1 Mutant in Tissue Culture and a Mouse-Skin Model

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Nucleoside analogues currently dominate HSV therapy and drug resistance is readily selected in cell culture (>10^{-4}) but rarely occurs in patients. Common resistant variants have defective thymidine kinase; although these mostly grow normally in tissue culture, they are often attenuated.

HPI, e.g. BAY 57-1293 are a newer class of antivirals, which have superiority over nucleoside analogues in animal infection models. BAY 57-1293-resistance could be selected by single passage in the presence of inhibitor. For example, BAYr1 is 70-fold resistant to BAY 57-1293 and occurred at a frequency of 10^{-6} in the well-characterized HSV-1 strain, SC16. There are previous reports that drug-resistant mutants of HSV-1 selected against BAY 57-1293 or other HPI have slower or near wild-type growth rates in vitro. However, we showed consistently that BAYr1 replicated faster in cell culture than its parent, SC16.

BAYr1 was fully pathogenic in a murine skin-infection model according to all the clinical parameters, including latency. BAYr1 was found to have two substitutions in the helicase protein (UL5: A4V and K356Q). Marker transfer revealed that K356Q alone is responsible for 70-fold resistance and faster growth in culture. Our results with BAYr1 support and extend previous reports: an HSV-1F mutant resistant to BAY 57-1293 (K356N) gave near wild-type mortality in a mouse survival test. Two mutants from HSV-1 KOS resistant to BILS 22 BS, a different HPI (K356N or G352V) grew normally in culture and were pathogenic in animal models. Our results and these two reports will be discussed in the relation to our recent surprising finding that some laboratory and clinical isolates of HSV-1 contain HPI-resistant mutants at high frequency.

doi:10.1016/j.antiviral.2007.01.026

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Evidence For In Vivo Inhibition of CMV Infection by the Quinazoline Class Protein Kinase Inhibitor Gefitinib

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Gefitinib (Iressa®) is a chemotherapeutic agent that exhibits activity against human and animal cytomegaloviruses (CMVs). We examined the activity of this agent against the guinea pig cytomegalovirus (GPCMV) both in vitro and in an in vivo disease model. In plaque reduction assays, gefitinib exhibited an IC_{50} of 3.3 μM, lower than the IC_{50} demonstrated against human CMV. The efficacy of gefitinib was next evaluated in an immunosuppression CMV disease model in outbred guinea pigs. Seronegative animals were treated intraperitoneally (i.p.) with cyclophosphamide, at a dose of 300 mg/kg at day −1 and 100 mg/kg at day +7, relative to i.p. challenge with an eGFP-tagged recombinant GPCMV, administered at a sublethal dose of 2×10^5 pfu. Beginning at day 1, guinea pigs were treated either with oral placebo ($n = 8$), or with either cyclic cidofovir at a dose of 20 mg/kg i.p. once weekly ($n = 8$), or gefitinib at a dose of 100 mg/kg once daily by gavage ($n = 8$). Antiviral therapy had a significant impact on weight loss. In placebo-treated animals, mean weights decreased in surviving animals by 17% at the endpoint of the experiment, compared to a 7% decrease in gefitinib-treated animals, and a 15% increase in

cidofovir-treated animals. Antiviral treatment also reduced mortality. In placebo-treated animals, mortality was 7/8 (87.5%). In the cidofovir group, mortality was 0/8 animals, compared to 3/8 (38%) animals in the gefitinib group. By qPCR, cidofovir therapy prevented DNAemia; gefitinib-treated animals had significantly lower viral loads than controls. In summary, gefitinib had in vitro activity against GPCMV and in vivo animals treated with gefitinib had improved weights and trends toward reduced mortality and reduced magnitude of DNAemia. These results derived from this initial trial support further evaluation of the potential for gefitinib as an anti-CMV antiviral in the guinea pig model.

doi:10.1016/j.antiviral.2007.01.027

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Successful Treatment in the Monkeypox and Variola Primate Models of Smallpox by the Oral Drug ST-246

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Based on activity in multiple small animal models, oral ST-246 was evaluated in our variola virus-cynomolgus monkey model of classical smallpox, which closely resembles human disease. The placebo group demonstrated typical disease with >1250 pox lesions and 33% mortality. Oral gavage with ST-246 begun 24 h after infection, when bone marrow, spleen, some lymph nodes and liver had $>10^8$ genomes/g and all tissues had 10^4–10^6 g^{-1}, eliminated disease as judged by complete lack of lesion formation, the best predictor of smallpox disease severity in humans, with no significant clinical or laboratory findings. Virus levels in blood did not increase over pretreatment levels (10^6 mL^{-1}) and was cleared in 6 days versus 16 days for placebo based on historical data. ST-246 was next evaluated using our monkeypox virus-cynomolgus monkey model of classical smallpox, which also closely resembles human disease. The placebo-treated group demonstrated typical disease with >1500 pox lesions and 100% mortality. Oral gavage treatment with ST-246 begun 1 day after infection, when bone marrow, spleen, some lymph nodes and liver had $>10^7$ genomes/g and all tissues have 10^5–10^6 g^{-1}, eliminated disease as judged by complete lack of lesion formation, with no significant clinical or laboratory findings. Virus levels in blood did not increase over pretreatment levels and was cleared in 4 days versus 16 days for placebo or IV Cidofovir™ based on historical data. Oral gavage treatment with ST-246 begun 3 days after infection, when bone marrow, spleen, some lymph nodes and liver had $>10^8$ genomes/g and all tissues had $>10^6$ g^{-1}, eliminated disease as judged by complete lack of lesion formation in 2/3 monkeys and <5% of control lesions in 1/3 that did not progress, with no significant clinical or laboratory findings. Virus levels in blood did not increase over pretreatment levels and was cleared in 6 days versus 16 days for placebo. ST-246 has been granted fast-track IND status and has not shown toxicity in phase I human single oral dosing at 2000 mg.

doi:10.1016/j.antiviral.2007.01.028

Oral Session IV: Hepatitis Viruses I

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Design and Characterization of R1626, A Prodrug of the HCV Replication Inhibitor R1479 (4′-Azidocytidine) With Enhanced Oral Bioavailability

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R1479 was identified as a selective inhibitor of HCV replication with high antiviral potency across HCV genotypes 1a and 1b isolates and a high barrier to resistance selection. Initial preclinical and clinical characterization of R1479 demonstrated suboptimal oral bioavailability because of limited absorption. A range of different types of R1479 prodrugs were synthesized and evaluated by characterization of physicochemical properties, Caco2 cell permeabilities and pharmacokinetics in rats and monkeys. Alkyl ester prodrugs were identified to substantially improve oral bioavailability of R1479, consistent with increased prodrug lipophilicity. R1626, the tri-isobutyrate ester prodrug of R1479 achieved a more than a five-fold increase in oral bioavailability and dose proportionality up to high dose levels. In concordance with dose dependent increases in plasma exposures of R1479, dose and time dependent mean viral load decreases of up to 3.7 $\log_{10}$ were observed in a 14 day multiple ascending dose monotherapy study in treatment naive patients with chronic HCV genotype-1 infection.

doi:10.1016/j.antiviral.2007.01.029