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Preclinical Development of the Amphipathic DNA Polymer REP 9AC for the Treatment of Hepatitis B Virus Infection

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Amphipathic DNA polymers (APs) are a novel class of nucleic acid-based medicines that have a broad spectrum activity against enveloped viruses and are effective *in vivo* against hepatitis C virus, HSV, CMV, influenza, RSV and Ebola infections. The assessment of the AP REP 9AC in the treatment of duck hepatitis B virus (DHBV) is presented as a model for human hepatitis B virus (HBV) infection. The antiviral assessment of APs was carried out in vitro using the infection of primary duck hepatocytes (PDH) with DHBV and *in vivo* using the Pekin duck DHBV model. The activity of APs and their analogs suggested a large amphipathic domain related to those found in type 1 fusion glycoproteins is involved in the entry or release of DHBV. For *in vivo* evaluation, 14-day-old ducks were infected with a defined dose of DHBV and treated with the REP 9AC (10 mg/(kg day) by IP injection) for 14 days. REP 9AC was well tolerated by the ducks with no detectable side effects. Liver biopsies collected on day 4 post-inoculation (p.i.) showed a small percentage of $DHBV^+$ hepatocytes in all REP 9AC treated and control ducks. However, by day 14 p.i. 100% of ducks treated with REP 9AC showed no evidence of DHBV infection in the liver. In contrast, in all control ducks treated with normal saline DHBV infection had spread to >95% of hepatocytes. REP 9AC was similarly effective at doses as low as 1 mg/(kg day). REP 9AC could be an effective treatment for HBV infection either as mono or combination therapy, which can likely be given once a week. More importantly, the novel mode of action of APs strongly suggest that treatment with REP 9AC will not result in the development of antiviral drug resistance. The compounds may also address the still uncertain role of virus spread in maintenance of chronic HBV infections as well as preventing the spread of antiviral drug-resistant HBV mutants in treated patients.

doi:10.1016/j.antiviral.2008.01.037

Oral Session 4: Retroviruses

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Characterization of a Novel Series of gp120 Inhibitors

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Despite recent advances in treatment of HIV infection, there remains a high need for new classes of antiretrovirals. Reports of virological and clinical efficacy of viral entry inhibitors suggest that this process may be an attractive point of intervention. A cell-based viral protein-mediated fusion assay was used to identify novel inhibitors of HIV entry. Subsequent assays were performed to identify the molecular target of the compounds, including gp120/CCR5 binding, gp120/CD4 binding and Biacore molecular interaction studies. Antiviral activity of compounds was assessed in a Phenosense™ assay (Monogram Biosciences) and in PBMC assays with primary isolates. The compounds were tested against a range of gp120 proteins containing mutations previously shown to confer resistance to small molecule gp120 inhibitors. Pharmacokinetic properties were assessed in rat and dog. A novel series of small molecule inhibitors of HIV entry was identified. The compounds inhibited the interaction of purified gp120 with soluble CD4 and were shown to bind to the gp120 protein. Antiviral activity was demonstrated against a range of clade B viruses, although activity against other clades was limited. The compounds were unable to inhibit fusion mediated by gp120 proteins containing mutations previously shown to confer resistance to gp120 inhibitors. Pre-clinical pharmacokinetic studies demonstrated favourable properties, including low protein binding, high oral bioavailability and low clearance. Our data indicate that the novel series of gp120 inhibitors we have identified is likely interacting with the viral protein in a similar way to previously described compounds. While our series may offer an attractive starting point for development of novel antiretrovirals, improvements in spectrum and resistance profile may be required prior to further progression.

doi:10.1016/j.antiviral.2008.01.038

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Novel Mechanism of Action of Pyrimidinediones Yields Enhanced Sensitivity to Multi-Drug Resistant Virus Strains

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A diverse series of 74 HEPT-like compounds with homocyclic substitutions at the N-1 of the pyrimidinedione have been evaluated in structure–activity relationship assays. Twelve congeners with subnanomolar efficacy and therapeutic indices greater than 1 million were evaluated to define their mechanism of anti-HIV action. The pyrimidinediones inhibit HIV-1 RT through direct interaction at the hydrophobic NNRTI-binding site. The compounds have no activity against HIV-2 RT and the selection of resistant HIV-1 results in typical NNRTI mutations with an accumulation of amino acid changes required to achieve high-level resistance. Unlike the NNRTIs, the pyrimidinediones possess a second mechanism of action which extends their range of action to include HIV-2 and results in inhibition of virus entry and/or maturation; this entry inhibition is more potent against clinical virus strains and prevents resistance mutations in the RT from accumulating to engender greater than 100-fold levels of resistance. Mechanistic assays with HIV-1 and HIV-2 suggest that the compounds act early to inhibit virus entry prior to fusion through interaction with a complex conformational

target which forms at 4 °C upon culture of cells and virus. Resistant viruses possess mutations in gp120 and gp41 consistent with the entry inhibition mechanism. However, the compounds do not block gp120-CD4 binding, chemokine receptor interactions, or the attachment of virus to target cells. They also do not inhibit SIV or SHIV-Env and do not inhibit entry or fusion in assays in which only isolated envelope glycoproteins and CD4 are involved (HL2/3 + MAGI cell fusion inhibition assay). Thus, inhibition of entry requires replication competent virus. Mutations in resistant viruses would suggest that gag and env proteins are included in the complex target recognized by the compounds. The contribution of gag proteins may also be responsible for the enhanced sensitivity of the pyrimidinediones (5–10-fold) to multi-drug resistant (MDR) viruses from experienced patients. The mechanistic results confirm that the pyrimidinediones represent a new and highly novel class of HIV inhibitors with an antiviral profile highly favorable for IND-directed investigation and clinical development.

doi:10.1016/j.antiviral.2008.01.039

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Development of the Dual-Acting Pyrimidinedione IQP0528 as a Vaginal Topical Anti-HIV Microbicide

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IQP0528 is a highly potent dual-acting HIV inhibitor targeting both reverse transcription and virus entry. The compound is non-toxic to all tested cell lines *in vitro*, including primary human cells, and the normal vaginal flora *Lactobacillus*. In standard *in vitro* assays, IQP0528 was active against all clinical strains of virus in the nanomolar to sub-nanomolar concentration range in PBMCs, dendritic cells and monocytes macrophages with therapeutic indices greater than one million. Equivalent or greater activity was observed when IQP0528 was evaluated in the presence of additives such as mucopolysaccharides or simulated vaginal and seminal fluids. The activity of the compound was not affected in cell-based entry assays mimicking the transition from low to neutral pH that occurs at the time of ejaculation. IQP0528 inhibited both cell-free and cell-associated virus transmission to CD4 expressing cells in virus transmission inhibition assays and was highly active in the microbicide transmission and sterilization assay (MTSA). IQP0528 was not active against several viral, bacterial or fungal STI-causing organisms. In preformulation studies, IQP0528 was soluble in a variety of solvents and was stable at ambient temperature and at pH's less than 8. Acute toxicology evaluations determined the compound to be non-toxic up to 1000 mg/(kg day) when dosed intravenously. Genotoxicology evaluations were all negative. IQP0528 is a novel candidate for a vaginal topical microbicide based on its dual mechanism of action, high level of potency, lack of toxicity, compatible formulation profile and toxicology profile. As a microbicide, IQP0528 would potentially inhibit two steps in virus replication which occur prior to reverse transcription and could be effectively used in combination with other microbicide products. IQP0528 is currently being formulated in an intravaginal ring for delivery of the compound.

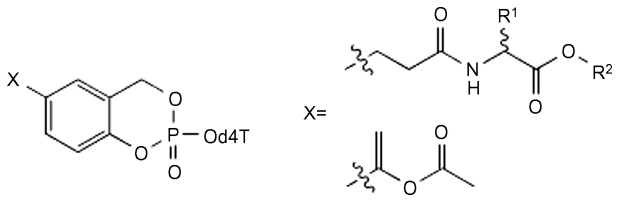

Fig. 1.

doi:10.1016/j.antiviral.2008.01.040

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Enzyme-triggered CycloSal-Pronucleotides

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CycloSal-nucleosylphosphate triesters are pronucleotides that deliver antiviral nucleotides into cells. The transport of these compounds across cellular membranes is basically achieved by passive diffusion, which is due to the high lipophilicity of the prodrug. As the release of the nucleotides is triggered by a pH-dependent hydrolysis cascade, cleavage may occur inside as well as outside cells. This unselective process may be influenced by attaching a trigger to the pronucleotide sensitive to esterases. The activated trigger is supposed to keep the prodrug inside the cell, leading to enrichment of the *cyclo*Sal-pronucleotide (trapping concept). To achieve this goal, different amino acid esters were linked to the aromatic ring of the *cyclo*Sal-masking unit via a carboxylic acid linker (see Fig. 1). In addition, enol esters were attached to the cycloSal-mask. In the presence of e.g. (carboxy)esterases, the aminoacyl or enol esters are transformed to the carboxylic acid or ketones, respectively, resulting in higher polarity, and thus decreasing ability of the prodrug to diffuse passively across membranes. In the case of the formed ketones, a fast hydrolysis reaction of the triesters released the nucleotide. The compounds were examined concerning their buffer stability, cell extract stability, cytotoxicity and antiviral activity in CEM/0 and TK-deficient cell lines.

doi:10.1016/j.antiviral.2008.01.041