Oral Session 6: Other Viruses, Veterinary Viruses and Late Breaker Presentations

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Use of Antivirals for Control of High Consequence Animal Diseases

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Design, Synthesis and Evaluation of 3-Ethynyl-Azole Nucleosides with Antiviral Activity Against Hantaviruses

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Hantaviruses are negative stranded RNA viruses that cause two acute febrile diseases in humans: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Currently there are no FDA approved drugs for the treatment of HFRS and HPS caused by hantaviruses. Clinical studies with HFRS patients have indicated improved prognosis from early treatment with the broad-spectrum antiviral drug ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), however, limited trials for HPS have not shown efficacy. We have explored chemical modifications of the 1,2,4-triazole scaffold in attempts to increase selectivity and activity for the viral L protein. We recently synthesized a new nucleoside analog with antiviral activity, 1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole (ETAR). ETAR showed an EC_{50} value of 10 and 4.4 mM for Hantaan virus (HTNV) and Andes virus, respectively. In order to define the structure–activity relationship and mechanism of action we designed, modeled and synthesized a series of isosteres, homologated analogs, and substituted derivatives that possess altered steric and hydrogen-bonding profiles. The antiviral activity of these compounds was evaluated in vitro against Hantaan virus and Andes virus. The potential for metabolic conversion of these compounds to the monophosphate by human adenosine kinase (hADK) was determined using an in vitro biochemical assay. Computational docking studies were used to characterize the binding of this series with hADK, and these results correlated with experimental values for hADK activity. These results provide a structural basis for the antiviral activity of this promising class of compounds against hantaviruses.

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A Derivate of the Antibiotic Doxorubicin Inhibits Dengue and Yellow Fever Virus Replication *In Vitro*

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Doxorubicin is an antineoplastic antibiotic obtained from *Streptomyces peucetius*. We report that doxorubicin exhibits *in vitro* antiviral activity against flaviviruses, i.e. the yellow fever virus (17D strain) ($EC_{50} < 0.78$ μg/ml) and dengue virus (type 2 NG strain) ($EC_{50} = 0.9$ μg/ml). However, doxorubicin proved also cytotoxic in the uninfected host cells and is thus not very selective as an antiviral agent. We identified a novel derivate of doxorubicin, SA-17 with excellent antiviral activity against DENV ($EC_{50} = 0.2$ μg/ml) and that was markedly less cytostatic than the parent compound. SA-17 also inhibited YFV-17D replication, although less efficiently than DENV replication. SA-17 proved inactive against viruses other than flaviviruses (bovine viral diarrhea virus, Coxsackie virus B3, HIV, and HSV-1: $EC_{50} > 100$ μg/ml). A dose-dependent anti-DENV activity was confirmed using a dengue reporter virus, i.e. infectious full-length dengue virus that expresses *Renilla* luciferase. Time-of-drug addition studies indicated that SA-17 acts at an early stage of the replication cycle. This hypothesis was confirmed in experiments using BHK cells harboring the DENV subgenomic replicon that only consists of the non-structural genes of the virus (NS1–NS5). SA-17 was unable to inhibit the replication of the replicon and thus, does not work at the level of the viral replication machinery. Further studies revealed that SA-17 exerts it activity *via* a virucidal effect, even when using very high titers of the virus as the inoculum. Likewise, doxorubicin was also shown to inhibit DENV replication by a virucidal mechanism, but the virucidal effect of the parent compound was less pronounced than that of SA-17. Further studies are ongoing to unravel the precise mechanism by which SA-17 exerts its virucidal effect on flaviviruses.

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One-third of the Surface of the Adenovirus Proteinase Contains Potential Drug Targets via a New Paradigm for Virion Maturation

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Late in adenovirus infection, inside young virions, the adenovirus proteinase (AVP) becomes activated by two viral cofactors enabling it to cleave virion precursor proteins thereby rendering the virus particle infectious. How this occurs reveals that more than one-third of the surface of AVP contains potential therapeutic targets. AVP is activated in part upon the binding

of pVIc, an 11-amino acid cofactor, via a series of contiguous structural changes occurring over a 54-amino acid long, bifurcated pathway. The other cofactor is the viral DNA. It has been a conundrum as to how 70 molecules of AVP-pVIc complexes can cleave multiple copies of six different virion precursor proteins at 3200 processing sites inside a nascent virion. Either the enzyme or its substrates must move, but these sequence independent DNA binding proteins cannot readily diffuse in three-dimensional space, because they remain bound to the highly concentrated (>500 g/L), tightly packed viral DNA. The conundrum may have been solved; AVP-pVIc complexes can slide along viral DNA via one-dimensional diffusion, thereby providing a way for AVP to locate and process the precursor proteins. AVP-pVIc complexes exhibited directionless sliding on viral DNA that could last more than one second and cover more than 20,000 base pairs via the largest one-dimensional diffusion coefficient observed for any protein moving along DNA, 21×10^6 bp^2/s. The ability of AVP via pVIc to exploit the DNA contour to guide it to its substrates may represent a new paradigm for virion maturation. Among potential therapeutic targets deduced from the activation mechanisms are: sites along the 54-amino acid long activation pathway, the DNA binding sites, the actin binding sites, the pVIc binding sites, as well as the active site. We determined crystal structures of these sites at high resolution (1.6 Å for AVP-pVIc and 0.98 Å for AVP). Structure-based drug design recently identified a compound predicted to bind to the pocket in which the N-terminus of pVIc binds and the active site; it has a K_i of 2.2 μM. Finally, because a number of sites on the enzyme interact with each other, a drug regimen may be designed that would prevent resistance to antiviral drugs from arising.

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Efficacy of T-1106 or T-705, Alone or in Combination with Ribavirin, in the Treatment of Hamsters Infected with Yellow Fever Virus

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Hamsters infected with an adapted Jimenez strain of yellow fever virus (YFV) have similar disease pathology to that seen in YFV-infected humans. The hamster model has been useful in the evaluation of antiviral compounds against YFV, including T-1106, which was shown to be effective in reducing disease parameters with a minimal effective dose between 10 and 32 mg/(kg d). The objective of the first study was to determine the efficacy of T-705, a fluorinated and non-ribosylated chemical similar to T-1106, in the treatment of YFV. Activity was observed in Vero cells with an EC90 of 418 ± 28 μM (SI > 9.6), which was lower than the EC90 for T-1106 of 677 μM (SI > 5.9). No significant improvement of disease parameters was seen with the oral administration 100 mg/(kg d) of T-705, although a trend towards improvement was observed. However, treatment of hamsters with 400 mg/(kg d) of T-705 was shown to be effective in significantly improving survival, serum ALT and AST levels, and weight change when treatment was started at 2 days post-virus inoculation (dpi). Significant improvement of survival was also seen with this dose of T-705, beginning as late as 3 dpi. The objective of the second study was to compare the activity of T-1106 and T-705 alone versus either of the two compounds in combination with ribavirin for the treatment of YFV disease. A synergistic effect was seen in cell culture when T-1106 or T-705 was combined with ribavirin. Treatment of hamsters with a combination of T-1106 or T-705 and ribavirin was superior to monotherapies. In summary, T-705 is efficacious in the treatment of YFV disease in hamsters, although a much higher dose (∼20-fold) is required as compared with T-1106. Superior activity was seen when T-1106 or T-705 was combined with ribavirin as compared with the administration of the monotherapies.

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Poster Session I: Retroviruses, Hepatitis Viruses, Respiratory Viruses, Emerging Viruses, and Antiviral Methods

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AlphaV Integrin-mediated Adhesion of Monocyte-derived Macrophages Influences HIV Infection

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Monocytes and macrophages are an important reservoir of human immunodeficiency virus (HIV) and may represent the largest reservoir of this virus in tissue. We have previously shown that an alphaV integrin blocking antibody inhibited HIV-1 infection in monocyte-derived macrophages (MDM), revealing an unexpected role of this integrin in HIV replication [Bosch et al., 2006. Antiviral Res.]. Integrins play a pivotal role in the interaction of cells with the extracellular matrix, with important implications for cell adhesion, migration and proliferation. To further characterize the role of alphaV integrin in HIV replication, MDM and HeLa-MAGI cells were infected using R5 or X4-tropic virus in the presence or not of a small heterocyclic non-peptide RGD mimetic (S36578-2) selective for avb3 and avb5 integrins. MDM are alphaV integrin positive cells. In MDM, the presence of S36578-2 inhibited HIV replication in a dose-dependent manner and in the absence of toxicity. Importantly, compounds from the same family showed an IC50 in correlation with in vitro measured affinity for avb3 and avb5, suggesting a strong specificity of its alphaV-dependent antiviral activity. Blockade of avb3 and avb5 integrins with S36578-2 also inhibited HIV replication in alphaV positive HeLa-MAGI cell line. In both cases, antiviral activity of S36578-2 is linked to a change in cellular morphology, thus giving further evidences of the integrin function's impairment. Supporting these data, S36578-2 antivi-