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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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-