141

Inhibition of Ebola Virus by S-Adenosylhomocysteine Hydrolase Inhibitors

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The filoviruses Ebola and Marburg cause the most severe viral hemorrhagic fevers with mortalities of 40-90% in sporadic human outbreaks, and can only be handled safely in maximum biological containment (BSL-4) facilities. Inhibitors of S-adenosylhomocysteine (AdoHcy) hydrolase inhibit Ebola virus replication *in vitro*, generally as predicted by their inhibition constants. AdoHcy is the product and feedback inhibitor of S-adenosyl-L-methionine (AdoMet)-dependent methyltransferases that play important roles in viral mRNA methylation. 3-Deazaadenosine (Cc3Ado) and 3-deazaneplanocin A (c3Nep) are potent *in vitro* inhibitors (EC_{50} 8 and 0.5 μg/ml respectively) of filovirus replication. Immune-deficient SCID mice infected with Ebola virus (Mayinga strain, 100 LD_{50}) die uniformly with a mean time to death of 27 days, but without hemorrhagic disease. Twice daily prophylactic administration to mice of Cc3Ado or c3Nep increased mean time to death, and suppressed virus replication both in circulation and in major organs. Studies comparing frequency of c3Nep dosing on lung titers at day 14 post infection demonstrated only a two $\log_{10}$ reduction with b.i.d. dosing compared to a 6.5 $\log_{10}$ reduction with t.i.d. dosing. The serum half-life in mice was 23 ± 3.2 min for Cc3Ado and12.8 ± 8.9 min for c3Nep. The more potent inhibitor c3Nep was evaluated first in a 100% lethal African green-Ebola primate model that closely mimics the clinical disease seen in man. Prophylactic t.i.d. dosing at the maximum tolerated dose (MTD) and 1/3 MTD beginning 24 hours prior to infection resulted in a significant increase in mean time to death in both groups (two fold with MTD/3) and reduction in the amount of viral antigen when compared to placebo. Later tissue distribution and metabolism studies in mice revealed that c3Nep, unlike Cc3Ado, is rapidly metabolized and c3Nep tissue levels were only 10% of Cc3Ado levels. Cc3Ado unlike c3Nep was > 80% bound to serum protein. The significant antiviral effect produced by c3Nep suggests that Cc3Ado should be investigated further in an immunocompetent guinea pig model.

142

Antiviral and Immunomodulating Activities of the Imidazoquinoline S-28463. L. M. Imbertson, J. M. Beaurline, J. F. Gerster, S. J. Gibson, V. L. Horton, R. L. Miller, P. E. Myhre, M. J. Reiter, C. B. Tamulinas, M. A. Tomai. 3M Pharmaceuticals, 3M Center, St. Paul, Minnesota 55144, USA.

Recently a new class of immunomodulating agents represented by the molecules imiquimod and R-842 has shown potent antiviral and antitumor activities in animal models. In this study another representative of this class, S-28463 (4-amino-2-ethoxymethyl-α,α-dimethyl-1*H*-imidazo[4,5-*c*]quinoline-1-ethanol) was evaluated for its antiviral and immunomodulating activities. S-28463 showed potent antiviral activity against Herpes Simplex virus-challenged guinea pigs when given topically, intravaginally or subcutaneously. Antiviral activity correlated well with the induction of serum 2'5' oligoadenylate synthetase activity. Doses of 0.03-0.3mg/kg sc given 24 hr before infection were completely protective. In addition, conditioned medium from S-28463 stimulated human peripheral blood cell cultures was effective at inhibiting Varicella zoster virus, Vesicular Stomatitis and Rhino virus infection but not Respiratory Syncytial virus infection of target cells. S-28463 (0.003 - 10mg/kg) was found to induce interferon-α (IFN), tumor necrosis factor (TNF), and interleukin-6 (IL-6) in mice upon oral administration. In rats, S-28463 induced increased serum levels of IFN and TNF. In addition, cynomolgus monkeys produced elevated levels of IFN, TNF, IL-1 receptor antagonist (IL-1RA) and IL-6 in response to S-28463. In human PBMC cultures, S-28463 at 0.03 - 10μg/ml induced increases in IFN-α, IL-1α, IL-1β, IL-1RA, IL-6, IL-8, IL-10, granulocyte macrophage colony stimulating factor (GMCSF), and granulocyte colony stimulating factor (G-CSF). Thus, S-28463 exhibits potent antiviral activity, which is mediated in part by the induction of cytokines such as IFN.