6

Effects of U-75875, A Peptidomimetic Inhibitor of Retroviral Protease, on Simian Immunodeficiency Virus (SIV) Infection in Rhesus Monkeys (*Macaca mulatta*). L. N. Martin[1], K. F. Soike[1], M. Murphey-Corb[1], R. P. Bohm[1], W. G. Tarpley[2], S. Thaisrivongs[2], T. Kakuk[2], T. J. Vidmar[2], and M. J. Ruwart[2]. Tulane Regional Primate Research Center[1], Covington, LA 70433, and Upjohn Laboratories[2], Kalamazoo, MI, 49001, USA.

U-75875 is a potent inhibitor of HIV-1, HIV-2 and SIV proteases, which blocks gag-pol protein processing, viral maturation and replication *in vitro*. Monkeys were treated with vehicle alone or with formulated U-75875 at 7 or 20 mg/Kg/day for 26 days by continuous intravenous (i.v.) infusion, beginning 6 hours prior to i.v. inoculation with 10 ID_{50} of SIV/Delta B670. The effects of antiviral treatment were assessed on SIV p26 antigenemia, serum infectious virus titer and level of proviral DNA in blood mononuclear cells evaluated by polymerase chain reaction. SIV infection of the control monkeys resulted in an initial viral antigenemia beginning on days 5 to 10 postinoculation (PI), reaching peak values on days 10-14, and lasting 15 to 22 or more days. Proviral DNA was detectable in PBMC by 7-11 days PI, reached the mean peak level by 11 days, and remained at high levels through day 24. Infectious virus was detected in serum from all of the controls by 24 days PI. Treatment with U-75875 for 26 days resulted in a dose-dependent delay in the day of peak antigenemia from a mean of 12.86 $\pm$ 1.95 days for the control group to a mean of 16.25 $\pm$ 3.37 days PI in the high dose treatment group. The level of proviral DNA in PBMC 11 days PI was significantly decreased in a dose-dependent fashion in the monkeys treated at 7 or 20 mg/Kg/day, and there was a delay in the attainment of the peak level of proviral DNA in these treated groups. The titer of infectious virus in the serum of the group treated with 20 mg/Kg/day was significantly decreased on day 24 PI, compared to controls. These results indicate an inhibitory effect of U-75875 on SIV infection in rhesus monkeys *in vivo*. (Supported in part by NIH, NO1-AI-62560 and The Upjohn Company).

7

Metabolism and *In Vitro* Anti-retroviral Activities of the bis(pivaloyloxymethyl) Prodrug of Acyclic 9-(2-Phosphonyl methoxyethyl) Adenine, R.V. Srinivas,[1] Y.-F. Gong,[1] B.L. Robbins,[1] M.C. Connelly,[1] N. Bischofberger,[2], A. Fridland,[1] Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN,[1] Gilead Sciences, Foster City, CA,[2] USA.

PMEA [9,2-phosphonylmethoxyethyl)adenine] is a novel antiviral agent active against several viruses including HIV and different herpesviruses. Due to its ionic properties PMEA has limited membrane permeability, which may limit oral bioavailability. Bis(pivaloyloxymethyl)-PMEA [bis(pom)PMEA], has been prepared as a potential membrane-permeable prodrug of PMEA. We have investigated the metabolism and anti-HIV activities of PMEA and bis(pom)-PMEA. Bis(pom)PMEA was found to be 10- to 20-fold more active than PMEA against different HIV-1 isolates, both in peripheral blood mononuclear cells and in cultured T-lymphocytic cell lines. Both PMEA and bis(pom)PMEA inhibited the synthesis of HIV proviral DNA, suggesting that the antiviral activities of these compounds may be mediated by their ability to inhibit HIV reverse transcriptase. Bis(pom)PMEA was readily taken up, and rapidly converted to PMEA and its active diphosphorylated metabolite PMEApp in the cells. A peak intracellular PMEA and PMEApp concentration of ~200 and ~80 pmols/10^6 cells respectively was obtained after 2 and 6 h incubation with 1 μM bis(pom)PMEA. In contrast, cells incubated with ten-times more PMEA (10 μM) showed peak intracellular PMEA and PMEApp concentrations of only ~15 and ~2 pmols/10^6 cells, respectively, after 4 h incubation thus suggesting a nearly 100-fold improvement in the cellular uptake of bis(pom)PMEA as compared to PMEA. Bis(pom)PMEA was unstable in the media and was broken down to mono(pom)PMEA which resembled PMEA in its metabolism and antiviral properties. These results show that bis(pom)-modified nucleoside analogs may be an effective approach to increase the bioavailability of free nucleoside and formation of active metabolites.