253

Inhibition of HIV-1 Infection of Macrophages and H9 Cells by Free or Liposome-Encapsulated L-689,502, an Inhibitor of the Viral Protease

E. Pretzer, D. Flasher and N. Düzgünes. Department of Microbiology, University of the Pacific, School of Dentistry, San Francisco, CA 94115, U.S.A.

The HIV-1 protease is responsible for cleavage of the gag-pol precursor protein at the time of budding. Since omission of this cleavage results in non-infectious virions, inhibitors of the viral protease have been investigated as therapeutic agents in HIV-1 infection. Liposome encapsulation may facilitate delivery of the inhibitor to target cells, decreasing side effects and the total dose required. We investigated the effects of L-689,502, a structure-based HIV protease inhibitor, on HIV-1 infection in human macrophages and in the human T cell line, H9. Macrophages were obtained from HIV-seronegative buffy coats by density gradient centrifugation and plastic adherence. Seven to 14 days after plating, the cells were infected with HIV-1_{BaL}, and then treated with the protease inhibitor. The free protease inhibitor was compared with inhibitor encapsulated in multilamellar vesicles (MLV) or in sterically stabilized liposomes with prolonged circulation. L-689,502 encapsulated in either type of liposome inhibited viral p24 production almost completely for periods of 7 to 18 days, if continuous treatment of macrophages with 100 nM inhibitor was started immediately after the two hour infection. Inhibition was dose-dependent, with no effect at 1-5 nM. Overnight treatment reduced infection to a lesser degree, although p24 levels remained below the control for up to 13 days. In both cases, the liposome-encapsulated inhibitor was more effective than the free drug, and MLV were slightly more effective than sterically stabilized liposomes. In H9 cells, continuous treatment with 10 nM free or 100 nM MLV-encapsulated inhibitor was able to block *de novo* infection with HIV-1_{IIIB} for up to 21 days (treatment was stopped after day 18). The inhibitor had much less effect on chronically infected H9/HTLV-IIIB cells. Our results indicate that liposomes enhanced the efficacy of L-689,502 in macrophages, and that the free inhibitor was more effective in H9 cells.

254

The Cyclic Congener of Cidofovir has Reduced Nephrotoxicity in Three Species.

M.J.M. Hitchcock[1], S.A. Lacy[1], J.R. Lindsey[2], and E.R. Kern[2], [1]Gilead Sciences, 353 Lakeside Drive, Foster City, CA 94404 and [2]University of Alabama at Birmingham, Birmingham, AL 35294, U.S.A.

Cidofovir (CDV), formerly HPMPC, is a potent antiviral agent with activity against a broad spectrum of viruses *in vitro* and *in vivo*. The dose-limiting toxicity in multiple animal species, including humans, is nephrotoxicity. The cyclic congener of CDV (cyclic-CDV) is equipotent to CDV in many *in vitro* and *in vivo* infection models, and acts as an intracellular prodrug for CDV. In rats (10 animals/dose group) dosed daily for 1 month with intravenous cyclic-CDV at doses of 2.5, 10 and 40 mg/kg, 10 mg/kg was not nephrotoxic and 40 mg/kg was nephrotoxic. By comparison, CDV in a similar study had no effect at 0.3 mg/kg and was nephrotoxic at 1 mg/kg. In cynomolgus monkeys (8 animals/dose group) dosed daily for 1 month with intravenous cyclic-CDV at doses of 0.5, 2.5 and 10 mg/kg, 0.5 mg/kg was a no-effect level and 2.5 mg/kg was nephrotoxic. By comparison, in a similar study with CDV, 0.1 mg/kg was a no-effect level and 0.25 mg/kg was nephrotoxic. Finally, a direct comparison study of the two agents was performed in guinea pigs, which are particularly sensitive to nephrotoxicity induced by CDV. Animals (3 per dose group) were dosed subcutaneously for 5 consecutive days and sacrificed on day 7. Nephrotoxicity average scores (out of 5) were 1.0, 2.0 and 5.0 for 5, 15 and 50 mg/kg of cyclic-CDV and 1.0, 2.7 and 3.3 for 0.5, 1.5 and 5 mg/kg of CDV, indicating that an equivalent dose of cyclic-CDV is less toxic than CDV and about ten-fold more cyclic-CDV is needed to cause an effect similar to CDV. Thus, cyclic-CDV in three species is about 10- to 20-fold less potent than CDV in inducing nephrotoxicity.