CYCLOTIDES ENHANCE THE ANTIVIRAL EFFECTS OF HIV-1 PROTEASE INHIBITORS

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The development of HIV-1 protease inhibitors (HPIs) in highly active antiretroviral therapy (HAART) has dramatically decreased AIDS mortality. However, HPIs exhibit suboptimal therapeutics in vivo due to their intrinsic low oral absorption and rapid efflux via ATP-binding cassette transporters. These attributes facilitate uninhibited viral propagation and rapid selection of drug resistant HIV-1 mutants and require administration of high HPI doses which often results in serious systemic side effects including cardiovascular dysfunctions and lipodystrophy. Considerable efforts to increase HPI bioavailability by developing drug-efflux inhibitors have had limited success. Therefore, we are investigating an innovative approach to enhance HPI bioavailability in acute and persistent HIV infection by capitalizing on the selective, dose-dependent, pore-forming properties of the plant cyclotide, cycloviolacin O2 from Viola odorata L. (Violaceae). Cyclotides, a relatively novel family of cyclic peptides, are isolated from plants in the Rubiaceae, Violaceae, and Curcurbitaceae families. Their knotted cystine structure imparts exceptional stability and resistance to thermal, chemical, and enzymatic degradation; features that enhance their therapeutic potential. Cyclotides display anti-HIV activity in vitro, and recent studies demonstrate one mechanism of bioactivity is the formation of multimeric pores with channel-like activity. In this study, we monitored CyO2-mediated cellular membrane disruption using SYTOX Green in uninfected (HuT78) and HIV-infected (HTLVIIIB) T-cell lymphocytes. CyO2 selectively disrupted HIV-infected membranes in a dose-dependent manner (0.5 - 5µM) and formed stable pores on lymphocytic cell membranes. Furthermore, we investigated the uptake of radio labeled (3H-) saquinavir, an HPI, in the presence of CyO2 and discovered CyO2 exposure enhanced the uptake of saquinavir in T-lymphocytes. Using HIV p24 Antigen ELISAs, we demonstrated that CyO2 significantly decreases viral load and combination regimens (i.e. nelfinavir + CyO2) improved antiviral efficacy in HIV-infected cells. Our findings indicate that CyO2, and possibly other cyclotides, can be used at sub-toxic concentrations (< 1uM) in order to chemosensitize HIV-infected lymphocytes to HPIs.

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