115

Expression and function of P-glycoprotein in HIV-1infected patients receiving protease inhibitor

JM Huraux[1], Ph Bossi[1,2], O Legrand[3], AM Faussat[3], F Bricaire[2], C Katlama[2], JP Marie[3], H Agut [1] & V Calvez[1]. Depts of Virology[1] & Infectious Diseases[2] Pitié-Salpêtrière Hospital & Hematology[3] Hôtel-Dieu, Paris-France.

The overexpression of the multidrug resistance (MDR-1) P-glycoprotein (PgP) has been implicated in multidrug resistance in human malignancies, and chloroquine-resistant Plasmodium falciparum infections. Recently, HIV-1 protease inhibitors (PI) have been described to be substrates of PgP. We searched for PgP overexpression in vivo in HIV-1 infected patients treated with a PI. We quantified the PgP expression and performed functional tests of PgP activity in samples of 18 HIV-1 infected patients. All patients were treated with a combination of reverse transcriptase inhibitors plus 1 or 2 PI (median duration: 33 months). HIV-1 RNA level was < 200 copies/mL in 10 patients and > 200 in 8 patients. Among the patients with virological failure, all had one or more resistance-mutations in the protease gene. Plasma protease concentrations were in the range of therapeutic values. The expression and function of PgP were tested on PBMCs with selected cells by UIC2, FITC, CD4 PE and CD14 PC5 antibodies. PgP expression was measured by flow cytometry and expressed as a continuous D value function using the Kolmorogov-Smirnov test. PgP was found to be positive in the CD4 cells of 6/18 patients (4 with and 2 without treatment failure). In these 6 patients, functional assays were performed by flow cytometry using rhodamine 123 as substrate, alone or in the presence of the PgP-modulator Cyclosporin A. PgP was found to be functional in the CD4 cells of all 6 patients. In conclusion, in HIV-1 infected patients treated with a combination containing a PI, the expression and function of PgP can be increased in patients with and without treatment failure.

116

Accumulation of HIV-1 Gag and Gag-Pol Processing Intermediates in the Presence of Non-peptidomimetic HIV-1 Protease Inhibitors.

R.R. SPECK*, X-F. YU, and C. FLEXNER.
The Johns Hopkins University, Baltimore, MD.

Introduction: Cross-resistance to HIV-1 protease inhibitors limits their utility in the long-term treatment of HIV-1. Non-peptidomimetic protease inhibitors may be less likely to promote cross-resistance with peptidomimetic inhibitors, and could become a useful new class of antiretroviral drugs. **Methods:** HIV-1 infected primary blood mononuclear (PBMCs) or transformed (H9) cells were incubated for three days with HIV-1 protease inhibitors in concentrations ranging from 0.03 to 500 times the IC_{50}. Viral proteins were separated by SDS-PAGE, transferred to nitrocellulose, and probed with either human anti-HIV-1 serum or murine HIV-1 reverse transcriptase (p51/p66)-specific monoclonal antibodies. **Results:** Treatment of HIV-1 infected cells with increasing concentrations of the non-peptidomimetic protease inhibitors: PNU-109112, PNU-140135, DMP-450, or DMP-323 resulted in the accumulation of Gag and Gag-Pol polyprotein processing intermediates. The specific intermediates that accumulated changed in a concentration-dependent manner. After correcting for differences in the IC_{50}'s for the drugs, the concentration-dependent accumulation observed in H9 cells was the same as that observed in PBMCs. The accumulation of intermediates was identical for all non-peptidomimetic drugs tested and was identical to processing intermediates produced by all previously tested peptidomimetic drugs. **Conclusions:** All competitive inhibitors of the HIV-1 protease that were tested, regardless of chemical structure, caused accumulation of the same Gag and Gag-Pol processing intermediates when concentrations were corrected for potency. This suggests a similar mechanism for inhibition of processing, and may suggest similar susceptibility to changes in enzyme secondary structure.