79

Characterization of HIV-1 Reverse Transcriptase Encoding Mutations at the Amino Acid Residues 161 and 208 Involved in Phosphonoformate Resistance

E. Tramontano[1], G. Piras[1], E. Congeddu[1], J. Mellors[2], H. Bazmi[2], P. La Colla[1]. *Dept. of [1]Biologia Sperimentale, Universita' di Cagliari, Italy. [2]University of Pittsburgh / VAMC, PA, USA.*

Mutations at the amino acid residues 161 (Gln to Leu) and 208 (His to Tyr) of reverse transcriptase (RT) have been identified to be involved in human immunodeficiency virus type 1 (HIV-1) resistance to phosphonoformate (PFA) (Mellors et al., this volume).

Highly purified recombinant RTs bearing either one or both these mutations were used in studing PFA susceptibility and enzyme kinetics. RT encoding both mutations (RT 161/208) showed the same dependence as the wild type enzyme (wt) for pH, KCl and $MgCl_2$, although it showed less preference for $MgCl_2$ than for $MnCl_2$.

Enzyme inhibition studies revealed that mutant RT 161/208 was about 4 fold less susceptible to PFA than wt RT (IC_{50} values were 1.45 μM and 0.37 μM, respectively). This result is consistent with cell culture data indicating that HIV-1 encoding both mutations is about 8 fold less susceptible to PFA. However, unlike PFA-resistant HIV-1, which is hypersusceptible to Nevirapine, TIBO R82150 and AZT, RT 161/208 was as susceptible to Nevirapine and TIBO R82150 as wt RT. In addition, RT 161/208 was 5 fold less susceptible to AZTTP compared with wt RT (K_I for AZTTP were 114 nM and 22 nM, respectively).

Interestingly, the K_m value for dTTP of RT 161/208 was about 3.5 fold higher than K_m of wt RT, suggesting a conformational change in the substrate binding site of the enzyme, leading to a decrease in the binding of dNTP and PFA.

Additional kinetic studies to verify whether the K_m for other substrates and the K_{cat} values are affected by these mutations, and to assess the effect of single mutations on the HIV-1 RT, will be reported.

This work was supported by ISS grant n. 9204-68.

80

In vitro selection of HIV-1 resistance to ddI is not as efficient as for other antiretroviral drugs and yields virus which shows reduced cytophatic activity.

F. Dianzani[1], G. Antonelli[2], D. Bellarosa, [1,3], F. Bambacioni[1], E. Riva,[1], A. Gentile[4], O. Turriziani[1];
1. Institute of Virology, University "La Sapienza", Rome; 2. Department of Biomedicine, University of Pisa, Pisa; 3. Menarini Ricerche Sud, Pomezia, Rome;4. Bristol Myers Squibb, Rome, Italy.

Mutants of HIV-1 resistant to most of the antiviral compounds have been isolated and characterized. This study, entirely conducted in vitro, was aimed to: i) verify whether different compounds (namely, AZT, TIBO R82913, ddI) could display different capability to select for drug resistance; and ii) to explore the effect of ddI on development of HIV-1 resistance to other drugs (namely, AZT and TIBO). Reduced sensitivity to all three compounds was obtained during serial passages in the presence of concentrations of the various inhibitors. However, the resistance to TIBO and AZT appeared earlier and was significantly higher (TIBO = 100 fold after 7 passages; AZT = 100 fold after 9 passages) when compared to the resistance to ddI (about 10 fold) which emerged only after 15 passages. Sequence analysis of the virus obtained in the different conditions reveals that AZT- , and TIBO- resistant strains contain some of the mutation associated to the resistant phenotype. In contrast, ddI-resistance mutations at codon 74, and 184 were not observed in the ddI-propagated virus. Interestingly, the kinetics of infection of the ddI resistant virus was different to those of the parental drug-sensitive and AZT-resistant virus. In fact preliminary results showed that this virus, although capable to induce a productive infection, display reduced cytophatic effect, probably through the development of a chronic infection.

Since ddI does not appear to be efficient in selecting for resistant mutants, its ability to prevent the appearance of AZT- and TIBO-resistant strains was evaluated. The results show that both AZT- and TIBO-resistance is less likely to occur when the compounds were used in combination with ddI. Specifically, while the virus passed in the presence of AZT or TIBO gradually acquired a significant level of resistance to the respective drug, the virus passed in the presence of AZT or TIBO plus ddI did not appear to acquire resistance. After 7 passages, ID 90 values were: AZT alone = 60.1+18 nM; AZT+ddI= 16.0+4.5 nM; TIBO alone= 650+30 nM ; TIBO + ddI= 30+7 nM). The latter finding could not be explained by the synergism of action of ddI with AZT or TIBO, which has been observed in our experimental conditions. Indeed, the combined concentration which displays the higher level of synergism (measured as Combination Index) is the less efficient in inducing the prevention of resistance development. Sequence analysis as well as biological characteristics of the virus selected during combined treatment are in progress. Taken togheter these findings demonstrates that ddI could be useful in combination therapy mainly because of its inability to induce ddI resistant strains.