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AN IN VITRO MODEL FOR DETERMINATION OF THE RATE OF RESISTANCE DEVELOPMENT IN HIV

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The development of resistance against different anti-HIV drugs is becoming a major problem in AIDS therapy. We have developed an in vitro system in order to determine the rate of resistance development against different compounds.

Virus growing in MT4 cells at the concentration of a compound giving 50 % of the RT activity of untreated infected cells was transferred to fresh cells without compound and with compound at 5-fold increasing concentrations. Cell free virus was transmitted once a week according to this scheme until virus with at least 100-fold decreased susceptibility to the compound emerged. The resistant virus was tested for cross resistance to other RT inhibitors and HIV-DNA was analysed for alterations in the RT gene (aa 1-220).

In this system no signs of high resistance (ED_{50} > 1 µM) was seen after 5 months in any of the infected cultures treated with nucleoside analog RT inhibitors like AZT or FLT or treated with the protease inhibitor U 75875.

In the presence of non-nucleoside inhibitors like 9-Cl-TIBO and L697,661 resistant virus could be isolated after 2 - 4 passages (2 - 4 weeks).

DNA sequence analysis showed aa changes in RT at positions 100, 103, 181, and 188 as single or multiple mutations after treatment with the non-nucleoside inhibitors.

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HIV REVERSE TRANSCRIPTASES WITH POINT MUTATIONS AS TOOLS FOR DRUG DEVELOPMENT.

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Mutants of human immunodeficiency virus (HIV) reverse transcriptase such as (RT), Tyr181 to Cys, Leu100 to Ile, Glu138 to Lys or Arg and Tyr188 to His, have been prepared and purified and used to characterize the effects of various inhibitors. As compared to wild type RT, the mutant RT's had lower K_{cat}/K_m values. The Km values were lower with heteropolymeric than with homopolymeric template-primers. Inhibition by phosphonoformate was non-competitive with both wild-type and mutant RT's and the Cys181 and Ile100 mutants were less sensitive to phosphonoformate than the wild type RT. The non-nucleoside RT inhibitors 9-Cl-TIBO and L-697,661 gave a non-competitive inhibition with respect to substrate of the wild type RT. Mutant RT's were inhibited at higher concentrations, showing a mixed type of inhibtion with respect to substrate. ddGTP caused a competitive inhibition of wild type and mutant RT's with respect to substrate. RT preparations with different mutations are useful in rapidly characterizing the interaction between various inhibitors and HIV RT and thus facilitate the development of new inhibitors.