Effect of efavirenz and nevirapine on HIV reverse transcriptase activity through a novel, PCR-based assay

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Amelioration of drug safety and emergence of drug resistance has greatly driven the process of new anti-HIV drug discovery, by targeting both novel and already identified viral objectives. Among these, renewed attention has been paid, to the identification of new molecules capable to act as reverse-transcriptase (RT) inhibitors. First generation methods for evaluating retrovirus RT activity, revealed to be complicated and not enough sensitive for screening potential, antiretroviral compounds. Thus, successive assays measured RT activity on the basis of its efficacy in generating cDNA and on PCR-based techniques. Previous studies from our laboratory demonstrated that a RT-PCR-based assay was applicable to the study of the inhibitory activity of compounds on HTLV-1 RT. Here we describe a novel, cell-free, RT-PCR-based assay specifically set up for evaluating the inhibitory activity of compounds towards HIV-RT. The assay utilizes RNase free, DNase- treated RNA from a stable transfectant cells expressing the HSV-1 envelope protein gD, as an RNA template. RNA template is reverse transcribed using US6 (gD gene) reverse primers in a reaction mix containing either commercial HIV-RT or viral lysate from H9 cells, as a source of RT, in the presence or in absence of the compounds to assay. cDNA products is then used for DNA PCR. In addition as source of RT viral lysate from HIV positive patients before and after therapy was used. Nevirapine and efavirenz were utilized as reference HIV-RT inhibitory compounds. A newly synthesized non-nucleoside, potential RT-inhibitory compound was also assayed. The sensitivity of our novel method was compared to that of commercial HIV reverse transcriptase assay. Results confirmed the suitability of our methods for the expected purpose, showing a higher sensitivity with respect to the commercial assay.