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# Different Properties of Wild Type and Drug-Resistant Mutants of Human Immunodeficiency Virus Type 1 Reverse Transcriptase *In Vitro*

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*Abstract*: **Drug-resistant mutants of human immunodeficiency virus type 1 (HIV-1) emerge during treatment with various reverse transcriptase (RT) inhibitors *in vitro* and *in vivo*. However, the virological nature and pathogenic importance of these mutants have not been fully elucidated. In this study, we have examined HIV-1 mutants resistant to 3′-azido-3′-deoxythymidine (AZT) and nonnucleoside RT inhibitors (NNRTIs) for their infectivity, RT activity, and replication in MT-4 cells. Although the infectivity of AZT- and NNRTI-resistant mutants was similar, the RT activity of AZT-resistant mutants was much higher than that of NNRTI-resistant mutants and their wild types. Furthermore, the RT activity of NNRTI-resistant mutants was significantly lower than that of the wild types. In contrast, the replication of NNRTI-resistant mutants was found to be greater than that of AZT-resistant mutants and the wild types. When HIV-1 proviral DNA (cDNA) synthesis was examined by PCR in MT-4 cells infected with the wild type, AZT-resistant mutant, or NNRTI-resistant mutant, the PCR signal of the NNRTI-resistant mutant was found to be much higher than those of the wild type and AZT-resistant mutant. These results suggest that the drug-resistant mutants differ from their corresponding wild types not only in drug sensitivity but also in other virological properties.**

*Key words*: **HIV-1, RT-resistant mutants, Infectivity, RT activity**

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of acquired immune deficiency syndrome (AIDS). Several nucleoside analogs, including 3′-azido-3′-deoxythymidine, 2′,3′-dideoxyinosine, 2′,3′-dideoxycytidine, and 2′,3′-didehydro-3′-deoxythymidine, have been licensed for clinical use in the treatment of patients suffering from AIDS or AIDS-related complex. These compounds are targeted at the HIV-1 reverse transcriptase (RT) after intracellular conversion to their 5′-triphosphate form (4, 6).

On the other hand, several compounds have been reported as nonnucleoside RT inhibitors (NNRTIs). These include tetrahydroimidazo-[4,5,1-jk][1,4]benzodiazepin-2(1*H*)-one and -thione derivatives (17), 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)-thymine (HEPT) derivatives (1, 3, 14), nevirapine (13), pyridinone derivatives (7), α-anilinophenylacetamide (α-APA) derivatives (17), and thiadiazol derivatives (8). These compounds act as highly potent and specific inhibitors of HIV-1 RT without requiring intracellular metabolism (5). Some of them are now under clinical evaluation.

The rapid emergence of drug-resistant mutants is a major concern in antiviral chemotherapy. In long-term treatment with AZT in AIDS patients, HIV-1 mutants with reduced sensitivity to this drug have frequently been isolated (9, 10). Viruses resistant to NNRTIs were also more easily isolated from patients treated with nevirapine (20). In accordance with this fact, drug-resistant

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*Abbreviations*: AIDS, acquired immune deficiency syndrome; α-APA, α-anilinophenylacetamide; AZT, 3′-azido-3′-deoxythymidine; $CCID_{50}$, 50% cell culture infectious dose; dGTP, 2′-deoxyguanosine-5′-triphosphate; $EC_{50}$, 50% effective concentration; E-EBU, 1-ethoxymethyl-5-ethyl-6-benzyluracil; EGTA, ethylene glycol bis (β-aminoethylether)-*N′, N′, N′, N′*-tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; HEPT, 1-[(2-hydroxyethoxy)-methyl]-6-(phenylthio)thymine; HIV-1, human immunodeficiency virus type 1; MOI, multiplicity of infection; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NNRTIs, nonnucleoside reverse transcriptase inhibitors; PCR, polymerase chain reaction; RT, reverse transcriptase.