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# Effects of HIV Q151M-associated multi-drug resistance mutations on the activities of (−)-β-D-1′,3′-dioxolan guanine

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**Abstract**

The multi-drug resistance HIV-1 genotype A62V/V75I/F77L/F116Y/Q151M is associated with resistance to many nucleoside reverse transcriptase inhibitors including AZT, ddI, ddC, d4T, abacavir, and 3TC. In this study, we evaluated the antiviral activity of (−)-β-D-1′,3′-dioxolan guanine (DXG) towards mutant HIV-1 containing V75I/F77L/F116Y/Q151M ($V75I_{complex}$) and A62V/V75I/F77L/F116Y/Q151M ($A62V_{complex}$) in MT-2 cells. We further investigated the mechanism of resistance by studying the incorporation of DXG 5′-triphosphate (DXG-TP) during DNA synthesis by mutant enzymes containing single mutations at Q151M or A62V, and the $V75I_{complex}$ and $A62V_{complex}$ using pre-steady state kinetic analysis. Our studies showed that mutant virus containing $V75I_{complex}$ and $A62V_{complex}$ were both more than 23-fold resistant to DXG, and this correlated with the 68- and 20-fold resistance changes observed in the enzymatic assay. Compared to the wild-type enzyme, DXG-TP was incorporated 39- and 21-fold less efficiently by the mutant enzyme containing $V75I_{complex}$ and $A62V_{complex}$, mainly due to decreases in the rate of incorporation. The A62V mutation significantly increased the rate of incorporation ($k_{pol}$) for both dGTP (3-fold) and DXG-TP (7.9-fold), while the binding affinity of A62V HIV-1 RT for DXG-TP was decreased 14-fold. At the enzyme level, the addition of the A62V mutation to V75I/F77L/F116Y/Q151M moderately (3.4-fold) reversed the resistance to DXG-TP.


*Keywords:* DAPD; Amdoxovir; DXG; Pre-steady state; HIV-1 reverse transcriptase; Multi-drug resistance; Q151M

## 1. Introduction

Ever since the outbreak the HIV epidemic over two decades ago, nucleoside reverse transcriptase inhibitors (NRTIs) have been key components of antiviral therapy. Once inside the cells, NRTIs are phosphorylated by cellular enzymes to their 5′-triphosphates in order to serve as alternative substrate inhibitors for HIV-1 reverse transcriptase (RT). These approved NRTIs are significantly diverse in their chemical structures, activation pathways and primary resistance profiles (Beach, 1998; Pillay et al., 2000; Stein and Moore, 2001). However, a constellation of A62V, V75I, F77L, F116Y, and Q151M has been shown to confer cross-resistance to most of the NRTIs. This set of mutation, called the Q151M complex or multi-drug resistance (MDR) mutations, was found in HIV-infected individuals receiving AZT plus ddI or ddC and other ddI-containing regiments (Kavlick et al., 1998; Schmit et al., 1996, 1998; Shafer et al., 1995; Shirasaka et al., 1993). MDR mutations resulted in high-level resistance to AZT, ddI, ddC, d4T, and abacavir (Deval et al., 2004; Kavlick et al.,

*Abbreviations:* AZT, 3′-azido-3′-deoxythymidine; $AZT^R$, AZT-resistant; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; d4T, 2′,3′-didehydro-3′-deoxythymidine; DAPD, (−)-β-D-2,6-diaminopurine dioxolane; DXG, (−)-β-D-1′,3′-dioxolane guanosine; D30/D45, DNA/DNA primer/template 30/45-mer; MP, 5′-monophosphate; MDR, multi-drug resistance; dNTP, 2′-deoxynucleoside 5′-triphosphate, a general term for the natural nucleoside 5′-triphosphates; ddNTP, 2′,3′-dideoxynucleoside 5′-triphosphate, a general term for the analog nucleoside 5′-triphosphates; RT, reverse transcriptase; 3TC, (−)β-L-2′,3′-dideoxy-3′-thiacytidine; TP, 5′-triphosphate; wt, wild-type

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1998; Pillay et al., 2000; Schmit et al., 1998; Shirasaka et al., 1995), low cross-resistance to 3TC (Deval et al., 2004; Garcia-Lerma et al., 1999; Schmit et al., 1998), and no resistance to tenofovir (Deval et al., 2004; McColl and Miller, 2003). The prevalence of the MDR mutations in treatment-experienced patients appears low (<2%), however, it is expected to rise in the future and could pose a serious treat to the efficacy of anti-HIV therapy (Pillay et al., 2000).

The emergence and the role for each individual mutation in the Q151M complex in viral resistance have been the focus of many studies (Iversen et al., 1996; Shafer et al., 1994; Shirasaka et al., 1995; Ueno et al., 1995). The Q151M complex develops in a sequential and cumulative pattern over the course of a 2–4-year observation period. Q151M is the first mutation to develop and it confers partial resistance to some of the nucleoside analogs tested (AZT, ddI, ddC and d4T). With time, mutations at position F116Y and F77L develop, followed by emergence of two other mutations A62V and V75I. The V75I, F77L and F116Y mutations have little or no effect on drug susceptibility by themselves, but their co-occurrence with Q151M results in high-level resistance to many NRTIs. The biochemical properties of RT carrying all or a subset of the Q151M complex have also been studied in detail. Steady state kinetic analyses by Ueno et al. demonstrated that the Q151M complex mutations (mainly Q151M) altered substrate recognition by the HIV RT and accounted for the resistance observed in vitro (Ueno and Mitsuya, 1997; Ueno et al., 1995). Pre-steady state kinetic studies by Deval et al. (2002) revealed that Q151M and Q151M complex confer resistance to 2′,3′-dideoxynucleotide analogs mainly through decreased rates of incorporation during DNA synthesis, while having no effect on pyrophosphorolytic and ATP-mediated excision of the incorporated ddNMP.

Amdoxovir ((−)-β-D-2,6-diaminopurine dioxolane, DAPD) (Fig. 1) is a selective inhibitor of HIV-1 replication in vitro. DAPD is deaminated by adenosine deaminase to the guanosine analog dioxolane guanine (DXG), which is subsequently phosphorylated to the corresponding 5′-triphosphate (DXG-TP) (Furman et al., 2001). DXG-TP is a potent alternative substrate inhibitor of HIV-1 reverse transcriptase (Furman et al., 2001). The two primary mutations associated with DXG-resistance are K65R and L74V, which have been studied in detail by recombinant HIV cell culture assays and enzyme kinetic studies (Bazmi et al., 2000; Gu et al., 1999; Jeffrey et al., 2003). A pre-steady state kinetic analysis showed that HIV-1 RT containing the Q151M mutation (alone or with the K65R or K103N) is 7–23-fold resistant to DXG-TP (Jeffrey et al., 2003). In the current study, we evaluated the antiviral activity of DXG against recombinant HIV containing the V75I/77L/116Y/151M ($V75I_{complex}$) and A62V/75I/77L/116Y/151M ($A62V_{complex}$) mutations. We also studied the incorporation of dGTP and DXG-TP by mutant HIV-1 RTs containing $V75I_{complex}$ and $A62V_{complex}$ using pre-steady state kinetic analysis.

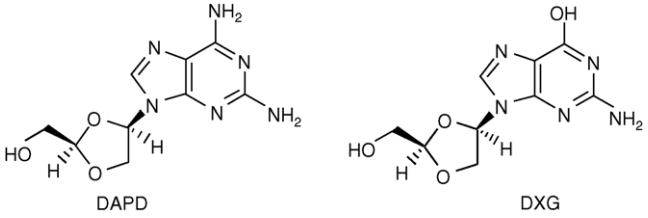


Fig. 1. Structure of DAPD and DXG.

## 2. Materials and methods

### 2.1. Construction, purification, and kinetic analysis

The wild-type RT gene construct p66RTB served as a template for directed mutagenesis using the QuikChange Kit (Stratagene, La Jolla, CA) to obtain the Q151M, $V75I_{complex}$, and $A62V_{complex}$ RTs. The recombinant RTs were co-expressed with HIV protease in *Escherichia coli* to get p66/p51 heterodimers and then purified by affinity chromatography as described (Boretto et al., 2001). All enzymes were quantified by active-site titration before biochemical studies.

### 2.2. Recombinant HIV-1 production and antiviral assay

The plasmid constructs described above were used as templates for the PCR amplification of the 1460-bp p66RTB fragment. The amplified RT genes were then cloned into a HIV-$1_{LAI}$ backbone (Shi and Mellors, 1997). The resulting recombinant proviral clones were electroporated into MT-2 cells for preparation of viral stocks as previously described (Mewshaw et al., 2002). Genotypic analysis of viral isolates was performed using di-deoxy sequencing on the ABI-377 system. Recombinant viruses were analyzed for phenotypic sensitivity to DXG, abacavir, ddI, AZT, D4T, and 3TC on MT2 cells using the XTT colorimetric assay (Weislow et al., 1989). MT2 cells were infected with either the mutant virus or wild-type HIV-$1_{LAI}$ at a multiplicity of infection of 0.03 in RPMI 1640 medium containing 10% fetal bovine serum, 20 μg of gentamicin/mL (Life Technologies), and 2 μg of polybrene/mL (Sigma) for 3 h at 37 °C. Following infection, cells were seeded into 96-well plates containing test compounds at $3 \times 10^4$ cells/well. Within each 96-well plate, test compounds were analyzed in triplicate at five-fold serial dilutions. The infected cells were cultured for 5 days in the presence of test compounds. On day 5, XTT {2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5[(phenylammino)carbonyl]-2*H*-tetrazolium hydroxide} was added, and the plates were incubated for 3 h at 37 °C and then analyzed for absorption ($A_{450}$). A dose-response curve for each individual compound was generated by using the absorption values of the uninfected cell controls as 100% protection and virus-infected cells not treated with drugs as 0% protection. From the dose-response curve, a 50% effective concentration ($EC_{50}$) was calculated and defined