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The effects of RNase H inhibitors and nevirapine on the susceptibility of HIV-1 to AZT and 3TC

Caroline A. Davis [a], Michael A. Parniak [b], Stephen H. Hughes [a,*]

[a] HIV Drug Resistance Program, National Cancer Institute at Frederick, Frederick, MD 21702-1201, USA
[b] University of Pittsburgh School of Medicine, Department of Microbiology and Molecular Genetics, Pittsburgh, PA 15219, USA

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ABSTRACT

It was recently proposed that HIV RT mutations that decrease RNase H activity increase zidovudine (AZT) resistance by delaying the degradation of the RNA template, allowing more time for AZTMP excision from the 3′ end of the viral DNA. This predicts that suboptimal concentrations of an RNase H Inhibitor (RNHI), which would decrease RNaseH activity, would decrease AZT susceptibility. Conversely, a suboptimal concentration of a nonnucleoside RT inhibitor (NNRTI) would decrease polymerase activity and increase AZT susceptibility. We determined the effect of several RNHIs and an NNRTI (nevirapine) on AZT and lamivudine (3TC) susceptibility with vectors that replicate using WT or AZT resistant RTs. Susceptibility to 3TC, which is not readily excised, did not change significantly. Nevirapine, and most RNHIs tested, had only small effects on the susceptibility of either HIV vector to AZT and 3TC. One RNHI, F0444-0019, increased the IC_{50} for AZT for either vector by ~5-fold, which may be a concern.

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Introduction

The reverse transcriptase (RT) of HIV is the enzyme that converts the single stranded viral RNA genome into the double stranded linear DNA that is subsequently integrated into the host genome. RT has two enzymatic activities that are essential for viral replication: a polymerase activity that can copy either an RNA or a DNA template and an RNase H activity that degrades RNA when it is part of an RNA/DNA duplex. RNase H degrades the RNA template during the synthesis of the first DNA strand, setting the stage for the synthesis of the second DNA strand. RNase H is also required for the two strand transfer events that are needed for the synthesis of the viral DNA and for the removal of the two RNA primers that are used to initiate minus and plus strand DNA synthesis. Due to its critical role in the HIV life cycle, RT is an important target for anti-HIV drugs. However, despite the fact that there are two essential enzymatic activities, all of the currently approved anti-RT drugs inhibit the polymerase activity. Although the RNase H activity of RT is an attractive target for the development of new anti-HIV drugs, no RNase H inhibitors (RNHI) have made it into clinical trials.

There are two classes of approved drugs that inhibit the polymerase activity of RT: nucleoside reverse transcriptase inhibitors (NRTI) and nonnucleoside reverse transcriptase inhibitors (NNRTI). NRTIs are dNTPs analogs that inhibit polymerization by being incorporated into the growing DNA strand. Unlike the natural dNTP substrates, all of the currently approved NRTIs lack a 3′ hydroxyl group; thus the incorporation of an NRTI blocks the extension of the viral DNA strand, a process called chain termination. Mutations in RT that confer resistance to NRTIs either increase the discrimination between the triphosphate form of the NRTI and the dNTPs during DNA synthesis, of which the best studied example is 3TC/FTC resistance caused by the M184V/I mutations (Gao et al., 2000; Sarafianos et al., 1999), or, as with most common AZT resistance mutations, increase the ATP-dependent excision of AZTMP from the end of the viral DNA (Arion et al., 1998; Boyer et al., 2001; Meyer et al., 1998, 1999). In contrast to NRTIs, NNRTIs bind to a site near to the polymerase active site, distorting the protein, and inhibiting the chemical step of polymerization (Andries et al., 2004; Ding et al., 1995; Kohlstaedt et al., 1992; Ren et al., 1995; Rittinger et al., 1995; Spence et al., 1995; Tantillo et al., 1994).

Resistance to NNRTIs usually involves mutations in or near the NNRTI-binding site, which interferes with the binding of the drugs in the NNRTI-binding pocket [see (Sarafianos et al., 2004) or (Sarafianos et al., 2009) for a review]. Although most of the primary mutations that give rise to NRTI and NNRTI resistance are relatively near the sites where the drugs bind, there are reports that mutations in the connection (CN) subdomain, which is not close to the binding sites for either NRTIs or NNRTIs, can enhance resistance to both NNRTIs and NRTIs (Brehm et al., 2007; Ehteshami et al., 2008; Gupta et al., 2010; Hachiya et al., 2008; Nikolenko et al., 2007, 2010; Yap et al., 2007; Zelina et al., 2008).

* Corresponding author at: HIV Drug Resistance Program, National Cancer Institute, NCI-Frederick, P.O. Box B, Building 539, Room 130A, Frederick, MD 21702-1201, USA. Fax: +1 301 846 6966.
E-mail address: hughesst@mail.nih.gov (S.H. Hughes).

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Modern highly active anti-HIV therapy (HAART) usually involves two different NRTIs and either an NNRTI or a protease inhibitor. While this combination therapy greatly decreases morbidity, long-term treatment often has undesirable side effects, and HIV can develop resistance to all of the available drugs. Given the emergence of drug resistant variants of HIV, it is important to continue to develop new inhibitors that would be effective against the existing resistant variants. Because drugs that inhibit the polymerase activity of RT are the backbone of most HAART therapies, it is important to consider whether a new class of inhibitor, such as the RNHIs, would be expected to enhance or diminish the effectiveness of any of the commonly used polymerase inhibitors, and whether a new inhibitor would be expected to have a positive or negative impact on resistance to the existing drugs.

Recent evidence suggests that HIV vectors that carry mutations that reduce the level of RNase H activity have a reduced susceptibility to AZT (Nikolenko et al., 2007); this result suggests that suboptimal concentrations of RNHIs could increase AZT resistance. Conversely, mutations that reduce the levels of the polymerase activity of RT are expected to increase susceptibility to AZT, which would suggest that a suboptimal concentration of an NNRTI should increase AZT susceptibility. However, we tested the NNRTI nevirapine (NVP) and found that suboptimal concentrations of NVP did not have a significant impact on the concentration of AZT required to inhibit HIV-1 replication by 50% (IC_{50}). Although a suboptimal concentration of most of the RNHIs we tested did not significantly affect the IC_{50} for AZT, the RNHI F0444-0019 did cause a significant decrease in AZT susceptibility (~5-fold), which is similar to the decrease in susceptibility to AZT seen when CN subdomain mutations were to added to NNRTI-resistant clinical isolates (Gupta et al., 2010) of HIV-1 and to the contribution to resistance made by CN subdomain mutations identified in clinical isolates (Lengruber et al., 2011).

Results

The addition of NVP has little effect on the IC_{50} for AZT for either WT or AZT-R vectors

We first added suboptimal amounts of the NNRTI NVP to cells that were subsequently infected with HIV to ask whether reducing the amount of polymerase activity would change the IC_{50} for AZT. The vectors used in these experiments either replicated using WT RT, which has a modest ability to excise AZTMP, or an excision proficient AZT-R RT (see Methods) (Boyer et al., 2001). The HIV vectors lack a functional Env coding region and were complemented with VSV-G, which limits the infections to a single cycle. The HIV vectors express a luciferase gene from the Nef reading frame; luciferase activity was used to measure ability of the virus to infect the cells. With these vectors, high level expression of luciferase requires that viral DNA is reverse transcribed and integrated into the host genome. Before studying the effect of NVP on AZT resistance, NVP was tested for its ability to block the replication of the vectors. The WT or AZT-R HIV vector was used to infect HOS cells in the presence of increasing concentrations of NVP. Supplemental Fig. 1 shows that NVP blocks HIV replication and has an IC_{50} of 36 ± 17 nM for a one-round vector expressing WT RT. The IC_{50} of NVP for blocking replication of the AZT-R HIV vector was 27 ± 1 nM (Supplemental Fig. 1). In subsequent drug combination assays, 40 nM NVP was used to reduce the infectivity of the virus stock by ~60%.

Using the same luciferase-based infectivity assay, the IC_{50} of AZT was measured in the presence and absence of 40 nM NVP to determine whether a decrease in polymerase activity would affect AZT resistance. The IC_{50} of AZT for WT HIV was 4 ± 3 nM in the absence of NVP and 3 ± 1 nM in the presence of 40 nM NVP, a negligible difference (Fig. 1 and Table 1). As shown in Fig. 1 and Table 1, using AZT-R HIV, the IC_{50} for AZT was 41 ± 38 nM and the IC_{50} was reduced to 21 ± 11 nM in the presence of 40 nM NVP, a difference of only 2 fold. Although the absolute value of the IC_{50} for AZT did show some drift over a period of several months, and this drift was seen in independent experiments performed by several different people in the laboratory (which accounts for the large standard deviations shown here), the fold change in AZT IC_{50} caused by the addition of either NVP or an RNHI (see below) was relatively constant.

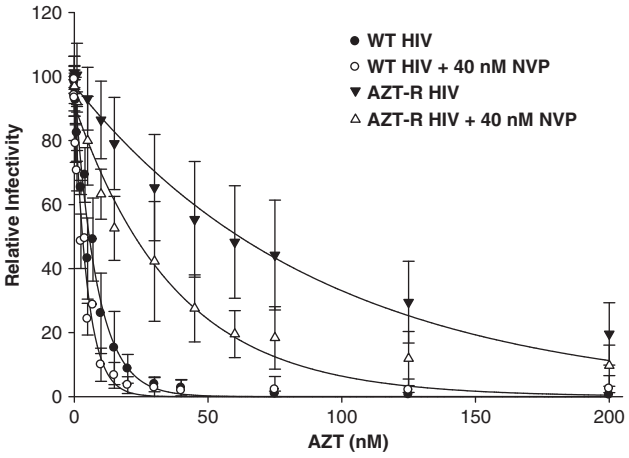

Fig. 1. Cell based luciferase assay measuring the effect of 40 nM NVP on the IC_{50} of AZT in HOS cells infected with WT or AZT-R HIV. The average IC_{50} values ± SD can be found in Table 1. Assays were performed 7 times with WT HIV and 5 times with AZT-R HIV.

Lamivudine (2′, 3′-dideoxy-3′-thiacytidine, commonly called 3TC), was used as a control in these experiments because it is not readily excised by RT (Boyer et al., 2001; Naeger et al., 2002). Because 3TC is not efficiently excised by either WT or AZT-R HIV, the addition of NVP would be expected to have a more modest effect on the IC_{50} for 3TC than the IC_{50} for AZT. Data presented here shows that the presence of NVP slightly lowered the 3TC IC_{50} for either WT or AZT-R HIV (~2-fold reduction for both, see Table 1 and Fig. 2), but the differences were not statistically significant. Thus, inhibiting the polymerase by the amount needed to reduce the infectivity by ~60% does not appear to have a large or specific effect on the susceptibility of the vectors that replicate using WT or the AZT-R RT to either 3TC or AZT.

Decreased RNase H activity may decrease AZT susceptibility

We next studied the effects of RNHIs on AZT susceptibility. To try to minimize the possibility that an effect on AZT susceptibility was due to an ancillary effect of the RNHI and not to the effect of the compound on RNase H activity, we tested several different RNHIs. The RNHI used in these experiments are shown in Fig. 3. All of these RNHIs have been tested with recombinant HIV-1 RT in vitro and have been shown to inhibit the RNase H activity, and are either less potent or inactive against the polymerase activity of RT (Table 2).

Because cytotoxicity can affect HIV replication and drug susceptibility, the RNHIs were tested for cytotoxicity in HOS cells using both XTT and ATP based assays (see Methods). XTT, a tetrazolium

Table 1
The Effect of NVP on the IC_{50} of AZT and 3TC in HOS Cells infected with WT and AZT-R HIV-1.

Virus	AZT IC_{50} (nM ± SD)		Fold decrease[a]	3TC IC_{50} (μM ± SD)		Fold decrease[a]
	−NVP	+NVP		−NVP	+NVP	
WT	4 ± 3	3 ± 1	1	8 ± 3	4 ± 1	2
AZT-R	41 ± 38	21 ± 11	2	24 ± 14	15 ± 3	~2

[a] Not statistically significant ($p > 0.05$).