Nucleoside reverse transcriptase inhibitors and HIV mutagenesis

Nancy A. Jewell1, Renxiang Chen2, Raquel Raices3 and Louis M. Mansky1-4*

1Molecular, Cellular and Developmental Biology Graduate Program, Ohio State University, Columbus, OH 43210; 2Ohio State Biochemistry Graduate Program, Ohio State University, Columbus, OH 43210; 3Integrated Biomedical Science Graduate Program, Ohio State University, Columbus, OH 43210; 4Department of Molecular Virology, Immunology, and Medical Genetics, Center for Retrovirus Research, and Comprehensive Cancer Center Ohio State University Medical Center, Columbus, OH 43210, USA

Keywords: NRTIs, resistance, evolution, mutagenesis, retrovirus

Introduction

Potent antiretroviral therapy (ART) of HIV-1 infection with antiretroviral drugs consisting of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) has dramatically reduced the rate of HIV- and AIDS-related morbidity and mortality. The lack of patient compliance to drug administration results in suboptimal therapy. Suboptimal drug therapy can lead to drug resistance, which limits the clinical benefit of drug treatment and can select for new variant viruses with altered virulence and tropism. In this leading article, we discuss literature that shows a correlation between the evolution of drug resistance and increased HIV and other pathogen mutation rates. In the case of HIV, NRTIs and NRTI drug resistance can increase HIV mutation rates, and can act together to further increase these rates. These increased mutation rates predict that drug failure during initial treatment could increase the probability of subsequent drug therapy failures due to the selection of mutator strains. However, increased mutagenesis of HIV by NRTIs could be viewed as an advantage in therapies directed at extinguishing virus infectivity by lethal mutagenesis.

NRTIs, drug resistance and HIV mutagenesis

The impact of NRTIs on HIV-1 mutation rates was first studied by testing how 3′-azido-3′-deoxythymidine (zidovudine; AZT) and (–)2′,3′-dideoxy-3′-thiacytidine (lamivudine; 3TC) (Figure 1), as well as AZT- and 3TC-resistance conferring mutations, influence the HIV-1 mutation rate.1 These analyses used the lacZα peptide gene as a mutation target, which has been used in previous mutation rate studies of HIV-1. AZT increased the HIV-1 mutation rate by 7.6-fold in a single round of replication, while 3TC increased the virus mutation rate by 3.4-fold (Table 1). AZT-resistant reverse transcriptase (RT) was also found to influence the mutation rate. In particular, HIV-1 replication with AZT-resistant RTs increased the mutation rate by as much as 4.3-fold, while replication of HIV-1 with a 3TC-resistant RT had no significant effect on the mutation rate. It was observed that only high-level, AZT-resistant RT variants could influence the in vivo mutation rate (i.e. those containing the mutations M41L/T215Y and M41L/D67N/K70R/T215Y).

Further studies of drug-resistant RTs have indicated that other amino acid residues in HIV RT associated with drug resistance can increase virus mutant frequencies when mutated. One example is the Y501F RT mutant, which leads to a four-fold increase in virus mutant frequencies.2 The Y501 residue is located in the RNaseH primer grip region of HIV RT and is associated with resistance to N-(4-tert-butylbenzoyl)-2-hydroxynaphthaldehyde hydrazone (BBNH), a potent inhibitor of RNaseH activity.

Recent studies with other NRTIs [didanosine (ddl), stavudine (d4T) and abacavir (ABC); Figure 1] indicate that NRTI drug treatment may generally lead to increased virus mutant frequencies during HIV-1 replication (R. Chen and L. M. Mansky, unpublished observations).3 The way in which NRTIs increase HIV-1 mutant frequencies may involve a similar mechanism, since it has been observed that virus mutant frequencies increase in an additive manner during virus replication in the presence of two NRTIs (e.g. AZT and 3TC, AZT and d4l, and 3TC and d4l).3 Hypotheses proposed to explain how NRTIs influence mutation rates include: (i) NRTIs alter nucleotide pools; (ii) NTRIs are incorporated into plus-strand DNA and may result in discontinuous DNA synthesis of viral DNAs with proper ends that integrate with subsequent error-prone repair by the host cell; and (iii) NRTIs may bind non-catalytically to RT and cause a conformational change that influences enzyme fidelity.4

These observations suggest that when virus replication occurs in the presence of suboptimal concentrations of drug, drug-resistant virus is selected for, and that replication of drug-resistant virus in the presence of drug could further increase the virus mutation rate. To test this hypothesis, the combined effects of drug and drug-resistant virus were analysed.3 It was found that the replication of AZT-resistant HIV-1 in the presence of AZT led to a multiplicative 24-fold increase in the virus mutant frequency compared with that observed with...
Leading article

This indicates that when drug failure occurs due to the evolution of drug resistance, replication of the drug-resistant virus in the presence of AZT significantly increases HIV-1 mutagenesis. In addition, it was observed that replication of an AZT/3TC dual-resistant virus in the presence of AZT and 3TC also led to a multiplicative 22.5-fold increase in mutant frequencies (Table 1). Thus, each of these drugs tested acted together with drug-resistant RT and increased virus mutant frequencies.

**Salvage therapy and increased HIV mutagenesis**

When HIV-infected individuals fail potent ART owing to the development of drug resistance, salvage therapy is necessary. Drug resistance commonly results from a lack of medication compliance or drug discontinuation. Therapy failure can be associated with poor drug tolerability, persistence of virus in immunological privileged sites, lack of drug potency and low drug plasma levels.

---

**Table 1.** Drugs and drug-resistant RTs that have been shown to increase HIV-1 mutant frequencies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug-resistant RT</th>
<th>Mutant frequency increase (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT</td>
<td>wt RT</td>
<td>7.6</td>
</tr>
<tr>
<td>3TC</td>
<td>wt RT</td>
<td>3.4</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>wt RT</td>
<td>4.5</td>
</tr>
<tr>
<td>Thymidine</td>
<td>wt RT</td>
<td>7.0</td>
</tr>
<tr>
<td>Thio guanine</td>
<td>wt RT</td>
<td>4.0</td>
</tr>
<tr>
<td>ddI</td>
<td>wt RT</td>
<td>6.0</td>
</tr>
<tr>
<td>–</td>
<td>AZT-resistant RT</td>
<td>4.3</td>
</tr>
<tr>
<td>–</td>
<td>3TC-resistant RT</td>
<td>1.0</td>
</tr>
<tr>
<td>AZT</td>
<td>AZT-resistant RT</td>
<td>24.0</td>
</tr>
<tr>
<td>3TC</td>
<td>AZT-resistant RT</td>
<td>13.6</td>
</tr>
<tr>
<td>AZT/3TC</td>
<td>AZT/3TC-dual resistant RT</td>
<td>22.5</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>AZT-resistant RT</td>
<td>21.8</td>
</tr>
<tr>
<td>Thymidine</td>
<td>AZT-resistant RT</td>
<td>16.7</td>
</tr>
</tbody>
</table>

wt, wild-type.
Interestingly, different drugs used in conjunction with the AZT-resistant virus led to a similar multiplicative increase in virus mutant frequencies. This indicates that when new drugs are added in drug therapy regimens, they could also act with the drug-resistant virus to further increase virus mutant frequencies, even though the drug-resistance phenotype is associated with another drug. For example, 3TC increased mutant frequencies of AZT-resistant virus to 13.6-fold compared with that of wild-type virus in the absence of drug (Table 1). Hydroxyurea, a well-documented drug used in HIV-1 treatment, is known to alter intracellular dNTP pools by inhibiting ribonucleotide reductase, and results in a depletion of all dNTPs. AZT-resistant HIV-1 replication in the presence of hydroxyurea resulted in a 21.8-fold increase in mutant frequencies compared with that observed in the absence of drug (Table 1). Like hydroxyurea, thymidine has also been shown to alter intracellular dNTP pools and in addition has been shown to increase retrovirus mutation rates. AZT-resistant HIV-1 replication in the presence of thymidine increased mutant frequencies by 16.7-fold (Table 1). Thioguanine (an anti-leukaemic agent that has been reported to inhibit RNaseH activity) has been shown to increase HIV-1 mutant frequencies by four-fold and to significantly alter mutant frequencies during virus replication with RTs containing mutations not associated with the drug.2 These data suggest that subsequent therapies could lead to increased HIV-1 mutagenesis even though the drug-resistant phenotype is not directed against the new drug(s) used in the drug therapy regimen.

**Therapeutic application of increased HIV-1 mutagenesis by NRTIs**

The data discussed above predict that when drug failure occurs during NRTI HIV-1 chemotherapy, there is an increased likelihood of further resistance evolving from subsequent drug regimens. As described below for antibiotic resistance with bacteria, mutators could lead to a shift in drug resistance from low-level to high-level resistance, and extend the spectrum of resistance. In addition, mutators could rapidly accumulate compensatory mutations, which increase the replicative capacity of the virus. In both instances, mutators can be considered to be a threat to successful drug therapy. Assays for mutator strains could potentially be used as an indicator for assessing the likelihood of therapeutic failure. Such strategies need to be tested for their utility.

An intentional increase in mutation rate has been speculated as a rational approach for antiviral treatment of RNA virus infections.3 RNA viruses have high mutation rates and are particularly vulnerable to increases in mutation rate that could extinguish virus replication by error catastrophe. There are few nucleoside analogues that are clinically effective in blocking RNA virus replication. Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a ribonucleoside analogue, has antiviral activity that can be mutagenic in some cases, but not in others.7

Promutagenic nucleoside analogues, which are incorporated into the viral genome during nucleic acid replication and result in a progressive accumulation of mutations that would ultimately lead to a drastic reduction in virus replication and fitness, have been used to extinguish HIV-1 replication.8 Given that the majority of mutations are deleterious, selection against such variants would reduce virus yield within a single cycle of replication and allow the maintenance of some significant level of virus fitness within the population. Based upon the analysis of HIV-1 mutation rates, an ∼30-fold increase in mutation rate would be necessary to extinguish infectious virus replication.9 The success of eliminating HIV-1 replication by this approach (called lethal mutagenesis, or error catastrophe), has yet to be tested clinically. Systematic analysis of NRTIs could identify NRTI combinations that increase HIV mutagenesis and enhance the likelihood of lethal mutagenesis.

**Antimicrobial drug resistance and increased pathogen mutation rates**

There is a growing body of literature indicating that mutator alleles are selected for in microbial populations, particularly in response to environmental stress. For instance, the emergence of antimicrobial resistance during drug therapy can increase the likelihood of selection for mutator alleles, as well as increase the probability of failure of subsequent drug therapies.10 The generation of drug resistance depends on the rate of emergence of resistant mutants, which is defined by the mutation rate. In bacteria, there are many examples indicating that antibiotic treatment not only selects for antibiotic-resistant bacteria, but also selects for mutator alleles that confer a higher mutation rate11 (Table 2). Correlations between mutation rate and the efficacy of antimicrobial drug treatment have recently been observed.12 Error-prone polymerases and mutations of the mismatch repair system, along with mutations of enzymes that protect DNA from DNA damaging agents and enzymes that degrade modified nucleotides, have been implicated as the mechanisms ultimately responsible for these mutator phenotypes (Table 3). A recent study of *Mycobacterium tuberculosis* has identified a DNA polymerase, DNA polymerase E2, which is upregulated when *M. tuberculosis* is exposed to DNA damaging agents (i.e. UV irradiation, mitomycin C and hydrogen peroxide).13 An *M. tuberculosis* DnaE2 mutant was severely attenuated for long-term murine infections, suggesting that...
continued repair of DNA damage during infection is essential for survival. An implication of this work is that DnaE2 contributes to the mutation rate of human immunodeficiency virus type 1. Journal of Virology 95, 9253–9.

Conclusions

The evidence discussed here reveals that increased HIV-1 mutation rates can be associated with the evolution of drug resistance. Such observations correlate with observations made in bacterial systems with antimicrobial drug resistance. The current management of HIV-1 infection involves combinations of NRTIs, NNRTIs and PIs that are changed over time when drug resistance occurs. The transmission of drug-resistant HIV-1, along with the development of drug-resistant virus, raises concerns about the efficacy of drug regimens, because of the presence of mutator phenotypes. Future studies should be directed at determining the risk of these mutator phenotypes with the potential for more rapid development of HIV-1 drug resistance to NRTIs. In addition, the unintentional increase in HIV mutagenesis by NRTIs could be used to improve the efficacy of drug therapy by the rational selection of NRTI combinations that either minimize the potential for HIV mutagenesis or intentionally increase HIV mutagenesis to induce (perhaps along with a mutagen) lethal mutagenesis.

Acknowledgements

Research in our laboratory is supported by the American Cancer Society (RPG0027801), the Campbell Foundation and the NIH (GM56615 and AI053155).

References


