Review article

Interactions between drug resistance mutations in human immunodeficiency virus type 1 reverse transcriptase

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Introduction

It has become apparent from recent studies that human immunodeficiency virus type 1 (HIV-1) has an enormous capacity to mutate in order to become resistant to antiretroviral agents (for a review see Larder, 1993). Monotherapy with nucleoside analogues or non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) has, without exception, resulted in the emergence of drug-resistant virus. This may take only a matter of weeks with NNRTIs (Richman et al., 1993; Saag et al., 1993), or months to years in the case of the most studied nucleosides 3'-azido-3'-deoxythymidine (AZT, zidovudine), 2',3'-dideoxyinosine (ddI) and 2',3'-dideoxycytidine (ddC; Boucher et al., 1990; Fitzgibbon et al., 1992; Japour et al., 1991; Land et al., 1990; Larder et al., 1989; McLeod et al., 1992; Richman et al., 1990; Rooke et al., 1989; St. Clair et al., 1991). Genetic characterization of these mutant strains has revealed that specific mutations in the RT coding region are responsible for the observed resistance (Fitzgibbon et al., 1992; Kellam et al., 1992; Larder & Kemp, 1989; Richman et al., 1993; Saag et al., 1993; St. Clair et al., 1991). In this review, the interactions occurring between these mutations will be highlighted in relation to the potential for the development of multi-drug resistance. Strategies for the therapeutic use of multiple RT inhibitors suggested by these observations will also be discussed.

Interactions between AZT resistance mutations

Of the licensed HIV-1 RT inhibitors, AZT has been in clinical use for the longest time (for a review see Wilde & Langtry, 1993). Consequently, many combination therapy trials have been designed where additional antiretroviral drugs are used with AZT. Since this is unlikely to alter significantly in the foreseeable future, it is appropriate to outline our current knowledge regarding AZT resistance mutations and their interaction.

Comparative DNA sequence analysis of the RT coding region derived from AZT-sensitive and -resistant strains has led to the identification of five specific amino acid substitutions that influence AZT susceptibility (at codons 41, 67, 70, 215 and 219; Kellam et al., 1992; Larder & Kemp, 1989; Larder et al., 1991). Detailed study of sequential HIV-1 isolates from a treated asymptomatic cohort has indicated that the accumulation of these mutations is not random but follows an ordered pattern (Fig. 1; Boucher et al., 1992; Kellam et al., 1994). The first mutation to appear is the replacement of a lysine by an arginine at codon 70 (abbreviated to K70R, where K and R are the one-letter codes for the amino acids lysine and arginine respectively). This is followed by a codon 215 mutation (usually T215Y). However, the K70R population seems to decline with the simultaneous appearance of the M41L mutation. Genetic linkage of the codon 41 and 215 mutations is a landmark in the development of significantly resistant strains. Subsequently, further therapy can result in the emergence of highly resistant virus strains with four or five of the resistance mutations.

To confirm the importance of the five mutations in AZT resistance, a large panel of HIV-1 variants have been constructed with defined combinations of these mutations in the RT gene (Kellam et al., 1992, 1994; Larder & Kemp, 1989; Richman et al., 1993; Saag et al., 1993; St. Clair et al., 1991). In this review, the interactions occurring between these mutations will be highlighted in relation to the potential for the development of multi-drug resistance. Strategies for the therapeutic use of multiple RT inhibitors suggested by these observations will also be discussed.
**Suppression of AZT resistance by other drug resistance mutations**

In view of the interactions observed between AZT resistance mutations, it might be anticipated that mutations in RT specific to other anti-retroviral agents could influence AZT sensitivity. Indeed, suppression of phenotypic AZT resistance due to the induction of other mutations in RT has been described and will now be considered.

The first example of AZT resistance suppression was seen during the analysis of ddI resistance (St. Clair et al., 1991). Individuals who had received prolonged AZT therapy and were subsequently switched to ddI were chosen for study. Sequential HIV-1 isolates from these individuals became progressively less resistant to AZT whilst simultaneously acquiring ddI resistance. DNA sequencing revealed that a new mutation, L74V, was induced during ddI therapy but, unexpectedly, pre-existing AZT resistance mutations did not necessarily revert to wild-type (St. Clair et al., 1991). In fact T215Y specifically persisted in the presence of L74V. Subsequent analysis of mutant virus created by site-directed mutagenesis confirmed that L74V alone conferred ddI resistance but when present with T215Y it completely suppressed AZT resistance (St. Clair et al., 1991). It should be noted that in this initial study additional AZT resistance mutations, namely M41L and K70R, did not persist after the L74V ddI resistance mutation emerged.

Similar observations of AZT resistance suppression have been made during *in vitro* resistance studies with NNRTIs. In experiments where pre-existing AZT resistant virus was exposed in cell culture to escalating concentrations of (+)-(S)-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo-[4,5,1-jk]-1,4-benzodiazepine-2(1H)-thione (chloro-TIBO) the virus rapidly acquired chloro-TIBO resistance, primarily due to the RT mutation Y181C (Larder, 1992). An associated decrease in AZT resistance was noted. Construction of HIV-1 variants containing defined combinations of AZT resistance mutations (including M41L plus T215Y) with Y181C confirmed that this NNRTI resistance mutation was responsible for the suppression of AZT resistance observed in the selection experiments (Larder, 1992). More recent site-directed mutagenesis studies in which different NNRTI resistance mutations were combined with AZT resistance mutations confirmed this effect with Y181C and also identified a further mutation, L100I, which has similar suppression properties (Byrnes et al., 1994).

In *in vitro* drug resistance studies have revealed yet another RT mutation, M184V, that suppresses AZT resistance. This mutation occurs in response to selective pressure exerted by a new generation of nucleoside

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**Fig. 1.** A model to illustrate the order of appearance of AZT resistance mutations in HIV-1 RT during therapy of asymptomatic individuals. The first mutation to appear is at codon 70, after which viruses with mutations at codons 41 and 215 also emerge and co-circulate. With further treatment, virus containing only the codon 70 mutation disappears and the dominant form of AZT-resistant virus has the 41 and 215 mutations linked on the same genome. Subsequently, high level resistant virus can appear with four or five of the recognized mutations in RT. These can be M41L/D67N/K70R/T215Y or D67N/K70R/T215Y or F/K219Q. The pathway whereby the virus acquires four AZT resistance mutations including the less common T215F is not as clear as that involving T215Y. The approximate increase in the IC_{50} level conferred by each single and multiple mutation illustrated is given in parentheses. These are based on IC_{50} values obtained with viruses derived from molecular clones with defined mutations in the RT gene (Kellam et al., 1992, 1994; Larder & Kemp, 1989; Larder et al., 1994).

Considerably more resistant than strains containing these mutations singly (an IC_{50} value of 0.6 to 0.7 μM, compared with 0.04 μM for M41L alone or 0.16 μM for T215Y alone; Kellam et al., 1992, 1994).

In this context, it appears that K70R has a negative or suppressive influence on T215Y. Owing to the relatively low level of AZT resistance conferred by K70R plus T215Y, it is not surprising that the virus strain with M41L plus T215Y subsequently becomes the dominant population during therapy (Kellam et al., 1994).
analogue inhibitors, namely \((-\cdot)2'\)-deoxy-3' thiacytidine (3TC) and \((-\cdot)2'\)-deoxy-5'fluoro-3' thiacytidine (FTC; Boucher et al., 1993; Gao et al., 1993; Schinazi et al., 1993; Tisdale et al., 1993). Cell culture experiments have shown that virus strains containing this mutation can appear very rapidly on exposure to 3TC or FTC and the single change has a profound effect on resistance to these inhibitors (conferring an approximately 500-fold increase in the IC\(_{50}\) value) as well as conferring a modest degree of resistance to ddI and ddC. In the M41L plus T215Y background, M184V completely reverses AZT resistance (Boucher et al., 1993; Tisdale et al., 1993). In a background of four AZT resistance mutations (D67N, K70R, T215F, K219Q) suppression was also seen although sensitivity did not completely return to wild-type levels (Tisdale et al., 1993).

The location of mutations that suppress AZT resistance together with the other known drug resistance mutations in the RT gene are shown in Fig. 2 and summarized in Table 1.

**Combinations of RT mutations that confer co-resistance**

It would seem attractive to propose specific combination therapies, based on reversal of resistance to AZT or other RT inhibitors. However, it is clear that certain combinations of RT mutations can result in multiple resistance. This was apparent when HIV-1 variants were constructed with the ddI resistance mutation L74V, plus Y181C. The resulting virus was co-resistant to ddI and NNRTIs (Larder, 1992). Thus, in the context of ddI resistance, Y181C did not show a suppressive effect. A recent study has also demonstrated that additional NNRTI resistance mutations do not reverse ddI resistance (Byrnes et al., 1994). In vitro selection experiments also confirmed that dual-resistant variants could occur. For example, when AZT-resistant strains were passaged in the presence of the NNRTI nevirapine (BRL-587), they rapidly developed nevirapine resistance and remained resistant to AZT (Larder, 1992). In this
Table 1. Mutations in HIV-1 RT that confer drug resistance*

<table>
<thead>
<tr>
<th>Amino acid substitution</th>
<th>Inhibitor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>M41L</td>
<td>AZT</td>
<td>Kellam et al. (1992)</td>
</tr>
<tr>
<td>K65R</td>
<td>ddC/3TC</td>
<td>Gu et al. (1994); Zhang et al. (1994)</td>
</tr>
<tr>
<td>D67N</td>
<td>AZT</td>
<td>Larder &amp; Kemp (1989)</td>
</tr>
<tr>
<td>T69D</td>
<td>ddC</td>
<td>Fitzgibbon et al. (1992)</td>
</tr>
<tr>
<td>K70R</td>
<td>AZT</td>
<td>Larder &amp; Kemp (1989)</td>
</tr>
<tr>
<td>L74V</td>
<td>ddI</td>
<td>St. Clair et al. (1991)</td>
</tr>
<tr>
<td>V75F</td>
<td>ddT/ddN</td>
<td>S. Lacey &amp; B. A. Larder, unpublished results</td>
</tr>
<tr>
<td>A98G</td>
<td>NNRTI</td>
<td>Byrnes et al. (1993); Richman et al. (1993)</td>
</tr>
<tr>
<td>L100I</td>
<td>NNRTI</td>
<td>Byrnes et al. (1993); Mellors et al. (1993); Richman et al. (1993)</td>
</tr>
<tr>
<td>K101E</td>
<td>NNRTI</td>
<td>Byrnes et al. (1993); Richman et al. (1993)</td>
</tr>
<tr>
<td>K103N</td>
<td>NNRTI</td>
<td>Nunberg et al. (1991)</td>
</tr>
<tr>
<td>V106A</td>
<td>NNRTI</td>
<td>Larder (1992); Richman et al. (1993)</td>
</tr>
<tr>
<td>V108I</td>
<td>NNRTI</td>
<td>Byrnes et al. (1993); Richman et al. (1993)</td>
</tr>
<tr>
<td>E138K</td>
<td>NNRTI</td>
<td>Balzarini et al. (1993)</td>
</tr>
<tr>
<td>V179D/E</td>
<td>NNRTI</td>
<td>Byrnes et al. (1993); Richman et al. (1993); Tisdale et al. (1993)</td>
</tr>
<tr>
<td>Y181C</td>
<td>NNRTI</td>
<td>Nunberg et al. (1991); Richman et al. (1993)</td>
</tr>
<tr>
<td>M184V/I</td>
<td>3TC/FTC/ddN</td>
<td>Boucher et al. (1993); Gao et al. (1993); Schinazi et al. (1993); Tisdale et al. (1993)</td>
</tr>
<tr>
<td>Y188C</td>
<td>NNRTI</td>
<td>Byrnes et al. (1993); Richman et al. (1993)</td>
</tr>
<tr>
<td>G190E</td>
<td>NNRTI</td>
<td>Klein et al. (1993)</td>
</tr>
<tr>
<td>T215Y/F</td>
<td>AZT</td>
<td>Larder &amp; Kemp (1989)</td>
</tr>
<tr>
<td>K219Q</td>
<td>AZT</td>
<td>Larder &amp; Kemp (1989)</td>
</tr>
<tr>
<td>P236L</td>
<td>NNRTI</td>
<td>Dueweke et al. (1993)</td>
</tr>
</tbody>
</table>

* All mutations listed in this table have been shown by site-directed mutagenesis of RT to induce a drug-resistant phenotype.

In one such study attempts were made to select, in cell culture, multi-drug-resistant HIV-1 (Larder et al., 1993). The AZT and ddI dual-resistant virus (M41L, T215Y, L74V) was exposed to increasing concentrations of nevirapine and AZT in the presence of ddI. In just three passages, virus appeared that was nevirapine-resistant. In addition, ddI resistance was maintained and the level of AZT resistance was actually enhanced (Larder et al., 1993). Thus, during a minimal amount of drug exposure, triply resistant HIV-1 had been selected. DNA sequence analysis of this strain revealed that the V106A mutation had appeared, which explained the observed nevirapine resistance. Construction of this genotype by site-directed

Convergent therapy or multi-drug resistance?

Given that HIV-1 can acquire dual resistance to different RT inhibitors, it might seem likely that the virus could become multi-drug-resistant. However, Chow et al. (1993a) reported that mutant virus containing substitutions conferring resistance to AZT (T215Y, K219Q) and ddI (L74V) became non-viable when an NNRTI resistance mutation (K103N) was added. This prompted speculation that so-called convergent combination therapy would not result in simultaneous resistance to AZT, ddI and an NNRTI such as nevirapine. However, recent reports have clearly demonstrated that this is not the case.

Table 2. Combinations of mutations in HIV-1 RT that confer multi-drug resistance

<table>
<thead>
<tr>
<th>RT genotype</th>
<th>AZT</th>
<th>ddI</th>
<th>Nevirapine</th>
<th>JTC</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>M41L/T215Y</td>
<td>R*</td>
<td>S†</td>
<td>S</td>
<td>S</td>
<td>Single</td>
</tr>
<tr>
<td>L74V</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Y181C or V106A</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>M41L/T215Y/L74V</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>Double</td>
</tr>
<tr>
<td>M41L/T215Y/V106A</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>L74V/Y181C</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>M184V</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>M41L/T215Y/L74V/V106A</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>Triple</td>
</tr>
<tr>
<td>M41L/T215Y/L74V/V106A/M184V</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

* Resistant.
† Sensitive.
mutagenesis resulted in virus with the expected triply resistant phenotype (Table 2; Larder et al., 1993).

How can we reconcile the above observations with those of Chow et al. (1993a)? One possibility was that the replication-defective triple mutant reported was non-viable for reasons other than the presence of the four resistance mutations. Compelling evidence that this was the case came in two reports from different laboratories where exactly the same variant had been constructed and found to be perfectly viable (Larder et al., 1993; Emini et al., 1993). Ultimately, Chow et al. conceded that further sequencing of their mutant revealed unintentional, lethal but drug-irrelevant, mutations in the RT coding region (Chow et al., 1993b). However, the exact nature of these errors was not described. Thus there is still no rational basis for convergent combination therapy, specifically in relation to the development of ‘replication incompatible’ mutations.

Conclusions and perspectives

It is tempting to believe that as HIV-1 is challenged with an increasing number of RT inhibitors, the most likely outcome is the development of multi-drug-resistant strains. Data discussed in this review suggest that this could well occur clinically with the use of certain combinations of inhibitors. The notion that the presence of multiple drug resistance mutations in the RT gene might result in a defective enzyme still remains a hypothesis and awaits specific proof. Indeed, the idea that multiple RT mutations could partially disable the virus is also controversial, as conflicting data exist in the literature (Kellam et al., 1994; Chow et al., 1993a; Larder et al., 1993).

The unexpected phenotypes caused by interactions between certain drug resistance mutations might however point the way towards more rational clinical use of multiple RT inhibitors in the future. It is conceivable that specific drug combinations could be used to reverse or delay AZT resistance, although situations have been described where the virus can utilize different combinations of mutations to become multiply resistant. Careful examination of HIV-1 isolates during AZT and 3TC combination therapy will be of interest since at present it does not appear that co-resistance to these inhibitors has been demonstrated. Further combinations of interest might be the concurrent use of nucleoside analogues together with an NNRTI. Owing to the fairly complex and relatively unpredictable interactions between RT mutations, it is obviously essential to monitor phenotypic sensitivity of HIV-1 in addition to obtaining DNA sequence data during clinical trials with multiple RT inhibitors. Phenotypic assessment of most clinical isolates can be achieved by culture in peripheral blood mononuclear cells (PBMC), although this procedure is rather cumbersome, costly and time-consuming (Japour et al., 1993). A more convenient alternative involves the production and assay of recombinant virus strains, which circumvents the requirement for PBMC co-culture (Kellam & Larder, 1994).

A functional understanding of the interactions observed between RT mutations will probably rely on more extensive genetic analyses, together with structural data. We have recently investigated the effect of a variety of amino acid substitutions in RT residues 74 and 215 to gain insight into their interaction (Lacey & Larder, 1994). Although we were able to define those residues which maintained RT activity and modulated inhibitor susceptibility, the precise way in which the amino acids interact could not be determined. Perhaps recent advances in defining the crystallographic structure of RT will provide the missing clues required to explain these observations (Kohlstaedt et al., 1992; Jacobo-Molina et al., 1993). Structural information from mutant enzymes might aid in the design of new inhibitors and suggest drug combinations that are likely to be more potent than those currently under evaluation or consideration.

References


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Rooke, R., Tremblay, M., Soudeyns, H., DeStefano, L., Yao, J., Fanning, M., Montaner, J. S. G., O'Shaughnessy, M., Gelmon, O.


