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The role of genotypic heterogeneity in wild type virus populations on the selection of nonnucleoside reverse transcriptase inhibitor-resistant viruses

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Abstract

Virus populations were selected in cell culture using two widely used protocols in order to evaluate the role of selection methodology on the genotype and phenotype of nonnucleoside reverse transcriptase inhibitor resistant viruses. Selection was performed by serial passage of virus in the presence of gradually increasing concentrations of antiviral compound or passage in the presence of a constant high concentration of compound. Using the CEM-SS cell line, the IIIB strain of HIV-1, and identical nonnucleoside reverse transcriptase inhibitors, resistant viruses were obtained and their phenotypic and genotypic properties were defined. Resistant virus populations containing the Y181C amino acid change in the reverse transcriptase were predominantly selected with each of the tested compounds. Several of the compounds selected secondary amino acid changes using both methods. A comparison of the resistant viruses selected in our laboratory using each of the two protocols with viruses reported by a second laboratory employing one of the two methods suggests that genotypic differences in the selected virus isolates may most likely result from variation in the genetic composition of the respective wild type virus pools, rather than the specific selection methodology employed. These results imply that HIV may select a wide variety of amino acid changes to avoid the inhibitory effects of the nonnucleoside reverse transcriptase inhibitors and the selection of compounds for clinical use in combination with agents possessing non-overlapping resistance phenotypes will require evaluation of the agents against virus isolates possessing each of the mutations known to confer drug resistance.

Keywords: HIV-1; Reverse transcriptase inhibitors; Resistance

1. Results and discussion

The human immunodeficiency (HIV) virus is dependent upon the viral reverse transcriptase

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(RT) for replication (Gendelman et al., 1987; Goff, 1990). Nonnucleoside reverse transcriptase inhibitors (NNRTIs) suppress HIV replication by binding at a hydrophobic pocket of the palm domain of the p66 subunit of the RT (Grob et al., 1992; Condra et al., 1992; Cohen et al., 1991, Kohlstaedt et al., 1992, Jacobo-Molina et al., 1993, Nanni et al., 1993). The pharmacologically diverse class of NNRTIs includes a number of compounds which are highly specific inhibitors of HIV-1 RT, possessing wide therapeutic indices. Representative compounds include tetrahydro-imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one and -thione (TIBO), 1-[(2-hydroxyethoxy)methyl]-6-(phenythio)thymine (HEPT), the pyridinones, the calanolides, the diphenylsulfones, nevirapine, and the oxathiin carboxanilide family of compounds (Buckheit et al., 1995b; Balzarini et al., 1995a; Buckheit et al., 1994; McMahon et al., 1993; Maass et al., 1993; Klunder et al., 1992; Kashman et al., 1992; Romero et al., 1991; Goldman et al., 1991; Bader et al., 1991; Pauwels et al., 1990; Merluzzi et al., 1990).

Although the NNRTIs are highly specific and potent inhibitors of HIV-1 replication, their utility is compromised by the rapid appearance of drug-resistant virus populations (Richman et al., 1991; Nunberg et al., 1991; Mellors et al., 1995; DeVreese et al., 1992; Balzarini et al., 1993a, Buckheit et al., 1995c). The potential benefits of therapy with a combination of NNRTIs necessitates continued investigation of the mechanisms of resistance selection in vitro and in vivo. We have recently demonstrated with a large group of structurally related UC compounds that the particular virus isolate selected in cell culture upon addition of a NNRTI and the degree of resistance of the virus to the selecting agent is dependent on the specific chemical structure of the compound. These studies have demonstrated that compounds might be defined based on their ability to preferentially select 'high resistance' or 'low resistance' phenotype viruses (Kinjerski et al., 1996). We have noted that selection of viruses in cell culture using identical compounds in different laboratories often yielded resistant virus populations with quite different amino acid changes in the RT (Kinjerski et al., 1996; Balzarini et al., 1995b,a, 1994; Balzarini et al., 1993a; Buckheit et al., 1995b). It has been suggested that the use of gradually increasing versus constant high drug concentrations during resistance selection may be responsible for the varied genotypes obtained. Our results clearly indicate that the methodology employed does not pre-determine the genotype of the isolate selected. The particular resistant genotype selected was, however, determined in part by the wild type virus pool from which it was derived. These results hold important implications for prediction of amino acid changes in the RT which will be selected from a highly heterogeneous wild type population in a patient and to the appropriate definition of effective combinations of NNRTIs to be used in patients.

The specific methods used to select virus populations resistant to NNRTIs vary between laboratories and include serial passage in the presence of gradually increasing drug concentration or passage in the presence of fixed drug concentration. To determine if the initial antiviral drug concentration influences the phenotype and genotype of the virus population obtained, we have selected resistant virus isolates using identical NNRTIs employing a method routinely used in our laboratory (Buckheit et al., 1995a; Buckheit et al., 1995b; Buckheit et al., 1995c) and a second commonly used method of selection (Balzarini et al., 1994; Balzarini et al., 1993b; Balzarini et al., 1995b). In addition, we have compared the virus populations selected by these two methods with those previously reported using identical compounds (Balzarini et al., 1995b; Buckheit et al., 1995c, Kinjerski et al., 1996). In each case, the wild type HIV-1 virus strain (IIIB) and the cell line (CEM-SS) were common factors in the selection process. The multiplicity of infection (MOI) employed to initiate infections in our comparative evaluations was held constant and the concentration of compounds used were similar to those reported by the other groups. All selections were performed at least twice and in some cases were performed up to ten times to evaluate the reproducibility of the selection process (Kinjerski et al., 1996).

Resistant viruses were initially selected in cell culture by serial passage of the IIIB strain of HIV-1 in CEM-SS cells in the presence of increasing concentrations of the antiviral compound. The initial passage was performed with a drug concentration two times the EC_{50} concentration of the compound as determined in the microtiter anti-HIV assay (Buckheit et al., 1993). The drug concentration was increased two-fold with each successive passage. Resistant virus populations were obtained within 4–6 passages and levels of resistance were evaluated using the microtiter anti-HIV assay. Resistance was defined as a greater than five-fold increase in EC_{50} value when compared to the EC_{50} obtained with the wild-type (IIIB) virus. Resistant virus populations were also selected as previously described in the presence of fixed drug concentration. Briefly, CEM-SS cells were infected with the IIIB strain of HIV-1 and were serially passaged in the presence of fixed concentrations of antiviral compounds (UC10: 0.2 and 1.0 μM; UC38: 0.5 μM; UC84: 1.7, 8.4 and 28.1 μM; costatolide: 0.8 and 4.1 μM; diphenylsulfone: 5.7 and 28.5 μM) until resistant virus populations were obtained. Selection of resistant virus isolates required approximately 5–12 passages using this method. Levels of resistance were again evaluated using the microtiter anti-HIV assay. Each of the resistant virus populations obtained were directly sequenced using eight overlapping primer oligonucleotides and the Sequenase PCR Product Sequencing Kit (USB-Amersham, Cleveland, Ohio) as previously described in detail (Kinjerski et al., 1996).

The NNRTIs UC10, UC38, UC84, costatolide, and diphenylsulfone (DPS) were used to select resistant virus populations using each of the two methods. Each of the antiviral agents were obtained from the Developmental Therapeutics Program, National Cancer Institute. The phenotypic and genotypic properties of the virus populations selected are presented in Table 1. With the exception of two viruses, each of the virus populations were completely insensitive to the selecting compound at the highest nontoxic drug concentration. Molecular characterization revealed that the UC10-, UC38-, UC84-, and DPS-resistant virus populations selected using either method contained the Y181C mutation in the RT. Several of the virus populations also contained secondary amino acid changes. The virus populations resistant to costatolide which were selected by either method contained the T139I amino acid change (Buckheit et al., 1995a). These data indicate that costatolide targets slightly different amino acid residues in the RT than the remaining compounds, resulting in the reproducible selection of a distinct mutation. This comparative study demonstrated that virus isolates with the Y181C amino acid change in the RT were readily selected using each of the two methods.

Table 1 also provides a comparison of the genotype of the virus isolates selected in our laboratory using both methods with the genotype of resistant viruses reported previously, using identical compounds. In the reported results, UC84 selected the E138K amino acid change, UC10 selected the K103N amino acid change, and UC38 selected the K101E and G190E amino acid changes (Balzarini et al., 1995a; Balzarini et al., 1995b). TSAO also selected the E138K amino acid change (Balzarini et al., 1993b; Balzarini et al., 1994). These previously reported amino acid changes were not identified in any of the virus isolates selected in our laboratory using either method. In the presence of a gradually increasing concentration of HEPT the resistant virus populations selected in our laboratory possessed a P236L amino acid change while a Y188H amino acid change was reported by another (Buckheit et al., 1995b; Balzarini et al., 1993a). Although some variability was observed in the secondary amino acid changes selected in our laboratory using both methods, comparison of the amino acid changes obtained with both methods were more reproducible than when these changes were compared to the amino acid changes reported by other groups.

It has been suggested that the amino acid changes selected in cell culture might be determined by the selection strategy employed, namely, that selection at gradually increasing concentration would preferentially yield Y181C-containing populations while selection at high concentration would yield other changes in the RT. Our data clearly indicate that the Y181C amino acid change

Table 1
Comparison of drug-resistant virus populations selected by multiple methods

Compound	Method of selection					
	Increasing concentration		Fixed concentration[a]		Previously reported amino acid changes[a]	
	Fold-resistance	Amino acid change	Fold-resistance	Amino acid change	Fold-resistance	Amino acid change
UC10 (NSC 645129)	>285	Y181C, K101E	30	Y181C (0.2, 1.0)	5	K103N[b] (0.3)
UC38 (NSC 629243)	>167	Y181C	>167	Y181C (0.5)	NP	K101E[b] G190E (NP)
UC84 (NSC 615985)	>270	Y181C, V106A, K101I/N/T, K103Q, G190A, T139I, A98S	>270	Y181C (1.7, 28.1) V108I (1.7) Y188H (8.4)	NP	E138K[b,c] (0.3, 1.4)
Costatolide (NSC 661122)	>54	T139I, L100I	>54	T139I (0.8, 4.1) Y188H (0.8)	ND	ND
DPS	>133	Y181C	11	Y181C (5.7, 28.5)	ND	ND
HEPT	>18	P236L	ND	ND	>33	Y188H[d] (20)
TSAO	>125	Y181C	ND	ND	>1500	E138K[e] (0.17, 1.7, 17)

[a]The concentration of the compound (μM) which selected the indicated amino acid change is indicated in parentheses.
[b]Balzarini et al., 1995b.
[c]Balzarini et al., 1995a.
[d]Balzarini et al., 1993a.
[e]Balzarini et al., 1993b.
ND, not done; NP, data not provided.

was selected in our assay system, irrespective of the initial drug concentration. In addition, since each of our infections was initiated at a standardized MOI and since we used drug concentrations similar or identical to those employed by others, these factors should not have influenced the selection process.

These data indicate that factors other than the initial drug concentration or the method of resistance selection define the outcome of the resistance selection process. It appears that the virus pool from which resistant viruses emerge plays a critical role in the selection process and confirms that a specific NNRTI may not be associated with one particular amino acid change in the RT. Rose et al. have recently demonstrated another means by which the virus pool may influence the selection of resistant viruses. They concluded that the particular viral backbone plays a role in determining virus breakthrough and cross-resistance patterns. Identical mutations in an NL4-3 genetic background and an RF background did not have the same effect on the activity of various antiviral compounds which inhibit the viral protease (Rose

et al., 1996). From the data reported here, it appears that differences also occur within the same virus strain maintained in different laboratories, resulting in substantial genetic divergence among separate virus pools. Such differences most likely may account for variations in the resistant virus populations selected by separate laboratories using identical antiviral compounds. A recent comparison of envelope sequence heterogeneity in various pools of IIIB virus obtained from different sites supports this hypothesis (Lockey et al., 1996), demonstrating substantial envelope sequence variation which resulted in changes in size, charge and hydrophobicity of the envelope glycoprotein. In this paper, 22 clones which originated from two parental HTLV-IIIB cultures were evaluated and the envelope sequence was compared with each other and with earlier reported sequence. These differences among the various HIV-1 virus pools must be considered when evaluations of resistance selection are used to define appropriate combinations of NNRTIs to use in patients. These data would suggest that the highly heterogeneous wild type populations found in patients would yield ample opportunity to select for a wide variety of amino acid changes, rendering effective NNRTI therapy difficult to achieve. Appropriate combinations of NNRTIs must be chosen for use in patients after extensive evaluation of the activity of the compound against each of the many amino acid changes in the RT which engender NNRTI-resistance.

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