Rapid biochemical assays for phenotypic drug resistance testing of HIV-1

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Background

Treatment of HIV-1-infected persons with antiretroviral drugs has significantly reduced the rate of HIV and AIDS-related morbidity and mortality. To date, the US Food and Drug Administration (FDA) has approved 19 antiretroviral drugs for treatment of HIV-1 infections, including 16 unique inhibitors of either HIV-1 reverse transcriptase (RT) or protease. Selecting a combination regimen that maximally suppresses virus replication is critical for treatment success, since failure to achieve viral suppression may result in the emergence of viruses carrying drug resistance mutations. Drug-resistant viruses may be selected from pre-existing viral quasispecies or may be generated de novo. The generation of drug-resistant variants is a consequence of the high mutation rates and replication of HIV-1. If an HIV-1-infected person produces an estimated $10^{10}$ virions daily and each genome contains an average of one mutation, every possible single mutation associated with drug resistance may be generated daily. Estimates based on the rate of emergence of nevirapine resistance in previously untreated persons indicate that $\sim 1$ in 1000 genomes carries the Y181C mutation associated with resistance to nevirapine resistance before treatment.

Mutations associated with resistance are classified as primary or secondary. Primary mutations usually appear early, are relatively specific for each drug and decrease drug susceptibility. Examples of such mutations are the M184V mutation associated with resistance to lamivudine (3TC), the T215Y/F and K70R mutations associated with resistance to zidovudine (AZT) and the G48V mutation associated with saquinavir resistance. Secondary mutations usually accumulate in viral genomes that already have some primary mutations or may occur as natural polymorphisms. Examples of secondary mutations are D67N, L210W and K219Q in the RT, and M36I and I54V/I in the protease. These secondary mutations confer little or no reduction in drug susceptibility by themselves, restore replication capabilities of viruses carrying primary mutations, and may also increase the level of resistance in such viruses. The clinical relevance of primary and secondary mutations in both RT and protease has recently been evaluated. The Drug Resistance Mutation Group of the International AIDS Society (IAS)-USA recommends suspension of the distinction between primary and secondary mutations for nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs), since the clinical distinction between these mutations is not clear. For protease inhibitors, the panel recommends the use of ‘major’ or ‘minor’ mutations instead of primary or secondary to avoid confusion regarding the order in which the mutations may occur.

Resistance testing

Data from various retrospective and prospective studies are accumulating to support the use of drug resistance testing in many clinical situations. Several agencies and expert panels, such as the IAS-USA panel, the EuroGuidelines Group for HIV Resistance and the US Department of Health and Human Services (DHHS), have now provided guidelines and recommendations for drug resistance testing. All panels recommend resistance testing to help guide the choice of new regimens after treatment failure, and agree that resistance testing should also be considered in primary HIV-1 infection. The IAS-USA panel and the EuroGuidelines Group also recommend resistance testing in pregnant women, whereas the US DHHS guidelines make no distinction between pregnant and non-pregnant patients. In other clinical situations, such as chronic infection and post-exposure prophylaxis, the usefulness of resistance testing is not clear.
Resistance can be determined genotypically by examining resistance-related mutations in the viral genome, or phenotypically by measuring susceptibility of HIV-1 to specific drugs. To date, several phenotypic and genotypic assays have been developed and are being used to monitor for drug resistance. All genotypic assays that are commercially available for genotypic testing are based on PCR amplification of viral pol sequences, primarily from plasma by RT–PCR. Amplified products can be analysed by hybridization or sequencing techniques. Hybridization techniques are illustrated by the commercially available Line Probe Assay (LIPA HIV-1 RT, Innogenetics, Gent, Belgium) (use of trade names is for identification only and does not constitute endorsement by the US DHHS, the Public Health Service or the Centers for Disease Control and Prevention), which detects key resistance mutations. The LIPA assay is available as a kit and is based on reverse hybridization of biotin-labelled amplified HIV-1 sequences from plasma with short, immobilized oligonucleotides. This assay detects both wild-type and resistant variants, and has high sensitivity for detecting a low proportion of resistant viruses. However, this method is available for genotyping only selected RT and protease mutations. In contrast, sequence analysis provides information on all positions associated with drug resistance. Several kits, including the TRUGENE HIV-1 Genotyping Kit (Visible Genetics, Inc., Toronto, Canada) and the ViroSeq Kit (Applied Biosystems, Inc., Foster City, CA, USA), have now been developed. The TRUGENE HIV-1 Genotyping Kit recently received FDA approval, becoming the first HIV drug resistance test to be approved for routine clinical use.

Rapid oligonucleotide ligation assays have now been developed for the detection of primary RT and protease resistance mutations. These investigational tools are based on the covalent joining of two adjacent oligonucleotide probes by a DNA ligase when they are hybridized to a PCR product. The increased ability of these assays to detect low proportions (~5%) of resistant viruses provides an advantage over sequence-based assays.

Phenotypic assays measure the ability of an HIV-1 isolate to replicate in the presence of a drug. Currently used phenotypic tests are based on the development of recombinant viruses (RVs). RVs are generated from patient-derived RT/protease sequences that are amplified by PCR and an RT/protease-deleted proviral laboratory clone. Therefore, the RVs retain the drug susceptibility of the RT and protease present in the clinical specimen. Susceptibility is determined by measuring the drug concentrations that inhibit 50% or 90% virus replication (IC50 and IC90, respectively). Comparison of the IC50 and IC90 values of these viruses with those from a reference wild-type HIV-1 provides a direct measurement of the fold changes in drug susceptibility.

To date, three RV assays are commercially available to measure susceptibility to RT and protease inhibitors: Antivirogram (Tibotec-Virco, Mechelen, Belgium), PhenoSense HIV-1 Drug Resistance Assay (ViroLogic, Inc., South San Francisco, CA, USA) and PhenoScript (VIRalliance, Paris, France). All these assays amplify HIV-1 RT and protease but differ in technical aspects related to recombinant virus construction and detection of virus replication. The results are interpreted based on cut-off values established for each assay. A comparative analysis of the Antivirogram and PhenoSense assays was recently carried out using plasma samples from drug-naive or drug-experienced HIV-1-infected persons. The result showed a 92% concordance in phenotypic category between both assays, with the majority of the discordant results seen among samples that had fold changes in IC50 values close to assay cut-off values. These findings indicate a good correlation between the results of both assays, despite the use of different testing strategies.

Despite the general agreement regarding the clinical utility of resistance testing, none of the current guidelines indicates what particular type of assay should be used. Whereas phenotypic assays provide a direct measurement of the susceptibility of RT and protease to antiretroviral drugs, clinically validated cut-off values that correlate virological response with fold changes in IC50 values are not completely defined. Genotypic assays are faster and cheaper than phenotypic assays but require expert clinical interpretation of results, especially since complex mutational patterns may have a significant impact on cross-resistance and resistance reversal among different drugs.

Rapid assays for phenotypic drug resistance testing

Since phenotypic assays currently used are complex, labour intensive and expensive, simpler and less expensive drug resistance assays are critically needed. Increased interest in developing biochemical assays for drug susceptibility testing of HIV RT and protease has emerged in recent years.

We have recently described a new approach to phenotypic drug resistance testing of HIV-1 to RT inhibitors that is not based on virus cultures but instead involves the use of rapid and simple biochemical assays that measure directly the susceptibility of RT activity in plasma to RT inhibitors. This testing approach was made possible by the use of Amp-RT, a PCR-based ultrasensitive RT assay that is capable of detecting very low level RT activity in plasma. The Amp-RT assay uses a known heterologous RNA template derived from the encephalomyocarditis virus (EMCV) RNA genome and a specific EMCV primer. The RT-derived EMCV cDNA is detected by PCR amplification and an ELISA-based hybridization with an internal EMCV-specific probe. Phenotypic susceptibility (i.e. IC50) to RT inhibitors can be determined from the RT signal generated in Amp-RT reactions containing different drug concentrations. Figure 1 illustrates the
principle of this testing approach. In contrast to conventional phenotypic assays, drug susceptibility testing by Amp-RT does not require virus isolation and culture, and, therefore, provides rapid information (1–2 days) on resistance.

Susceptibility testing of HIV-1 RT by Amp-RT is done by measuring the IC50 of the RT enzyme to NNRTIs or the triphosphate form of NRTIs. Amp-RT IC50 values determined in several wild-type and nevirapine- or 3TC-resistant isolates have been shown to correlate well with those measured by culture-based phenotypic assays. A simple testing strategy using one drug concentration in the Amp-RT assay was also found to be useful for rapid screening of resistance in plasma samples. We have demonstrated the validity of this testing approach for detecting resistance to 3TC and nevirapine. Both assays have the ability to detect low proportions (~10%) of resistant viruses, and, therefore, may be used for early detection of resistance to these drugs. Amp-RT-based assays have been developed for other RT inhibitors, including didanosine, zalcitabine and efavirenz.

The ability of Amp-RT to detect RT activity associated with any retroviruses, including HIV-1 and HIV-2, provides a unique tool that is strain or subtype independent. Such assays can also be used to monitor for drug resistance in persons infected with divergent HIV-1 subtypes. For instance, we have recently demonstrated the ability of this assay to detect RT from HIV-1 group O in plasma. In addition, we showed biochemical resistance to 3TC in HIV-1 group O-infected patients who had failed 3TC-containing regimens and had detectable M184V mutation. Therefore, these assays may provide a valuable tool to monitor for drug resistance in non-subtype B viruses and in HIV-2.

Rapid biochemical assays for susceptibility testing of HIV-1 protease are also being developed. Among these assays, a bacteriophage lambda-based assay and an in vitro transcription/translation system have been used successfully to evaluate protease activity and to discriminate between wild-type and drug-resistant protease. Whereas these assays have shown promising preliminary results in a limited number of clinical samples and in site-directed mutants, validation with large numbers of clinical specimens is needed.

In summary, the need for rapid and simple drug resistance assays is becoming more critical as drug resistance testing is increasingly integrated into patient management. Such assays may also provide a rational approach for resistance testing in resource-poor settings where the use of antiretroviral drugs is expected to increase in the near future.

References


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