Genotypic Resistance in HIV-infected Patients Failing a d4T/3TC/NVP Regimen

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Abstract

The objectives of this study were to determine the genotypic patterns of resistance in antiretroviral-naïve patients failing initial therapy with a stavudine (d4T)/lamivudine (3TC)/nevirapine (NVP) regimen. There were 16 patients (10 females and 6 males) with the mean age of 30.3±6.5 years. Mean duration from the initiation of d4T/3TC/NVP regimen to virological failure was 20.0±13.5 months. Mean CD4 cell counts and median plasma HIV RNA at the date of virological failure were 367.6±340.1 cells/mm³ and 5,650 copies/ml (range, 949-49,086), respectively.

Twelve of 16 (75%) patients had genotypic resistance to both nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI). NRTI key mutations were M184V (87.5%), D67N (18.8%), K70R (12.5%), T215Y (12.5%), and K219Q (12.5%). No patient developed mutations associated with didanosine (ddI) or tenofovir disoproxil fumarate (TDF) resistance (K65R or L74V). NNRTI key mutations were K103N (50%), Y181C (18.8%), G190A/S (18.8%) and V108I (12.5%). Selection of NVP and 3TC resistance often with other thymidine analogue mutations (TAMs) was frequently associated with virological failure in our patients. Genotypic resistance testing is helpful for selection of the next regimen in patients failing d4T/3TC/NVP regimen. The prospective genotypic resistance study with a larger number of patients is required to confirm our results. (J Infect Dis Antimicrob Agents 2004;21:61-7.)

Introduction

The use of highly active antiretroviral therapy (HAART) has dramatically changed the course of human immunodeficiency virus (HIV) disease, with a substantial reduction in morbidity and mortality.1 However, long-term suppression of HIV is not easy to achieve because of the adverse effects, complexity of drug regimens and drug costs, which adversely impact adherence, leading to selection of resistant mutants and...
treatment failure.

In April 2002, the Thai Government Pharmaceutical Organization (GPO) produced a generic combined pill of three anti-HIV drugs, stavudine (d4T), lamivudine (3TC), and nevirapine (NVP), called GPO-VIR. The cost of GPO-VIR is lower than the original pills, and is much more affordable for HIV-infected patients in Thailand. In 2004, 60,000 HIV-infected people are expected to use this combined pill. Thus, a large number of patients who will fail from this single regimen in the near future is anticipated.

HIV resistance to antiretroviral agents is a major contributory cause of treatment failure. The dynamics of HIV replication, together with patient-, physician-, and drug-related factors, lead to emergence of HIV-resistant strains in some patients. GPO-VIR, an NVP-containing regimen, is simple and well tolerated but have a low genetic barrier that may facilitate the development of high-level resistance in only one step of viral mutation. HIV genotypic resistance testing, which detects resistance mutations in the reverse transcriptase and protease genes by comparing the gene sequences of resistant virus to those of a wild-type strain that has been previously described. The efficacy of each antiretroviral class and each individual antiretroviral drug is threatened by specific mutations and resistance mechanisms. Genotypic resistance testing is most useful in patients failing an initial regimen and in identifying the presence of resistance to drugs in the currently failing regimen. However, the cost of the test limits the usefulness of resistance testing in clinical practice in Thailand. Our study aimed to demonstrate the genotypic resistance patterns in patients who failed from the specific regimen of d4T/3TC/NVP, and suggest the options of salvage therapy in patients who failed GPO-VIR and cannot afford genotypic resistance testing.

**PATIENTS AND METHODS**

**Patients**

From January to December 2003, HIV-infected patients who had virological failure from an initial regimen of d4T/3TC/NVP and were tested for HIV genotypic resistance in Ramathibodi Hospital were included into the study. According to the US and British treatment guidelines, the definition of virological failure includes no achieving undetectable HIV RNA (<400 copies/mL) at 24 weeks of treatment or rebound HIV RNA during treatment. The patterns of drug-resistant viral genotypes, mutations critical to the HIV-1 protease and reverse transcriptase sequences were reviewed from the results of genotypic resistance tests. Individuals who had been on drug holidays for longer than 2 weeks before the time of genotypic resistance tests were excluded.

**Genotypic Resistance Testing**

HIV RNA was extracted from plasma samples, using the QIAamp viral extraction kit (Qiagen, Inc., Chatsworth, CA, USA). The TRUGENE HIV-1 Genotyping Assay was used in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics Inc., Toronto, Canada) to sequence the protease and reverse transcriptase (RT) regions of the HIV-1 cDNA. Testing involved simultaneous clip sequencing of protease and codons 35-244 of the RT from the amplified cDNA in both the 3’ and 5’ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software. We focused on mutation at positions in the polymerase gene known to be associated with the commonly used drugs against HIV-1. Interpretation of the genotype in terms of drug resistance was based on an algorithm established by the Stanford HIV RT and Protease Sequence Database.

**RESULTS**

There were 16 patients with 10 (62.5%) females and 6 males. The mean age was 30.3±6.5 years. Ten patients had received GPO-VIR, the others had separate pills of d4T, 3TC and NVP. Mean duration from the initiation of d4T/3TC/NVP regimen to virological failure was 20.0±13.5 months. Mean CD4 cell counts and median plasma HIV RNA at the date of virological failure were 367.6±340.1 cells/mm³ and
5,650 copies/ml (range, 929-49086), respectively.

All patients had genotypic resistance to nucleoside reverse transcriptase inhibitor (NRTI) or non-nucleoside reverse transcriptase inhibitor (NNRTI). Twelve of 16 (75%) patients had genotypic resistance to both NRTI and NNRTI. NRTI key mutations were recognized in 14 (87.5%) patients. These included M184V (87.5%), D67N (18.8%), K70R (12.5%), T215Y (12.5%), and K219Q (12.5%) (Figure 1). No patients developed mutations associated with didanosine (ddI) or tenofovir disoproxil fumarate (TDF) resistance (K65R or L74V). NNRTI key mutations were recognized in 14 (87.5%) patients. These included K103N (50%), Y181C (18.8%), G190A/S (18.8%) and V108I (12.5%) (Figure 2). Genotypic resistance to protease inhibitor (PI) was not detected.

DISCUSSION

HIV drug resistance results mainly from the interplay of HIV replication and antiretroviral drug selection pressure. When antiretroviral therapy does not completely suppress viral replication, the replicating HIV population has the opportunity to develop new mutations. Figure 3 shows the number of patients with different genotypic mutations.

Figure 1. Genotypic resistance to NRTI key mutations.

Figure 2. Genotypic resistance to NNRTI key mutations.
on the number of viral mutations required to overcome the antiviral activity of the regimen (genetic barrier) and the rate of replication (fitness) of drug-resistant virus. A regimen with high genetic barrier, e.g. PI-based regimen, would require the virus population to acquire multiple mutations before development of virological failure. In contrast, NNRTI-based regimens have a low genetic barrier and allow the development of high-level resistance in one step of mutation.  

A regimen of d4T/3TC/NVP, currently the most commonly prescribed regimen in Thailand, contains 2 low-genetic-barrier drugs, NVP and 3TC. HIV acquires high resistance for these agents with only a single mutation.success of treatment from this regimen requires a strict adherence.

Our study showed that 75 percent of the patients failing a d4T/3TC/NVP regimen had key mutations to both NVP and 3TC. Twelve of 16 (87.5%) patients had key mutations to NVP. K103N occurred in a half of our patients. Other studies to determine the genotypic and phenotypic patterns of resistance in patients failing initial therapy with NVP-based regimens also showed a high prevalence of NVP resistance and demonstrated K103N as the most common mutation.

The K103N mutation indirectly inhibits the binding of NNRTI to the binding pocket of RT and results in a 20-fold resistance to NVP and efavirenz (EFV). Therefore, mutations conferring resistance to one drug in this class generally confer cross-resistance to all other NNRTIs. Normally, NNRTI resistance mutations occur in 2 clusters in the RT gene at codons 100-108 and at codons 179-190. The most common changes selected by NVP involve a K103N mutation and a Y181C mutation. However, our study showed that only 19 percent of the patients had Y181C.

Another low-genetic-barrier drug in the d4T/3TC/NVP regimen is 3TC. Resistance to 3TC is conferred by a point mutation at RT codon 184, producing M184V or M184I. In a study of 3TC monotherapy before the HAART era, the entire wild-type population of HIV in the plasma is replaced by variants carrying the M184V mutation within weeks of initiating treatment with 3TC. 3TC resistance is rapidly acquired if the other components in the regimen are not effective. It is not surprising that 88 percent of our patients had M184V. A previous study of genotypic resistance in 83 HIV-1 infected Thai patients who had been treated with any antiretroviral drug also showed that M184V/I was the most common mutations.

Our study also showed that there were other thymidine analogue mutations (TAMs), including D67N, K70R, T215Y, and K219Q, from the failure of d4T/3TC/NVP regimen. One patient (6.3%) had 4 TAMs (data not shown). Studies from several groups clearly demonstrates that d4T can select mutations typically associated with zidovudine (AZT) resistance (eg. K70R, T215Y). There is substantial evidence of cross-resistance between AZT and d4T. Presence of these mutations was significantly associated with a poor response to d4T therapy. Multinucleoside drug resistance mutation including the Q151M complex and insertion mutation at codon 69, were not observed in our study, because every patient in our study had failed only one regimen containing d4T/3TC/NVP for a short period of treatment. Higher experience with multiple regimens increases the risk of multinucleoside drug resistance.

According to the results of our study, patients who failed a d4T/3TC/NVP regimen would also have a very high rate of resistance to all NNRTI and 3TC, and low rate of resistance to other NRTIs including d4T and AZT. The salvage regimens for these patients could be boosted PI-based regimens with two reliable NRTIs, such as ddI or TDF. However, TDF is not currently available in Thailand. Thus, abacavir (ABC) may be used. ABC retained efficacy against resistant HIV with the M184V genotype alone. However, 6.3 percent of our patients had 4 TAMs. The chance of ABC failure in this salvage regimen is possible. In addition, 5 of 16 (31.3%) patients had D67N, K70R, T215Y or K219Q. The chance of failure when using of AZT or d4T should also be relatively high.

The limitation of our study includes a retrospective fashion and low number of patients. We did not demonstrate the sequences of mutations occurring
in our patients. These results are also based on an early detection of virological failure on the basis of HIV RNA testing every 3-6 months. In most medical centers, HIV RNA is not easily performed and virological failure is usually detected late after treatment. There could be a higher rate of NRTI resistance including multinucleoside resistances. We did not determine the adherence of patients in this study due to the retrospective fashion.

In conclusion, selection of NVP and 3TC resistance often associated with other TAM mutations was frequently observed in patients failing a d4T/3TC/NVP regimen. Genotypic resistance testing is helpful for selection of the next regimen in patients failing this regimen. A well-designed prospective study to investigate the genotypic resistance pattern in patients failing initial therapy with GPO-VIR is required.

ACKNOWLEDGEMENT

The authors would like to thank all the attending staffs and fellows in Division of Infectious Diseases who had cared the patients in this study, and the staffs in Molecular Virology Unit, Department of Pathology, Ramathibodi Hospital.

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