Resistance analyses in HIV infected patients with a history of multiple antiretroviral treatment regimens

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Objective: To assess HIV-1 isolate based resistance profiles from extensively pretreated patients and effects of a resistance guided switch of antiretroviral therapy.

Methods: In a prospective study phenotypic and genotypic resistance analyses were performed on HIV infected individuals with failure of the current therapy and history of at least three antiretroviral regimens. Antiretroviral therapy was changed according to the results. Viral load and CD4 lymphocyte counts were measured at baseline, after 10 (SD 2), and 24 (2) weeks.

Results: All patients (n=52) failed their actual regimen. Currently versus ever previously taking the specific drug, resistance associated mutations and phenotypic resistance to AZT and 3TC were found in over 80% of individuals; resistance to DDI and D4T was detected in less than 10% of cases. A resistance guided switch of therapy was followed by a median decrease of viral load of 0.5 log10 units after 24 weeks. Individuals resistant to two or more drugs compared with patients with resistance to less than two drugs of ongoing treatment, were switched to a regimen containing DDI, D4T, and a PI or NRTI. After 10 (SD 2) weeks viral load decrease was pronounced in patients with resistance to at least two drugs in the previous regimen.

Conclusions: Among different RTI, the profile of clinically relevant resistance indicates pronounced differences when looking at separate drugs. Regarding virological response, in the context of available drugs, resistance tested with currently used methods is of limited value in extensively pretreated patients and seems to have its value primarily in first or second switch of therapy.

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Keywords: HIV drug resistance; antiretroviral therapy; HIV diagnostic tests

Introduction

Advances in antiretroviral therapy (ART) have led to dramatic decrease of morbidity and mortality in HIV positive patients, but in studies and in everyday clinical practice viral rebounds are observed. The frequency of this phenomenon increases with duration of treatment. Recently some studies were published reporting clinical benefits from genotypic and phenotypic resistance testing; however, many important questions in everyday clinical management remain unresolved. Performing resistance tests is a routine procedure in study settings while in everyday clinical life cost and insurance issues can cause significant delay. This evaluation was performed to learn more about relevance of genotypic and phenotypic resistance tests in extensively pretreated patients under everyday conditions.

Materials and methods

Data were collected from HIV positive patients in outpatient clinics of the General Hospital St Georg in Hamburg, Germany, between September 1997 and December 1998. Resistance testing was performed after failure of at least three different regimens of ART before resistance analysis. Viral load (Amplicor Test, Hoffmann LaRoche) and CD4 lymphocyte counts were determined at the time antiretroviral therapy was changed after resistance testing, as well as 10 (SD 2) and 24 (2) weeks afterwards. For genotypic analysis of resistance associated mutations, the Amplicor HIV-1 monitor test (Roche Diagnostics) ultrasensitive protocol was used. Each gene (RT and PR gene) was cycle sequenced in forward and reverse direction and the raw data were analysed and edited by Sequence Navigator Software (Perkin Elmer, Weiterstadt, Germany). To identify relevant mutations for drug resistance, forward and reverse sequence of the RT and PR gene were aligned versus the HIV-1 reference strain pNL4-3, edited and loaded into the Complign module of the Mac Molly Tetra Software for further mutation analysis. All resistance associated mutations were reinterpreted using HIV-1 resistance mutation compilation (December 1998 version). Phenotyping drug resistance testing was performed using the recombinant virus technology (Antivirogram, Virco, Belgium) according to the method previously described.

Results

BASELINE CHARACTERISTICS

A total of 52 HIV positive patients (47 males, five females) were analysed between July 1997 and March 1999 (median age 44 years; range 29-76 years). According to CDC classification, 73% fulfilled group C criteria, 19% group B, and 8% group A. A median of CD4 lymphocytes was 173 cells × 10^9/l (range 3–470
cells × 10^6/l). ART had been changed in all patients at least three times before resistance testing. The median number of changes during therapy was six (three to nine) with median duration of ART before resistance analysis of 30.5 months (13–75 months).

Viral load change was separately observed for patients who were able to add at least two susceptible drugs, according to genotypic or phenotypic test in new therapy (n = 20), versus patients with one or less new susceptible drugs (n = 24); however, for both groups a median decrease of viral load of 0.5 log10 was observed after 10 (SD 2) and 24 (2) weeks (fig 1A). For phenotypic tests a more than 10-fold resistance was defined as relevant. Fourfold to 10-fold resistance was assumed when apparent in at least two new regimen: group 1 with isolates resistant to >2 drugs compared to group 2 with isolates resistant to <2 drugs.

Follow up of patients with resistance

Guided Switch of Antiretroviral Therapy

Individuals who were able to switch to a regimen with at least two new susceptible drugs (group 1) versus those with no or one new susceptible drug (group 2) were followed up for evaluation: 15 of 20 patients (75%) at week 10 (SD 2) and week 24 (2) and two patients were lost to follow up. Two patients from the first group

Table 1  Cross resistance: distribution of individuals according to the number of drugs within one class to which their viral isolates showed >10-fold phenotypic or genotypic resistance. NNRTI are omitted because of insufficient data

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and most of them were previously treated with DDI and/or D4T. A similar finding was reported by Shafer et al who reported four heavily pretreated patients: all patients were taking their respective antiretroviral therapy with AZT and later added or substituted other drugs over a period of 4–9 years. In all four cases high level resistance to AZT and 3TC but low level resistance against DDI, DDC, and D4T were reported. Two facts may be considered for explanation: AZT had been given as the first antiretroviral drug to most of our patients and duration of treatment was more than twice as long as for the other drugs.

It is conceivable that the resistance pattern against AZT developed first and persisted in the further course of infection, and further mutations eventually appearing with DDI or D4T therapy were no longer drug specific. Furthermore, it should be discussed whether the use of modified cut-off values could explain discrepancies between resistance test results and clinical success of antiretroviral therapy. Only recently new cut offs for phenotypic resistance analysis have been introduced (for example, 3.5 instead of 4.0 for DDI and DDC; 3.0 instead of 4.0 for D4T). In a recent publication, analysis of 5000 clinical samples using these cut-off values demonstrated that resistance to D4T increases by 5.4%, to DDI by 3%, and to DDC by 2%. Additional investigations should be performed to clarify impact and effect of new cut offs regarding response to antiretroviral therapy. Another explanation could be, that resistance to DDI, D4T, and DDC is not detectable with resistance tests used in this study. Earlier studies indicate that resistance to these substances is difficult to detect and that mutations, commonly associated with AZT resistance, recently called TAMs or NAMs, appear during therapy with these drugs. Susceptibility to nucleosides is known to depend on inconstant levels of cellular phosphokinases, an effect that is not taken into account in resistance tests. Addition-ally, it is possible that AZT and 3TC fail because resistance develops, while other RTIs fail because of adherence or for pharmacokinetic reasons. In recently performed studies at our institution we found self reported adherence not different between patients taking AZT and/or 3TC and those taking DDI, D4T, and/or DDC (data not shown). Generally, pharmacokinetic reasons are difficult to assess because therapeutic drug monitoring procedures for RTIs are not fully adequate. This is because of difficulties with the measurement of active metabolites (intracellular triphosphates), which would be the relevant measurement targets.

Resistance guided switch of therapy led to better results in patients who had resistant isolates against two or more drugs in the previous regimen, compared to those with less than two drugs, when both were switched to a regimen containing three susceptible drugs after testing (fig 1B).

Absolute reduction of the viral load was limited even in patients switched from a regimen with two or more resistant drugs to three
susceptible drugs—that is, DDI, D4T, and a PI or NNRTI. It must be noted that subjects switched to a regimen with two or more susceptible drugs after testing remained on the same regimen considerably longer than patients who were not able to switch to a regimen with at least two new drugs. Taking all these results into account, it seems that patients will benefit most when resistance tests are performed at earlier disease stages—that is, after first failure of therapy. In therapy naive patients, resistance testing can detect primary resistance mutations. After failure of initial therapy, in many cases, only few resistance mutations are present and the virus population is relatively homogeneous; in these cases resistance tests offer a good chance for selecting a suppressive regimen. By increasing the number of consecutive therapy regimens and increasing the number of resistance mutations, the mutational landscape in clinical isolates becomes more complex, so does interpretation of resistance test results. Guidance taken from treatment history is highly valuable at this stage. In later stages of disease the virus has accumulated broad cross resistance to nearly all available drugs. The goal of complete suppression of viral replication may be less important at this stage; it might be more important to maintain a certain selection pressure towards less aggressive mutants.

In summary, resistance analyses seem primarily of value in first or second switch of therapy or to select a primary regimen.

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