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Short communication

Virological and immunological stability in HIV infected patients undergoing partial-treatment interruption

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ABSTRACT

Background: Partial-treatment interruption in patients with drug-resistant viremia has been associated with stable HIV RNA levels suggesting that interruption of protease inhibitors may be an effective strategy for patients without other therapeutic options while waiting for the development of new drugs.
Objective: Our goal was to maintain virological and immunological stability in patients experiencing virologic failure with multiresistant HIV to allow access to newly developed antiretroviral drugs, and to characterize the impact of partial-treatment interruption on replication capacity and resistance profile.
Study design: From 2003 to 2004, a group of 12 heavily treated patients was studied. Protease inhibitor treatment was interrupted and patients were treated with nucleoside analog retrotranscriptase inhibitors (Trizivir) and the fusion inhibitor Enfurvirtide to establish the therapeutic benefit and the virologic response.
Results: Both, CD4 T-cell counts and viral load remained stable for a period of time that enabled all the patients to access rescue treatments (median = 13.5 months; IQR: 9–19). The replication capacity of the patient-derived viruses significantly decreased or remained stable during the partial-treatment interruption. The decrease in replication capacity was mainly attributable to the selection of viruses carrying at least two fewer minor mutations in the protease. As of December 2008 10 of 12 patients maintained undetectable HIV RNA levels.
Conclusions: Study results indicate that a partial-treatment interruption regimen based on Trizivir with Enfurvirtide augmentation allows for a loss of protease inhibitor resistance mutations as well as for a decrease in the replication capacity of patient-derive HIV protease gene recombinants.

1. Background

Virologic failure is associated with progressive immunological deterioration. However, short-term immunological stability can be maintained despite ongoing viral replication when a less fit virus population is selected.[1,2] Resistance mutations allow the virus to replicate in the presence of drugs with a decreased replication capacity.[3,4] There is a correlation between replication capacity and treatment outcome,[5] and it may predict disease progression.[6]

Therapeutic options for heavily treated patients with multiresistant viruses are limited. Discontinuation of all antiretroviral drugs is detrimental to clinical outcome, leading to the emergence of drug-sensitive viruses with increased replication capacity, and subsequent immunologic deterioration and progression of the disease.[7–9] Partial-treatment interruption of the protease inhibitors (PIs) in patients with drug-resistant viremia is associated with stable HIV RNA levels in most subjects[3,10,11] suggesting that partial-treatment interruption of PIs may be effective for patients without other therapeutic options.

2. Objectives

We conducted a study with the objective of maintaining heavily treated patients in virological and immunological stability to allow for further access to new antiretroviral drugs. Therapeutic benefits and virological responses in HIV-infected individuals who continue to receive a controlled partial-treatment interruption of PIs were characterized. To date, no study has addressed the effect of partial-treatment interruption of PIs performed at predetermined intervals using both genotypic and phenotypic tests.

Abbreviations: HIV, human immunodeficiency virus; PI, protease inhibitor; RTIs, retrotranscriptase inhibitors; AZT, zidovudine; 3TC, lamivudine; ABV, abacavir; TZV, zidovudine plus lamivudine plus abacavir; T20, Enfurvirtide; TDF, tenofovir; PR, protease; RT, retrotranscriptase.

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3. Study design

3.1. Patients

This prospective, observational study was performed at the Virgen del Rocío University Hospital between 2003 and 2004. Patients were included if they: (i) failed multiple regimens of highly active antiretroviral therapy and experienced virological failure (viral load above 500 copies/ml) during the preceding 6 months; (ii) had multiple resistance mutations; (iii) had 12 months of previous protease inhibitor-based therapy. Exclusion criteria were active opportunistic infection, poor adherence, or treatment interruption of more than 15 days before the study. After partial-treatment interruption, patients were maintained on a combination of TZV [zidovudine (AZT) plus lamivudine (3TC) plus abacavir (ABV)] with enfurvirtide (T20). The strategy was stopped if viral load increased by 1 $\log_{10}$ or if CD4 T-cell counts decreased by 50% from baseline levels. Rescue treatments were administered based on the availability of new antiretrovirals, the patientís pharmacological history and resistance tests. This study was approved by the Institutional Ethics Committee and patients gave signed informed consent.

3.2. Clinical and laboratory evaluations

Plasma samples were obtained for determinations of the viral load (Amplicor HIV Monitor, Roche, Basel, Switzerland) and CD4 T-cell counts. Information was collected from patients at baseline (time 0), after 6 months and at the end of the interruption. In samples were a sufficient quantity of plasma remained after the above testing, replication capacity and genotyping testing were performed (5 of 12 patients). Genotyping was performed using the TruGene Genotyping System (Bayer Healthcare) and resistance was determined according to the 2008 International AIDS Society recommendations.

3.3. Recombinant viruses

Plasma viral RNA isolation was performed using the Viral RNA Kit (QIAGEN, CA, USA) and used for reverse transcriptase-PCR using SuperScriptIII System (Invitrogen Corporation, CA, USA) with specific primers for POL, PR and RT.[12] PCR products were cloned into the proviral vectors pNL4-3LacZ/polRen, pNL4-3LacZ/PRRen and pNL4-3LacZ/RTRen, sequenced (Secugen, Madrid, Spain), and used for transfection to produce recombinant viruses as previously described.[12]

3.4. Replication capacity assay

Recombinant viruses replication capacity was determined using a multiple-cycle assay as described previously.[12] Relative replication capacity was determined by comparing luciferase levels of recombinant viruses and the wild type virus (control NL4-3Ren).[13]

3.5. Statistical analysis

For all statistical tests a p-value ≤ 0.05 was considered significant. Statistical analyses were performed using SPSS version 14.0 (SPSS, Inc., Chicago, IL).

4. Results

4.1. Patient characteristics and follow up

Baseline characteristics of the patients are summarized in Table 1. For all patients, previous antiretroviral therapy before partial-treatment interruption and interrupted PIs are shown in Table 2. As new antiretrovirals became available, patients were changed to new regimens (Fig. 1), and as a result, in December 2008, 10 had undetectable viral loads. Patient R5 died 8 months after initiation of partial-treatment interruption due to pneumonia secondary to the treatment of a cytomegalovirus infection. Throughout the 8 months of follow up the patient remained virologically and immunologically stable.

Table 1
Demographic and clinical characteristics of the patients at the time of inclusion.

Characteristic	$n = 12$
Age, years [median, (IQR)]	42 (38–50)
Male [no. (%)]	12 (100%)
Risk factor for HIV [no. (%)]	
Sexual contact	7 (58.3%)
Infection drug user	5 (41.6%)
CDC clinical category (no.)	
2/3	2/10
Time on HAART [median months, (IQR)]	83.5 (60.5–86.5)
CD4/μl [mean (range)]	293 (91–628)
HIV-RNA $\log_{10}$ copies/ml [mean (range)]	5.38 (4.17–6.08)
Lowest CD4/μl [no. (%)]	
350–200	2 (16.6%)
199–50	5 (41.6%)
<50	5 (41.6%)
Highest recorded HIV RNA level ($\log_{10}$ copies/ml) (median; IQR)	5.39 (5; 5.79–16)
NRTIs ever prescribed (median, IQR)	7 (6–7)
NNRTIs ever prescribed (median, IQR)	1.5 (1–2)
PIs ever prescribed (median, IQR)	5 (4.75–6)

Abbreviations: IQR, interquartile range; CDC, Centers for Disease Control and Prevention definition and staging; category 2, no AIDS-defining conditions and either CD4+T-lymphocyte count 200–499 cells/μl or CD4+ T-lymphocyte percentage of total lymphocytes 14–28; category 3, CD4+ T-lymphocyte count of <200 cells/μl or CD4+ T-lymphocyte percentage of total lymphocytes of <14 or documentation of an AIDS-defining condition.

4.2. Dynamics in CD4 T-cell counts and viral load

Patient characteristics at baseline, 6 months and at the end of the partial-treatment interruption and treatment are shown in Table 2. There were no statistically significant changes in either CD4 T-cell counts or viral load, indicating that patients remained virologically and immunologically stable after partial-treatment interruption.

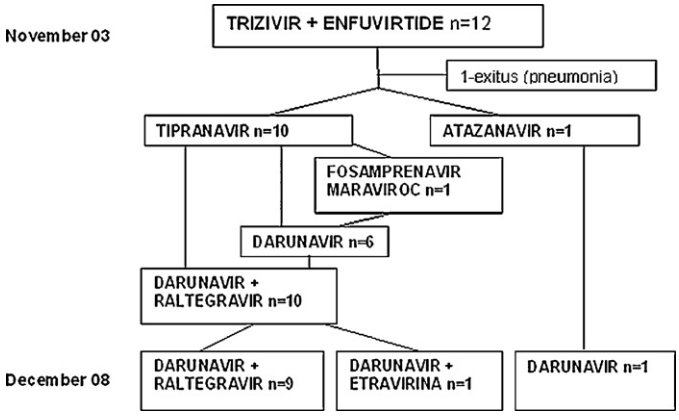

Fig. 1. Patient treatment follow up from November 2003 until December 2008.