-DUAL NUCLEOSIDE THERAPY FOR HIV INFECTION: ANALYSIS OF RESULTS AND FACTORS INFLUENCING VIRAL RESPONSE AND LONG TERM EFFICACY

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We performed a retrospective analysis of our experience with dual nucleoside regimens to look for predictors of long term benefit. We evaluated a cohort of 68 HIV-infected patients treated at 3 Italian hospital-based facilities. The results were analysed using univariate and multivariate statistical analyses. Fourty-three males and 25 females were treated for 22 ± 14 months. Sixty three patients (92.6%) suffered no or low-grade side-effects. Thirty-four patients (50%) reached a viral load <400 copies/ml (undetectable). Viremia remained persistently undetectable in 9 cases (13.2%). Relapsing-remitting of viremias were seen in 13 patients (19.1%) even though their therapies were not modified. Eight patients (11.8%) showed relapsing viremias persistently around or below 10,000 copies/ml. All patients reaching undetectable viremia but one showed increasing or stable CD4+ cell counts. Factors predicting favourable response were: pre-treatment CD4+ T-cells >150/μl pre-treatment viremia <50,000 copies/ml, pre-treatment lymphocytes >1,500/μl, and no previous exposure to NRTI. Total lymphocyte counts and CD4+ T-cells showed a significant correlation. Dual NRTI regimens may be still considered for patients unable to tolerate HAART regimens and presenting with favourable predictors of response.

Maximal long-term suppression of HIV replication in HIV-infected patients represents the major goal of antiretroviral therapy. It allows progressive and sustained recovery of immune function, thus preventing disease progression, AIDS-defining events and death (1,2). Persistently undetectable viremia have been achieved in 40% to 50% of patients on highly active antiretroviral therapy (HAART) regimens, which included three or more drugs: one or two protease inhibitors (PI) and/or one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) (3,4). The same result has also obtained in a smaller proportion (10% to 20%) of patients treated with a dual nucleoside regimen: associations of currently available Nucleoside Reverse Transcriptase Inhibitors (NRTIs) (5-9).

An increasing body of evidence indicates that HIV eradication is still out of reach even when the most powerful drug combinations are used (10-12). A residual low-level of viral replication can be detected with highly sensitive molecular techniques and/or at specific sites, such as lymph nodes. Therefore, the probability of selecting drug-resistant HIV strains is increased thus favoring high-level viremia relapses in the long run.

Long-term compliance with HAART regimens

Key words: dual nucleoside therapy of HIV infection, nonadvanced HIV infection, relapsing-remitting HIV viremia

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is difficult because of their complexity and frequent side effects (13). Furthermore, they cause metabolic disturbances (lipodistrophy, dislipidemia, and hyperglycemia) more frequently than dual NRTI regimens (1-2, 14-15). Side-effects may hamper adherence in a large proportion of patients, allowing selection of multi-drug resistant HIV strains and jeopardizing the long-term efficacy of powerful associations of drugs.

Under such circumstances, antiretroviral therapy with two NRTIs may still represent an option to control HIV infection in patients who are deemed unable either to adhere to or to tolerate more complex therapeutic regimens and, at the same time, likely to reach sustained control of viral replication with simpler regimens.

In the present study we retrospectively evaluated the outcome of 68 patients infected with HIV-1 who were treated with dual NRTI regimens at our Institutions. We looked for factors predicting long-term virological as well as clinical efficacy of dual regimens in this cohort.

MATERIALS AND METHODS

We reviewed the medical records of all patients under routine clinical management in three Infectious Disease Units located in central Italy. We considered the records of all patients who started their first-line combination antiretroviral therapy with a dual NRTI regimen from October, 1996 through December, 1999 and analysed all those who fulfilled the following inclusion criteria: chronic HIV-1 infection confirmed by Western Blot testing, at least one measurement of total peripheral lymphocytes, CD4+ and CD8+ peripheral T-lymphocytes and plasma HIV viral load in the 12 weeks preceding the start of dual NRTI therapy, and data relative to at least two of the 3-month follow-ups.

Cell counts were performed according to standard techniques. Plasma HIV RNA quantification was performed by PCR using standardized technology (Amplicore® Roche), with a lower limit of quantification of 400 copies/ml.

Specific nucleoside combinations were prescribed on the basis of drug availability and the patient's decision after appropriate counselling by caregiver. The patients were not randomized for the treatment programs nor did they participate in a prospective study. Most of the patients started dual NRTI regimens at a time when it was the only available treatment strategy at the study centres and preferred to continue even after PI and NNRTI became available. Seven patients were prescribed a dual NRTI regimen during 1998 and 1999 as they refused a 3-drug regimen. All patients were free to shift to more complex regimens at any time of follow-up and were frequently offered counselling about alternative treatment options. Compliance was assessed during the three-month clinical checks by counting the number of capsules that were not assumed (if >15% of the capsules were not taken then the compliance was rated as poor) (16).

Disease progression was defined as the occurrence of one major AIDS-defining event or death.

Demographic factors (age, sex, duration of infection, socio-economical status, type of employment), behavioural factors (intravenous drug addiction and adherence to therapy), as well as CDC status, previous exposure to NRTI, and coinfection with chronic hepatitis viruses were evaluated as possible factors influencing long-term efficacy of dual nucleoside therapy. The thresholds for the quantitative variables were determined in accordance with current international literature (1-2).

Statistical Analysis

Previous exposure to antiretroviral agents, CDC status, adherence to treatment, pre-treatment viral load, pre-treatment total lymphocyte counts, CD8+ T-cell counts, CD4+ T-cell counts and hepatic comorbidity were studied to see whether they were associated with viremia.

Univariate and multivariate statistical analyses of data were carried out. Qualitative variables were described as relative frequency and percentage of responders (viremia <400 copies/ml at least once) and non-responders (viremia >400 copies/ml) groups. The χ^2-test corrected for continuity, χ^2-test for trend and Fisher's exact test were used for comparing proportions of two groups. The multiple linear logistic regression model was used to assess which modalities of the pre-treatment variables studied influence the positive response to dual NRTI regimens. The model included the presence of specific demographic, behavioural, clinical and analytical features as indipendent variables, and the outcome variable, responders or non-responders (0 and 1, respectively) as dependent variable. This analysis was used to estimate the odds ratio (OR) and its 95% confidence interval (95% CI), adjusted for the other variables in the model. Data analysis was performed using the SPSS® statistical package.

RESULTS

The distribution of demographic and clinical data and relevant results of statistical analysis according to NRTI antiretroviral therapy response (viremia) for the 68 patients are summarized in Tables I and II.

Fourty-three were males and 25 females, 31 patients were intravenous drug users (IVDU). Forty six patients (67%) were stage A (asymptomatic), 13 (19%) stage B (symptomatic) and 9 (14%) stage C (severe sign and symptoms) of the CDC classification.

Patients were treated with dual NRTI regimens for an average of 22 ± 14 months (range from 6 to 44 months). Treatment–combinations were Zidovudine + Didanosine in 15 patients (22.1%), Zidovudine + Lamivudine in 38 patients (55.9%), Zidovudine + Zalcitabine in 12 patients (17.6%), Stavudine + Lamivudine in 2 patients (2.9%) and Stavudine + Didanosine in the remaining patient (1.5%).

Eleven patients modified their NRTI treatment due to gastro-intestinal intolerance or other early low-grade toxicity within the first three months of treatment, and continued their second assigned combination for the rest of their follow-up period. Sixty-three patients (92.6%) suffered no or only low-grade side-effects at any time thereafter. In no case was dual therapy interrupted due to drug-related adverse events.

Forty-four patients (64.7%) were rated compliant and 24 (35.3%) as poorly compliant. The latter group was composed more frequently by IVDUs (10 patients, 77%).

Sex, socio-economic status, type of employment, and IVDU status did not have a statistically significant influence on virologic response. Subjects with a history of infection lasting >10 years included a statistically insignificant

Variables (pre-treatment)	Viremia <400 copies/ml (n=34) N (%)	Viremia ≥400 copies/ml (n=34) N (%)	χ^2-test p-value
Age[a]	38.9 (25-65)[a]	39.1 (26-64)[a]	
Sex			
Male	20 (58.8)	23 (57.6)	0.253*
Female	14 (44.2)	11 (32.4)	n.s.
Duration of infection			
<5 yrs	16 (47.1)	10 (29.4)	6.22†
5-10 yrs	15 (41.1)	11 (32.3)	p<0.05
10-15 yrs	3 (8.8)‡	13 (38.3)	
Socio-economic status			
High income	10 (29.4)	16 (47.1)	1.36†
Average income	21 (61.8)	15 (44.1)	n.s.
Low to very low in.	3 (8.8)	3 (8.8)	
Employment			
Autonomous	11 (32.4)	14 (41.2)	0.17†
Dependent	10 (29.4)	7 (20.6)	n.s.
Other	13 (38.2)	13 (38.2)	
IVDU status			
Yes	16 (47.1)	15 (44.1)	0.01*
No	18 (52.9)	19 (55.9)	n.s.
CDC status			
A	23 (67.6)	23 (67.6)	0.19†
B	7 (20.6)	6 (17.6)	n.s.
C	4 (11.8)	5 (14.8)	

Tab. I. *The association between responders (<400 copies/ml) and non-responders (≥400 copies/ml) to dual NRTI antiretroviral therapy and demographic variables for the 68 HIV-1 infected patients.*
*[a]expressed as mean (range), *χ^2-test corrected for continuity; †χ^2-test for trend; ‡p<0.05 Fischer's exact test (10-15 yrs versus 5-10 and <5 yrs of infection).*

lower proportion of patients reaching <400 c/ml (see Tab. I).

Twenty-eight patients (41.2%) had never been exposed to any antiretroviral drug prior to starting a dual NRTI regimen. Thirty-nine of the remaining 40 patients had been treated with Zidovudine for an average of 28.7 months. Eight patients had also been treated with Didanosine or Zalcitabile as a second line monotherapy for an average 12.5 months. When a dual NRTI regimen was started, a second NRTI was associated to ongoing or re-prescribed Zidovudine in 36 cases, whereas both NRTI were replaced in 4 cases.

Thirty-four patients (50.0%) gained a viral load <400 copies/ml after starting their dual NRTI treatment. Twenty four patients (70.6%) became aviremic by month 6 of treatment, 5 more patients by month 9, whereas the remaining 5 patients took up to 33 months to reach undetectable viremia. Therapeutic regimens were neither changed nor intensified whenever clinical and immunological parameters indicated a stable or improving condition even in the presence of low-level, detectable viremia.

Overall, 3 patients (4.4%) had disease progression during follow-up (2 cases of wasting and one case of esophageal candidiasis. A steep decrease in CD4+ T-cell counts was observed in 4 additional patients during follow-up.

Being naive for antiretrovirals, having a viral load <50,000 copies/ml, having a total lymphocyte

Clinical Variables	Viremia		χ^2-test p-value
	<400 copies/ml (n=34) N (%)	≥ 400 copies/ml (n=34) N (%)	
Pre-treatment viral load			
<50,000	21 (61.8)	11 (32.4)	4.78*
>50,000	13 (38.2)	23 (67.5)	p<0.05
Pre-treatment lymphocyte count			
<1,500	17 (50.0)	26 (85.3)	4.05*
>1,500	17 (50.0)	8 (14.7)	p<0.05
CD4+ T-cell count			
<150	1 (2.9)‡	12 (35.3)	7.17†
150-400	26 (76.5)	20 (58.8)	p<0.01
>400	7 (20.6)	2 (5.9)	
CD8+ T-cell count			
<500	7 (20.6)	10 (29.4)	0.82†
500-900	17 (50.0)	14 (41.2)	n.s.
>900	10 (29.4)	10 (29.4)	
Previous exposure to NRTI			
No	19 (55.9)	9 (26.5)	4.92*
Yes	15 (44.1)	25 (73.5)	p<0.05
Compliance with treatment			
Full (>85%)	24 (70.6)	20 (58.8)	0.58*
Inadequate (<85%)	10 (29.4)	14 (41.2)	n.s.
Co-infection with HCV			
Yes	22 (54.7)	19 (55.9)	0.25*
No	12 (35.3)	15 (44.1)	n.s.

Tab. II. *The association between responders (<400 copies/ml) and non-responders (≥400 copies/ml) to dual NRTI antiretroviral therapy and clinical variables for the 68 HIV-1 infected patients.*
χ^2-test corrected for continuity;
† *χ^2-test for trend;*
‡ *p<0.05 Fischer's exact test (CD4+ T-cell counts : >400 versus <150 and 150-400).*

Variable (Pre-treatment values)	OR* (95% CI)
CD4+ T-cell counts	
>400†	1
150-400	1.64 (0.61-2.81)
<150	17.54 (2.03-51.83))
viral load	
<50,000†	1
>50,000	8.48 (1.81-18.52)
total lymphocyte count	
>1,500†	1
<1,500	5.09 (1.69-37.24)
Previous exposure to NRTI	
No†	1
Yes	3.52 (1.16-26.60)

Tab. III. *Multiple logistic regression model for risk factors associated with lack of response to dual NRTI therapy.*
** adjusted for sex, duration of infection, CDC status, CD8+ T-cell count, and compliance to treatment.*

count >1,500 /μl, and having a CD4+ T-cell count >150/μl at baseline had a statistically significant association with favourable virologic response (Tab. II).

A multiple logistic regression model confirmed the statistical significance of predictors revealed by univariate analysis (Tab. III). The highest odds ratio for a lack of virologic response was associated with having very low CD4+ T-cell counts (<150 / μl) (OR= 17.54; 95% CI= 2.03-51.83), followed by having a viral load >50,000 copies/ml at baseline (OR= 8.48; 95% CI= 1.81-18.52), having total lymphocyte counts <1,500 /μl (OR= 5.09; 95% CI= 1.69-37.24) and having been exposed to previous antiretroviral therapy (OR= 3.52; 95% CI= 1.26-26.6).

DISCUSSION

Optimal antiretroviral treatment of HIV infection is still a matter of open debate (1,2,16). Present international guidelines suggest that regimens should be aimed at maximal efficacy whenever instituted. This means the use of 3 or more drugs for all viremic patients with >30,000 – 60,000 copies/ml and a CD4+ T-cell count of <350/μl (1-2). These indications are based on evidence that maximal suppression of HIV replication gives the best chances of long term control of HIV infection (1-2). Until recently, such an approach was reinforced by the expectation that HAART might ultimately lead to HIV eradication in treated patients (1-2). World-wide experience with current therapeutic options, however, has led to the conclusion that HIV has the potential of eluding all current efforts aimed at its eradication (1-2,10-12).

Complex therapeutic regimens including 3 or more drugs have been proven to have a reduced efficacy in a clinical setting compared to an experimental setting. This is due to the difficulty that most patients face in maintaining multidrug regimens: daily life activities and relevant metabolic side effects hamper long-term compliance (13-15). As a matter of fact, present HAART regimens seem to hold the potential for maximal control of viremia only in one half of treated patients, rather than in 70% to 90% of patients, as indicated in many clinical trials after 24 or 48 weeks of treatment (1-2).

On the other hand, a parallel line of evidence has recently gathered remarkable information as to the role of HIV-specific CD4+ and CD8+ T-cell responses in controlling HIV infection. Patients successfully on HAART, when exposed to recurrent bursts of relapsing viremia, as during planned or unplanned interruptions of antiretroviral therapy, may gain strong CD4+ T-cell responses against to endogenous HIV-1 in a significant proportion of cases (17-20). This HIV-1 specific cell-mediated immunity seems to be crucial in controlling HIV-1 replication and is very low or absent both in patients who progress towards end-stage immunodeficiency and in those who remain persistently aviremic while on HAART (17-20).

Finally, data from various sources around the world show that a considerable proportion of patients gain progressive quantitative as well as qualitative reconstitution of immune function, as evidenced by T-cell subset measurements and functional assays, in spite of persistently detectable viral replication under antiretroviral treatment (21, 22, 23).

In view of such considerations, we decided to

perform a retrospective analysis of our experience with dual NRTI therapy in a cohort of 68 HIV-infected patients treated for nearly two years at our Institutions. Data on prospective follow-up from similar cohorts of patients on dual nucleoside regimen have been recently reported in the literature (5,6,8,9,23-25). Most of these patients had non-advanced HIV infection and were observed for comparable lenght of time.

Our results are in good keeping with those reported by others. First, some 15% of patients reached undetectable viremia 3 to 6 months after the start of dual therapy and remained persistently aviremic thereafter (5-6, 8-9).

Second, 50% of patients reached undetectable viremia at least at one checkpoint after starting therapy. For some of them, however, this took much more than the expected 3 to 6 months, and in one case undetectable viremia was obeserved 33 months after starting therapy. Progressive improvement of clinical status as well as of CD4+ T-cell counts pushed us to monitor these patients over time without changes of therapy, which has allowed such an observation.

Third, pre-treatment viremia <50,000 copies/ml, CD4+ T-cell counts >150/μl and lack of previous exposure to antiretroviral drugs at the time of starting dual NRTI regimens were all conditions independently associated with virologic response, as documented by results of the logistic regression model of multivariate statistical analysis and in keeping with other authors (5-6,8-9,23-25). Interestingly, CD4+ T-cell counts <150 /μl were at least as relevant predictors of poor outcome as high pre-treatment viremias, in line with recent evidence that cell mediated immunity may play a relevant role in controlling HIV replication (17-20) and that in very advanced immune disruption HAART regimens including PIs may offer additive and still unexplained potential for immune recovery (1-2,26).

We also looked for the potential predictive value of total lymphocyte counts as a surrogate marker for CD4+ T-cell counts. Total lymphocytes indeed were quite informative and tightly correlated to CD4+ T-cell counts, which may be of relevance for the management of HIV-infected patients in resource-poor settings, where complicated or expensive cytometric analyses of T-cell subsets may be yet unaffordable (27).

Our observation, however, highlights other interesting information. Most of the responders whose viremia did relapse fell into 2 categories. A relevant proportion of them re-gained undetectable viremia without any change of antiretroviral therapy. Their CD4+ T-cell counts were stable or increased at the end of follow-up, and their clinical status similarly stable or improved. To the best of our knowledge, although limited to a small number of patients, this is the first report of such an outcome. The second category is represented by relapsers who constantly showed low-level viremias after relapse, again with stable or improved clinical and immune parameters. This peculiarity in our series is likely due to the fact that most clinicians would have shifted such patients to a three or four-drug second line regimen as soon as viremia relapsed (1,2). Our conservative attitude proved safe, however, as only 1 patient among those who reached undetectable viremia progressed to a more advanced HIV-related disease during follow-up. To further assess the safety of this conservative management of HIV infection we are planning to evaluate to what extent patients still on dual NRTI regimens at our Institutions (23 out of 68, 33.8%) may have accumulated mutations in RT (and PI) transcripts hampering or jeopardizing the choice of a second-line therapeutic regimen (28).

As to the biological and pathogenetic meaning of such findings, they may well be related to HIV-1 specific T-cell responses. Relapsing HIV replication during dual NRTI therapy may booster HIV-1 specific T-cell defences, ultimately leading to more efficient control of viral replication (17-20, 29-30). This has been hypothesized when therapy was interrupted after early treatment of acute infection by HIV (30).

In conclusion, dual NRTI regimens may still represent a valuable option for a low-risk subset of HIV infected patients, unexposed to antiretroviral agents, with preserved CD4+ T-cell counts and low viremia, if unable to adhere to more labour-intensive associations of drugs or at high risk of drug-related toxicity. Availability of such a choice may be particularly relevant in resource-poor settings around the world.

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