Unexpected CD4 cell count decline in patients receiving didanosine and tenofovir-based regimens despite undetectable viral load

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\textbf{Background}: We recently observed a significant CD4 cell count decline in patients receiving didanosine (ddI) 400 mg, tenofovir (TDF) and nevirapine (NVP), despite virological suppression.

\textbf{Methods}: We identified from our computerized patient database subjects who initiated combinations containing ddI and/or TDF for reasons other than virological failure, including simplification or intolerance. Changes in total, CD4+ and CD8+ lymphocyte counts since the initiation of therapy were analysed retrospectively. Plasma concentration of ddI was prospectively determined in eight of these patients receiving ddI 400 mg + TDF + NVP and 3 weeks after a ddI dosage reduction.

\textbf{Results}: A total of 302 patients were studied. A significant decrease in CD4 and CD8 and in total lymphocyte counts was only seen in subjects receiving ddI standard dose + TDF-containing regimens, despite the maintenance of viral suppression. More than 50% of these patients showed a decline of more than 100 CD4 cells at 48 weeks. In contrast, subjects not receiving ddI + TDF together experienced the expected progressive increase in CD4 T-cell counts. Plasma levels of ddI were elevated in all patients receiving the standard ddI dose + TDF. DdI plasma levels significantly decreased when patients weighting > 60 kg reduced ddI dose to 250 mg, achieving similar levels to those generated by ddI 400 mg without TDF.

\textbf{Conclusions}: Co-administration of ddI at standard doses plus TDF appears to exert a deleterious effect on CD4 and CD8 counts. Although lymphocyte toxicity related to excessive ddI plasma levels could explain our findings, other mechanisms cannot be excluded. Pharmacokinetic data suggest ddI dose reduction when coadministered with TDF.

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Introduction

Tenofovir disoproxil fumarate (TDF) is the first commercialized acyclic nucleotide reverse transcriptase inhibitor. Administered as a single tablet once daily, TDF has activity against wild-type and most nucleoside-resistant HIV-1 and against HIV-2 [1]. In addition, in vitro studies with TDF have shown a weak inhibition of mitochondrial polymerase γ, suggesting a low mitochondrial toxicity [2]. Finally, TDF has additive or synergic in vitro activity with other antiretrovirals, permitting its use as a part of different antiretroviral approaches. Co-administered with other once daily antiretroviral agents such as didanosine (ddI), it is a good alternative as simplification strategy.

Recently, pharmacokinetic interactions have been reported between TDF and ddI [3]. When 400 mg of this analogue is co-administered in combination with TDF and food, a 60% increase in plasma ddI area under the curve (AUC) has been reported [3]. Accordingly, there is a concern about both short- and long-term toxicities associated with ddI + TDF-containing regimens, such as peripheral neuropathy or pancreatitis. However, neither changes in intracellular ddI levels, nor the ddI-related toxicity of this combination are well known [4].

We have observed a significant decrease in CD4 cell counts during the clinical follow-up of patients receiving the standard dose of ddI + TDF + nevirapine (NVP), despite the maintenance of viral suppression. This finding prompted us to investigate the effect of different TDF and/or ddI-containing regimens on CD4 cell counts.

Methods

Patients

We identified, from our patient computerized database, patients who initiated any of the following combinations for reasons other than virological failure (mainly simplification or intolerance):

Group 1 (n = 86): ddI 400 mg + TDF + NVP

Group 2 (n = 23): ddI 400 mg + TDF + efavirenz (EFV)

Group 3 (n = 20): ddI 400 mg + TDF + lopinavir (LPV)/ritonavir (rtv)

Group 4 (n = 21): ddI 250mg + TDF-based regimens (all patients weight < 60 kg).

Group 5 (n = 33): TDF-based regimens not including ddI.

Group 6 (n = 69): ddI 400 mg (250 mg/day in subjects weighting < 60 kg) + lamivudine (3TC) + NVP

Group 7 (n = 50): ddI 400 mg (250 mg/day in subjects weighting < 60 kg) + stavudine (d4T) + NVP

Patients exclusion criteria were: viral load rebound during therapy, concomitant therapies with immune suppressors such as hydroxyurea, interferon and ribavirin, or with immune modulators like interleukin-2, as well as improper adherence, and interruption or discontinuation of treatment.

Data collection

Changes in total lymphocytes, CD4 and CD8 T cells, leukocytes, neutrophil, haemoglobin and platelets, 48 weeks before and during the 48 weeks after the initiation of therapy, as well as antiretroviral-related adverse events reported during the study, were analysed retrospectively.

In a subgroup of eight patients receiving ddI + TDF + NVP and not co-infected by hepatitis viruses, plasma ddI concentrations were prospectively determined just before the drug intake (administered together with meal) and at seven different time-points after drug administration (AUC12). The ddI dose in these patients was afterwards reduced from 400 to 250 mg/day. Three weeks after dose reduction, the pharmacokinetic analysis was repeated. Changes in CD4 T cells during the following 12 weeks were also determined.

Statistical methods

Standard descriptive statistics were performed. Differences in each group during follow-up were analysed with standard parametric (t-test, for equality of means), and non-parametric tests (Mann-Whitney test), as needed.

Results

A total of 302 patients out of 2000 HIV-1 infected subjects from our database, were included into the study. More than 75% of them achieved at least 48 weeks of follow-up. All participants in the current study maintained viral suppression during the follow-up since viral failure was an exclusion criteria.

During the 48 weeks before the initiation of ddI and/or TDF therapy, CD4 lymphocyte counts were similarly maintained or even increased in all study arms. After the initiation of therapy, only patients receiving standard ddI dose + TDF showed a significant decrease
in the mean absolute and percentage CD4 T-cell counts \((P = 0.05\) for group 1, \(P = 0.05\) for group 2, \(P = 0.048\) for group 3 and \(P = 0.035\) for group 4) (Fig. 1). Subjects in the remaining groups, not receiving ddl + TDF, experienced the expected progressive increase in the CD4 T-cell counts.

The percentage of patients decreasing, increasing or maintaining CD4 cell counts in each group is shown in Table 1. More than 50% of patients in groups including ddl + TDF experienced a decline in CD4 lymphocyte counts of more than 100 cells; up to 30% decreased more than 200 cells. Only eight subjects (15%) showed an increase. Conversely, up to 85–90% of those in ddl- or TDF-containing regimens remained unchanged or increased their CD4 T-lymphocyte counts.

Mean absolute and percentage CD8 cell count did not significantly vary except in groups 2 \((\text{ddl} + \text{TDF} + \text{EFV}, P = 0.038\) and 4 \((\text{ddl} 250 \text{mg} + \text{TDF}, P = 0.036\) (Fig. 1). Total lymphocyte count also decreased in groups 1 \((P = 0.04\), 2 \((P = 0.029\), 3 \((P = 0.034\) and 4 \((P = 0.05\). No significant changes in haemoglobin, total leukocyte, neutrophil and platelet counts were seen during follow-up.

Only 4% of patients receiving any ddl + TDF combination presented asymptomatic grade 2 (>1.5–2 fold limit superior normality) amylase increases, leading to ddl discontinuation. Polineuropathy was found to be mild in 25% of subjects but moderate to severe in only 2% and led to ddl withdrawal. Unexpectedly, xerostomia appeared in 10% of patients under ddl + TDF-containing regimens; it was severe in only 5% and significantly ameliorated with ddl discontinuation. Among patients from the remaining groups, namely those groups not including the combination ddl + TDF, no cases of xerostomia were reported whereas one patient interrupted ddl due to pancreatitis and 4% of patients reported moderate-severe symptoms of ddl or d4T-related polineuropathy, leading to treatment discontinuation in 2%.

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**Fig. 1. Mean changes before and after the antiretroviral switch in absolute CD4 and CD8 T cells in different groups.** (a) Didanosine \((\text{ddl}) + \text{tenofovir (TDF)}\)-containing regimens: ddl + TDF + nevirapine \((\text{NVP})\) \((\text{G1}, n = 86)\); ddl + TDF + efavirenz \((\text{EFV})\) \((\text{G2}, n = 23)\); ddl + TDF + lopinavir \((\text{LPV})/\text{ritonavir (rtv)}\) \((\text{G3}, n = 20\) and ddl 250 mg + TDF-based regimen \((\text{G4}, n = 21)\) and (b) ddl or TDF-containing regimens: TDF-based regimen, without ddl \((\text{G5}, n = 33)\); ddl + lamivudine \((3\text{TC})\) + NVP \((\text{G6}, n = 69)\); ddl + stavudine \((\text{d4T})\) + NVP \((\text{G7}, n = 50)\).
Pharmacokinetic analysis demonstrated high ddI plasma levels when given at 400 mg dose + TDF + NVP, which exceeded those achieved when ddI was administered without tenofovir [4]. Didanosine dose reduction to 250 mg implied ddI plasma levels similar to those achieved with ddI 400 mg fasted without TDF. Briefly, median AUC decreased from 5220 ng/ml (range, 1760–8280) to 2340 ng/ml (range, 1590–4700). Median maximum concentration (Cmax) diminished from 1390 ng/ml (range, 640–3070) to 750 ng/ml (range, 300–1560).

Prior to ddI dosage reduction, mean CD4 cell count in these eight subjects decreased by 163 $\times$ 10^6 cells/l. Three months after initiating ddI 250 mg/day, mean CD4 counts increased 60 $\times$ 10^6 cells/l. In more detail, after ddI dose reduction, CD4 count significantly increased in six patients (mean increase of 136 $\times$ 10^6 cells/l), remained stable in two patients, and persisted decreasing in the remaining two subjects (mean decrease of 72 $\times$ 10^6 cells/l).

### Discussion

The present study shows that patients receiving regimens containing TDF and ddI at standard dose (400 mg/day in patients weighting $\geq$ 60 kg and 250 mg/day in subjects weighting < 60 kg) experience a significant decrease in the absolute and percentage CD4 and CD8 cell counts, despite the maintenance of viral suppression. More than 50% of these patients showed a decline of more than 100 $\times$ 10^6 CD4 cells/l at 48 weeks follow-up, and up to 30% of them of more than 200 $\times$ 10^6 cells/l. Importantly, CD4 cells depletion was also seen in patients weighing less than 60 kg who received ddI 250 mg/day and TDF. Other combinations including either TDF or ddI did not show such a decrease in the CD4 lymphocyte counts.

In our patients, CD4 cell counts remained stable or progressively increased during the 48 weeks before the switch to the ddI + TDF-based regimen. Indeed, the CD4 cell decline began to be evident 24 weeks after the switch. On the other hand, patients receiving TDF without ddI or vice versa did not show such decrease. Such results, together with other published data [5], discount the possibility that either TDF or ddI alone could be the causative agent of the lymphocyte decrease found in our study. Furthermore, extensive prior experience with ddI-containing regimens also refutes the suggestion that ddI alone may cause such CD4 cell decline [6,7].

The maintenance of the viral suppression despite the progressive CD4 cell reduction observed in these subjects, argues against the lack of antiviral potency of ddI + TDF-containing regimens. Such discordant response regarding virological and immunological results has previously been described in HIV-infected patients on highly active antiretroviral therapy [8,9], but it has been reported in less than 10%. However, a significant decrease in CD4 cell counts was observed in half of our patients.

Therefore, a plausible explanation for the observed decline in CD4 and CD8 counts could be some kind of lymphocyte toxicity that may be specifically related to the combination of ddI and TDF. In favour of this hypothesis is the fact that, only CD4 counts, CD8 and total lymphocyte counts decreased, whereas no significant changes were observed in other blood series.

According to our pharmacologic measurements, the excessive ddI plasma levels seen in patients receiving ddI + TDF may have exerted an influence on such lymphopenia, although other explanations cannot be discarded at present. Didanosine dose reduction lead to a decrease in ddI plasma levels, such as other authors have also recently reported [10,11], that was followed...
Our findings have a number of important clinical implications. First, ddI dosage adjustment should be considered when combined with TDF. Patients weighing >60 kg may reduce the ddI dose from 400 mg/day to 250 mg/day. Those weighing <60 kg may benefit from receiving 125 to 200 mg/day instead of 250 mg/day. In fact recent data has shown the equivalence of ddI 200 mg + TDF administered with food, with ddI 250 mg alone [11]. Such dose-reductions should diminish the degree of lymphopenia. Second, the decrease of CD4 counts related to the combination of ddI standard dose + TDF may have limited the immunological outcome achieved in previous salvage [12] or intensification approaches. Finally, a decrease in CD4 cell count could be perceived dramatically by the patient, because it is often the main reference of the activity of therapy, putting a proper adherence on risk.

Prospective controlled studies with a larger sample are needed to complement our results as well as to discern the pathogenic mechanism of such lymphocyte changes. Other nucleoside reverse transcriptase inhibitor combinations including TDF, should be also investigated in more detail. In addition, it seems important to understand the mechanism by which the co-administration of ddI and TDF leads to the important increase in plasma ddI currently observed. In fact, it is not clear at present, if this results from an increase in the absorption of ddI, a decrease in clearance or a modification of tissular distribution. Furthermore, the consequences of this pharmacokinetic interaction on the intracellular levels of ddI and TDF and their phosphorylated metabolites must be examined in detail. This will require extensive studies on the transmembrane transportation of both drugs and appropriate measurement of their intracellular triphosphate moiety in peripheral blood mononuclear cells from HIV-infected patients.

References