

Inflammatory effects of atazanavir/ritonavir versus darunavir/ritonavir in treatment naïve, HIV-1-infected patients

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RUNNING TITLE: Chronic immune activation and inflammation in HIV infection

Abstract

Limited studies have compared the impact of different antiretroviral regimens on soluble markers of inflammation with discordant results. In this prospective study, treatment naïve HIV-1-infected patients were included if they started their current regimen with atazanavir/ritonavir (ATV/r)(N= 73, Group 1) or darunavir/ritonavir (DRV/r) (N=85, Group 2) plus tenofovir/emtricitabine. The analysis of IL-6, MCP-1, sCD163, VCAM-1, ox-LDL and adiponectine was performed on two stored plasma samples, the first prior to antiretroviral therapy initiation and the second one year after initiation. The results of our analysis show a difference in ox-LDL between the two groups with higher mean (SD) values in ATV/r based group 608.5 ± 137.4 vs 519.1 ± 119.6 in DRV/r group , after controlling for baseline levels of ox-LDL as well as other potential confounding factors controlled by means of matching design or linear regression modelling. Our analysis provides further data examining the association between the modulation of vascular inflammatory and of activation markers with specific protease inhibitors-based treatments over one year of exposure to these drugs. The data show little evidence for an association, supporting the notion that antiretroviral regimens has generally poor efficiency in downregulating these soluble markers.

Key Words: vascular inflammation; activation markers; first-line antiretroviral therapy; atazanavir; darunavir; HIV

Introduction

Chronic immune activation and inflammation are a hallmark of human immunodeficiency virus (HIV) infection and represent a key component of HIV pathogenesis [1,2]. Such persistent immune activation has been shown to increase non-AIDS complications such as cardiovascular risk, loss of bone mineral density and neurocognitive disorders [3-7]. Antiretroviral therapy (ART) is able to induce the control of HIV replication, but immune activation persists in people living with HIV (PLWHIV) [8-11]. Limited studies have compared the impact of different ART on residual immune activation and soluble markers of inflammation with discordant results [12-19]. However, the effects of different ART on cellular or soluble markers of activation are unclear.

With this analysis we aimed to compare changes in soluble biomarkers of vascular inflammation/activation and the impact of an increase in bilirubin level in patients who started tenofovir/emtricitabine (TDF/FTC) plus atazanavir/ritonavir (ATV/r) or darunavir/ritonavir (DRV/r) based as their first-line ART. In particular, some markers that have not been previously thoroughly investigated in the literature.

Patients and methods

In this prospective study, treatment naïve HIV-1-infected patients enrolled in the ICONA (Italian Cohort Naïve Antiretrovirals) Foundation Study cohort were included if they started their current regimen with ATV/r (Group 1) or DRV/r (Group 2) plus TDF/FTC. Participants also need to have two stored plasma samples, the first prior to ART initiation and the second one year after initiation (time window: +9; +15 months) to be included. Exclusion criteria were patients with HIV-2 infection and HCV-RNA.

Patients receiving ATV/r or DRV/r were matched by age (± 5 years), gender, mode of HIV transmission, race, T CD4+ absolute cell count (groups as ≤ 200 , 201-350 and > 350), and a CD4/CD8 ratio (grouped as ≤ 0.3 , 0.4-0.7 and > 0.7).

Soluble markers

From frozen plasma the concentrations of Interleukin-6 (IL-6) pg/ml, Monocyte Chemoattractant Protein-1 (MCP-1) pg/ml, soluble (s) CD163 pg/ml, Vascular Cell Adhesion Molecule- 1 (VCAM-1) pg/ml and adiponectin pg/ml were measured by a bead-based immunoassay (AimPlex Premixed Multiplex Kit Human Custom 4-Plex for IL-6, MCP-1, sCD163, VCAM-1 determinations, Co. AimPlex Assay Kit Human Adiponectin respectively, from YSL Bioprocess Development) according to the manufacturer's instructions [20] using the FACS Canto II flow cytometer (Becton Dickinson, BD) equipped with the FACS DIVA version 6.0 software (BD). Plasma cytokine concentration was expressed in picograms per milliliter by FlowCytomix Pro Software (BD) [21]. Circulating plasma oxidized-low density lipoprotein (ox-LDL) measurements were performed by the enzyme-linked immunosorbent assay Merckodia Oxidized LDL ELISA, according to the manufacturer's instructions (Merckodia AB). Plasma ox-LDL concentration was expressed in mU/L.

Statistical analyses

Patients' characteristics were compared according to the type of third PI/r drug initiated (ATV/r vs. DRV/r) using Wilcoxon chi-square (for categorical variables) and nonparametric tests for comparison of median (for numeric variables). The 12-month biomarkers values were compared in the ATV/r vs. DRV/r recipient group by means of an analysis of covariance (ANCOVA) adjusted for baseline values. Markers were modelled on the 10-logarithmic scale to

satisfy the normal distribution assumption. The multivariable ANCOVA model included the following potentially confounding factors: age, sex, smoking, diabetes, HIV-RNA, and T CD4 baseline counts. The model with ox-LDL as the response variable was further adjusted for baseline LDL cholesterol and for statin use. A 'sensitivity analysis' was also performed after restricting the regression analysis to patients who had maintained the same pair of NRTIs (TDF / FTC) for the entire 12-month period.

Instead of removing the non-matched individuals we kept them in the analysis and performed an additional adjustment by including patients' characteristics as covariate in the ANOVA model. A repeated-measures one-way analysis which correctly handles the matched structure of the data are used.

Results

We enrolled 73 patients (62 males, 85%) in Group 1 (ATV/r) and 85 patients (77 males, 90%) in Group 2 (DRV/r). At treatment initiation (baseline) the median (IQR) T CD4+ cell count was 413 (281-589), and 332 (110-510) in Group 1 and 2, respectively ($p=0.05$). A statistically significant difference ($p= 0.005$) was found between two groups in the median (IQR) HIV-RNA log₁₀ copies/ml, higher in the DRV/r group: 4.67 (4.06-5.26) and 5.17 (4.73-5.49), respectively. At the baseline no difference was found ($p= 0.63$) in average levels of total bilirubin 0.5 (0.41-0.72) and 0.53 (0.38-0.7), proportion with a history of cardiovascular diseases: 2 (2.4%) in group 1 and 4 (5.5%) in group 2 ($p=0.31$), and proportion with a history of hypertension: 9 (10.6%) vs. 5 (6.8%), respectively ($p=0.41$) (Table 1). At the second time-point 132/158 (84%) patients had undetectable HIV-RNA load. During the study follow up only 17 patients modified the original NRTI-pair (6 of those who started ATV/r and 11 of those who started DRV/r).

Mean unadjusted biomarkers, CD4 count, HIV-RNA and bilirubin values are shown in Table 2. In this ANCOVA analysis (univariate and multivariate analysis for soluble biomarkers in the two groups), adjusted for age, gender, smoking, diabetes, HIV-RNA and T CD4+ cell count, plasma LDL and use of statins at baseline, we found that after 1 year of exposure to DRV/r or ATV/r-based regimens, ox-LDL were lower in the group of patients treated by DRV/r. We did not find evidence for a difference between the two groups for any of the other soluble markers evaluated.

We performed also a univariable sensitivity analysis of soluble biomarkers after restricting to patients who remained on TDF/FTC up to the date of collection of the second plasma sample. Again, only for ox-LDL, the association was statistically significant ($p= 0.007$) with higher values in ATV/r based group. No statistically significant differences were found for IL-6 ($p= 0.708$), MCP-1 ($p=0.864$), sCD163 ($p=0.977$), sVCAM-1 ($p= 0.257$) and adiponectin ($p= 0.389$).

Discussion

The rationale for comparing biomarkers profiles in patients receiving ATV/r or DRV/r-based regimens was dictated by the fact that the possible impact of these specific PI drugs on levels of soluble markers of inflammation has not been previously thoroughly investigated.

In the ATADAR Study no major differences between ATV/r and DRV/r in cholesterol fractions, over 96 weeks were observed, however, ATV/r led to higher triglycerides and more total and subcutaneous fat than DRV/r. Fat gains with ATV/r were associated with insulin resistance. In contrast with what occurred in the ATV/r arm, the LDL subfraction phenotype improved with DRV/r at week 48. This difference was associated with a lower impact on plasma triglycerides with DRV/r [22,23]. In a substudy of ATADAR, there was a trend for a faster Carotid-Intima media thickness progression in people treated with DRV/r as compared to those on ATV/r

[24]. In the D:A:D. Study, cumulative use of DRV/rtv, but not of ATV/rtv, was independently associated with a progressively increasing risk of centrally validated cardiovascular events [25]. In our study the only biomarker showing a difference between the studied anchor drugs was ox-LDL but only when restricting the analysis to patients who did not modify their NRTI-pair over time, levels being lower in people who started DRV/r. The results for other biomarkers were similar in the intention to-treat and per-protocol analyses restricted to people remaining on TDF/FTC. Ox-LDL expresses an inflammatory vascular damage correlated also with activation of innate immunity subsets [11]. In the group of patients treated with DRV/r we found a reduction of level of this marker compared to other group of ATV/r treated patients. The levels of bilirubin, as well as the therapy with TDF/FTC, were not associated with the studied soluble markers, except for ox-LDL in the group with a stable backbone of antiretroviral therapy, with lower levels in DRV/r group of patients.

Furthermore there was no evidence for an association between change in bilirubin over the study period and any of the changes in retrospectively measured biomarkers, except for adiponectine values (positively correlated).

Our analysis has some limitations. First, this is not a randomized comparison and therefore we cannot rule out that results might be affected by unmeasured confounding. Second, it is a limited comparison restricted to two specific PI-based regimens with no comparison with other type of regimens frequently used in first line (INSTI-based) or in simplifications strategies (NRTI-sparing regimens). Moreover, we did not correlate the soluble markers with parameters of activation of innate and adaptive cellular immunity, that probably playing a leading role in the inflammation process [26].

In conclusion, our analysis provides further data examining the association between the modulation of vascular inflammatory and of activation markers with specific PI-based

treatments over one year of exposure to these drugs. The data show little evidence for an association, supporting the notion that ART has generally poor efficiency in downregulating these soluble markers [14-17]. In contrast, ox-LDL appeared to be elevated in people who received ATV/r as compared to DRV/r. Further analyses should be conducted to examine the trends in soluble markers in people receiving other antiretroviral therapeutic strategies.

CONFLICT OF INTEREST

On behalf of the co-authors I confirm that there are no known conflicts of interest.

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References

- [1.]Hunt PW. HIV and inflammation: mechanisms and consequences. *Curr HIV/AIDS Rep* 2012; **9**:139-147.
- [2.]Hunt PW. Role of immune activation in HIV pathogenesis. *Curr HIV/AIDS Rep* 2007; **4**: 42-47.
- [3.]Deeks SG, Phillips AN. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. *BMJ* 2009; **338**:a3172.
- [4.]Dauby N, De Wit S, Delforge M, Necsoi VC, Clumeck N. Characteristics of non-AIDS-defining malignancies in the HAART era: a clinico-epidemiological study. *J Int AIDS Soc* 2011; **14**:14-16.
- [5.]Benjamin LA, Bryer A, Emsley HC, Khoo S, Solomon T, Connor MD. HIV infection and stroke: current perspectives and future directions. *Lancet Neurol* 2012; **11**:878-890.
- [6.]Gazzola L, Bellistri GM, Tincati C, et al. Association between peripheral T-Lymphocyte activation and impaired bone mineral density in HIV-infected patients. *J Transl Med* 2013; **11**:51
- [7.]Kaplan-Lewis E, Aberg JA, Lee M. Aging with HIV in the ART era. *Semin Diagn Pathol.* 2017; **34**:384-397
- [8.]Guihot A, Dentone C, Assoumou L, et al. Residual immune activation in combined antiretroviral therapy-treated patients with maximally suppressed viremia. *AIDS* 2016; **30**:327-330
- [9.]Hileman CO, Funderburg NT. Inflammation, Immune Activation, and Antiretroviral Therapy in HIV. *Curr HIV/AIDS Rep* 2017; **14**:93-100

- [10.] Gandhi RT, McMahon DK, Bosch RJ, et al. Levels of HIV-1 persistence on antiretroviral therapy are not associated with markers of inflammation or activation. *PLoS Pathog* 2017; **13**:e1006285
- [11.] Zidar DA, Juchnowski S, Ferrari B, et al. Oxidized LDL Levels Are Increased in HIV Infection and May Drive Monocyte Activation. *J Acquir Immune Defic Syndr* 2015; **69**:154-160.
- [12.] Kelesidis T, Tran TT, Stein JH, et al. Changes in Inflammation and Immune Activation With Atazanavir-, Raltegravir-, Darunavir-Based Initial Antiviral Therapy: ACTG 5260s. *Clin Infect Dis* 2015; **15**:651-660
- [13.] Estrada V, Monge S, Gómez-Garre D, et al. Comparison of oxidative stress markers in HIV-infected patients on efavirenz or atazanavir/ritonavir-based therapy. *J Int AIDS Soc* 2014; **17**:19544
- [14.] Estébanez M, Stella-Ascariz N, Mingorance J, et al. Inflammatory, procoagulant markers and HIV residual viremia in patients receiving protease inhibitor monotherapy or triple drug therapy: a cross-sectional study. *BMC Infect Dis* 2014; **14**:379.
- [15.] Hattab S, Guihot A, Guiguet M, et al. Comparative impact of antiretroviral drugs on markers of inflammation and immune activation during the first two years of effective therapy for HIV-1 infection: an observational study. *BMC Infect Dis* 2014; **14**:122.
- [16.] McComsey GA, Kitch D, Daar ES, et al. Inflammation markers after randomization to abacavir/lamivudine or tenofovir/emtricitabine with efavirenz or atazanavir/ritonavir. *AIDS* 2012; **26**:1371-85.

- [17.] Miro JM, Manzardo C, Ferrer E, et al. Immune Reconstitution in Severely Immunosuppressed Antiretroviral-Naive HIV-1-Infected Patients Starting Efavirenz, Lopinavir-Ritonavir, or Atazanavir-Ritonavir Plus Tenofovir/Emtricitabine: Final 48-Week Results (The Advanz-3 Trial). *J Acquir Immune Defic Syndr* 2015; **69**:206-215.
- [18.] Hileman CO, Kinley B, Scharen-Guivel V, et al. Differential Reduction in Monocyte Activation and Vascular Inflammation With Integrase Inhibitor-Based Initial Antiretroviral Therapy Among HIV-Infected Individuals. *J Infect Dis* 2015; **212**:345-354.
- [19.] Ketlogetswe KS, Post WS, Li X, et al. Lower adiponectin is associated with subclinical cardiovascular disease among HIV-infected men. *AIDS* 2014; **28**:901-909.
- [20.] Suda J, Dara L, Yang L, et al. Knockdown of RIPK1 Markedly Exacerbates Murine Immune-Mediated Liver Injury through Massive Apoptosis of Hepatocytes, Independent of Necroptosis and Inhibition of NF- κ B. *Journal Immunology* 2016; **197**:3120-3129
- [21.] Parodi A, Kalli F, Svahn J, et al. Impaired immune response to *Candida albicans* in cells from Fanconi anemia patients. *Cytokine*. 2015;**73**:203-7
- [22.] Martinez E. Gonzalez-Cordon A, Ferrer E, et al. Differential body composition effects of protease inhibitors recommended for initial treatment of HIV infection: a randomized clinical trial. *Clin Infect Dis* 2015;**60**:811-820
- [23.] Saumoy M. Ordóñez-Llanos J, Martínez E, et al. Atherogenic properties of lipoproteins in HIV patients starting atazanavir/ritonavir or darunavir/ritonavir: a substudy of the ATADAR randomized study. *J Antimicrob Chemother* 2015;**70**:1130-8

- [24.] Gonzales-Cordon A, Domenech M, Camafort M, et al. Subclinical CV disease in patients starting contemporary protease inhibitors. *HIV Medicine* 2018 May 10
- [25.] Ryom L, Lundgren JD, El-Sadr W, et al. Cardiovascular disease and use of contemporary protease inhibitors: the D:A:D international prospective multi cohort study. *Lancet HIV* 2018 May 3.
- [26.] Lagathu C, Cossarizza A, Bereziat V, Nasi M, Capeau J, Pinti M. Basic science and pathogenesis of ageing with HIV: potential mechanisms and biomarkers. *AIDS* 2017; **31**: S105-119