Effects of rosuvastatin versus pravastatin on low-density lipoprotein diameter in HIV-1-infected patients receiving ritonavir-boosted protease inhibitor

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Objective: HIV infection is associated with an atherogenic lipoprotein profile, and ritonavir-boosted protease inhibitors exacerbate this phenotype. We evaluated the effect of 45 days of rosuvastatin versus pravastatin on the low-density lipoprotein (LDL) size and the distribution of LDL subfractions in HIV-1 patients receiving boosted protease inhibitors with elevated LDL levels.

Design: Substudy of the randomized double-blind multicentre ANRS 126 VIHstatine trial.

Setting: Twenty clinical centres in France.

Patients: HIV-infected patients receiving boosted protease inhibitors with dyslipidaemia (LDL cholesterol > 4.1 mmol/l and triglycerides < 8.8 mmol/l).

Intervention: Rosuvastatin 10 mg/day ($n = 39$) or pravastatin 40 mg/day ($n = 37$) for 45 days.

Main outcome measure(s): LDL size and distribution of LDL subfractions blindly assessed by gradient gel electrophoresis at baseline and at day 45.

Results: Rosuvastatin was more effective than pravastatin in increasing the diameter of the LDL peak. The LDL diameter change was 0.33 ± 0.59 nm in the rosuvastatin group versus -0.01 ± 0.52 nm in the pravastatin group ($P = 0.021$). Rosuvastatin was also more effective in increasing significantly the percentage of large LDL (LDL1, $P = 0.038$; LDL2, $P = 0.031$) and in decreasing the percentage of small LDL (LDL3, $P = 0.009$).

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Received: 6 April 2012; revised: 4 June 2012; accepted: 15 June 2012.

DOI:10.1097/QAD.0b013e328357063c

Conclusion: Rosuvastatin was more effective than pravastatin in normalizing LDL size and LDL subfraction distributions, leading to a less atherogenic phenotype.

AIDS 2012, **26**:1801–1805

Keywords: atherogenic phenotype, HIV-1, low-density lipoprotein subfractions, pravastatin, protease inhibitor, rosuvastatin

Introduction

Dyslipidaemia in HIV-1 patients under combined antiretroviral therapy (cART) including ritonavir-boosted protease inhibitors consists of hypertriglyceridaemia (HTG), increased low-density lipoprotein (LDL) cholesterol (LDL-c), decreased high-density lipoprotein (HDL) cholesterol (HDL-c) and decrease in the size of LDL particles, with different magnitudes according to the different protease inhibitors [1–5]. Part of atherogenicity of the HTG is due to the formation of small dense LDL (sdLDL) particles, leading to an increased risk of cardiovascular disease [6]. The sdLDL are thought to be very atherogenic particles, because of their high ability to infiltrate the arterial wall, to link the LDL receptors and of their high oxidizability [6].

Pravastatin and rosuvastatin are the most widely used statins in HIV-1-infected patients in France; they possess potent lipid-lowering effects with limited metabolic interactions with protease inhibitors [1]. The aim of our study was to evaluate the evolution of LDL size and LDL subfraction distribution after a 45-day course of rosuvastatin or pravastatin in dyslipidaemic HIV-1-infected patients on a ritonavir-boosted protease inhibitor with elevated LDL-c levels and participating in the French National Agency for AIDS and Viral Hepatitis Research (ANRS) 126 VIHstatine trial [1].

Methods

The VIHstatine randomized, double-blind, multicentre trial (NCT00117494) was designed to assess the impact of a 45-day course of rosuvastatin 10 mg/day or pravastatin 40 mg/day on lipid values in dyslipidaemic (LDL-c $>$ 4.1 mmol/l and triglycerides (TG) $<$ 8.8 mmol/l) HIV-1-infected patients receiving ritonavir-boosted protease inhibitors [1]. The Pitié-Salpêtrière Hospital institutional review board approved the protocol and all the patients gave their written informed consent. The present substudy focused on patients with available frozen samples both at baseline and at day 45.

Fresh serum lipids were measured by routine enzymatic methods at baseline and day 45 [1]. Blind determination of lipid parameters (total cholesterol, triglycerides, LDL-c and HDL-c) was performed on fresh sera at baseline and day 45 of statin treatment in a central laboratory [1]. LDL subclasses were determined blindly in serum by gradient gel electrophoresis as explained previously, after isolation of LDL by sequential ultracentrifugation [7,8]. The LDL from the two frozen sera (day 0 and 45) was run simultaneously on the same gel, together with the LDL isolated from a normolipidaemic donor [4]. To calculate the percentage distribution of LDL subclasses, areas under the scanned curves were integrated within the size limits on an optical densitometer Hyrys2 (Sebia, Evry, France) at 570 nm, and the surface of each subfraction was divided by the total area under the curve to provide estimates of the percentage of each fraction. The usual distribution of the different LDL subclasses measured in 63 healthy individuals in our laboratory was as follows: 10–16% LDL-1 (28.5–27.0 nm), 30–50% LDL-2 (27.0–25.5 nm), 30–50% LDL-3 (25.5–24.2 nm) and 6–12% LDL-4 (24.2–22.0 nm) [7,9]. The diameter of the main LDL peak was calculated (25.49 ± 0.49 nm) as described elsewhere [10,11]. A diameter of less than 25.5 nm for this peak classically identifies an atherogenic phenotype [8,12].

Variables were summarized by using proportions for categorical variables, the median and interquartile range for age, time since HIV diagnosis, the CD4 cell count and the time on cART; the mean and SD for continuous variables used as endpoints, that is, LDL diameter, percentages of LDL subfractions (LDL1, LDL2 and LDL3), total cholesterol, LDL-c, HDL-c and triglycerides. As the LDL4 subfraction percentage can be deduced by subtraction of LDL1, LDL2 and LDL3 percentages to 100%, no test was performed for LDL4 subfraction.

Changes between baseline and day 45 were compared between the two statin arms by using the Mann–Whitney nonparametric test. All reported *P* values are two-tailed, with a significance level of 0.05. The SPSS software package version 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and SAS statistical software version 9.3 (SAS Institute Inc., Cary, North Carolina, USA) were used for analyses.

Results

Seventy-six of the 83 patients enrolled in the VIHstatine trial with available frozen samples at both baseline and day

Table 1. Baseline characteristics of the population studied.

	Pravastatin ($n=37$)	Rosuvastatin ($n=39$)
Sex (male)	30 (81%)	30 (77%)
Age (years), median (IQR)	49 (42–52)	47 (42–56)
Transmission groups, *n* (%)		
MSM	21 (57)	20 (51)
Heterosexual	13 (35)	14 (36)
Unknown/others	3 (8)	5 (13)
Ethnic origins, *n* (%)		
Whites	32 (86)	35 (90)
Sub-Saharan Africans	5 (14)	4 (10)
Time since HIV diagnosis (years), median (IQR)	12 (5–16)	11 (6–15)
CD4 cell count (cells/μl), median (IQR)	448 (315–598)	482 (313–659)
Plasma HIV-RNA < 400 copies/ml, *n* (%)	32 (86)	35 (90)
Prior AIDS event, *n* (%)	10 (27)	7 (18)
Number of previous ARVs, median (IQR)	7 (6–9)	7 (5–9)
Time on cART (years), median (IQR)	9 (4–13)	9 (5–13)
Ritonavir boosted protease inhibitors treatment, *n* (%)		
Lopinavir/r	13 (35)	10 (26)
Fosamprenavir/r	10 (27)	7 (18)
Atazanavir/r	7 (19)	15 (38)
Others (TPV, IDV, SQV)	7 (19)	7 (18)
Current smoking or stopped less than 3 years, *n* (%)	10 (27)	16 (41)
Total cholesterol (mmol/l), mean (SD)	7.24 (0.94)	7.31 (1.22)
LDL cholesterol (mmol/l), mean (SD)	4.94 (0.90)	5.05 (1.13)
HDL cholesterol (mmol/l), mean (SD)	1.38 (0.35)	1.38 (0.38)
Triglycerides (mmol/l), mean (SD)	2.57 (1.70)	2.69 (1.29)
LDL diameter (nm), mean (SD)	25.6 (0.8)	25.4 (1.0)
LDL subfractions		
LDL1 (%), mean (SD)	7.1 (4.3)	8.0 (7.9)
LDL2 (%), mean (SD)	46.8 (20.5)	41.5 (20.1)
LDL3 (%), mean (SD)	35.7 (16.7)	35.9 (15.1)

ARV, antiretroviral; cART, combined antiretroviral therapy; HDL, high-density lipoprotein; IDV, indinavir; IQR, interquartile range; LDL, low-density-lipoprotein; SQV, saquinavir; TPV, tipranavir.

45 were eligible for this substudy: 37 in the pravastatin arm and 39 in the rosuvastatin arm, with the same demographic, immunovirological and lipidic parameters as the total population analysed in the VIHstatine trial [1].

The characteristics of the patients of this substudy were well balanced between the rosuvastatin and pravastatin groups (Table 1). Seventy-nine percent of the patients were men, and the median age was 48 years. Fifty-four percent were MSM and 12% were Africans. The median CD4 cell count was 475 cells/μl, and the plasma HIV-1 RNA level exceeded 400 copies/ml in seven patients (9%) (maximum 5238 copies/ml). Twenty-two percent were in AIDS Centers for Disease Control and Prevention (CDC) stage, and 34% were active smoker or past smokers who had stopped at less than 3 years. The median duration of ART exposure was 9 years, with a median of seven different drugs (four nucleoside reverse transcriptase inhibitors, one nonnucleoside reverse transcriptase inhibitor and two protease inhibitors). Protease inhibitor therapy was well balanced between the two arms and mainly consisted of lopinavir (30%), atazanavir (29%) and fosamprenavir (22%).

After 45 days of statin therapy, we observed, as in the full trial [1], significant changes from baseline (day 0 to 45) in the between group comparison (pravastatin versus rosuvastatin) for LDL-c levels (mean ± SD, -1.05 ± 0.74 versus -1.68 ± 0.89 mmol/l, $P < 0.001$), for total cholesterol (-1.16 ± 0.86 versus -1.92 ± 0.99 mmol/l, $P < 0.001$) and for TG (-0.23 ± 1.01 versus -0.73 ± 1.01 mmol/l, $P = 0.034$). No difference was observed for HDL-c levels (0.05 ± 0.23 versus 0.01 ± 0.19 mmol/l, $P = 0.750$).

Rosuvastatin was more effective than pravastatin in increasing the diameter of the LDL peak: mean ± SD of LDL diameter change was 0.33 ± 0.59 nm in the rosuvastatin arm versus -0.01 ± 0.52 nm in the pravastatin arm (Fig. 1a, $P = 0.021$). Rosuvastatin was also more effective in increasing significantly the percentage of large LDL (Fig. 1b, LDL1 1.7 versus 0.5%, $P = 0.038$; Fig. 1c, LDL2 6.1 versus −1.1%, $P = 0.031$) and in decreasing the percentage of small LDL (Fig. 1d, LDL3 −4.4 versus 1.5%, $P < 0.009$). There was no association between the change of LDL size between day 45 and baseline and the type of protease inhibitors (0.10 ± 0.55, 0.09 ± 0.66, 0.29 ± 0.61 and 0.16 ± 0.47 in patients receiving lopinavir/r, fosamprenavir/r, atazanavir/r or another boosted protease inhibitor, respectively, $P = 0.916$).

Discussion

In a substudy of the randomized double-blind multicentre ANRS 126 trial in HIV-1-infected patients with elevated

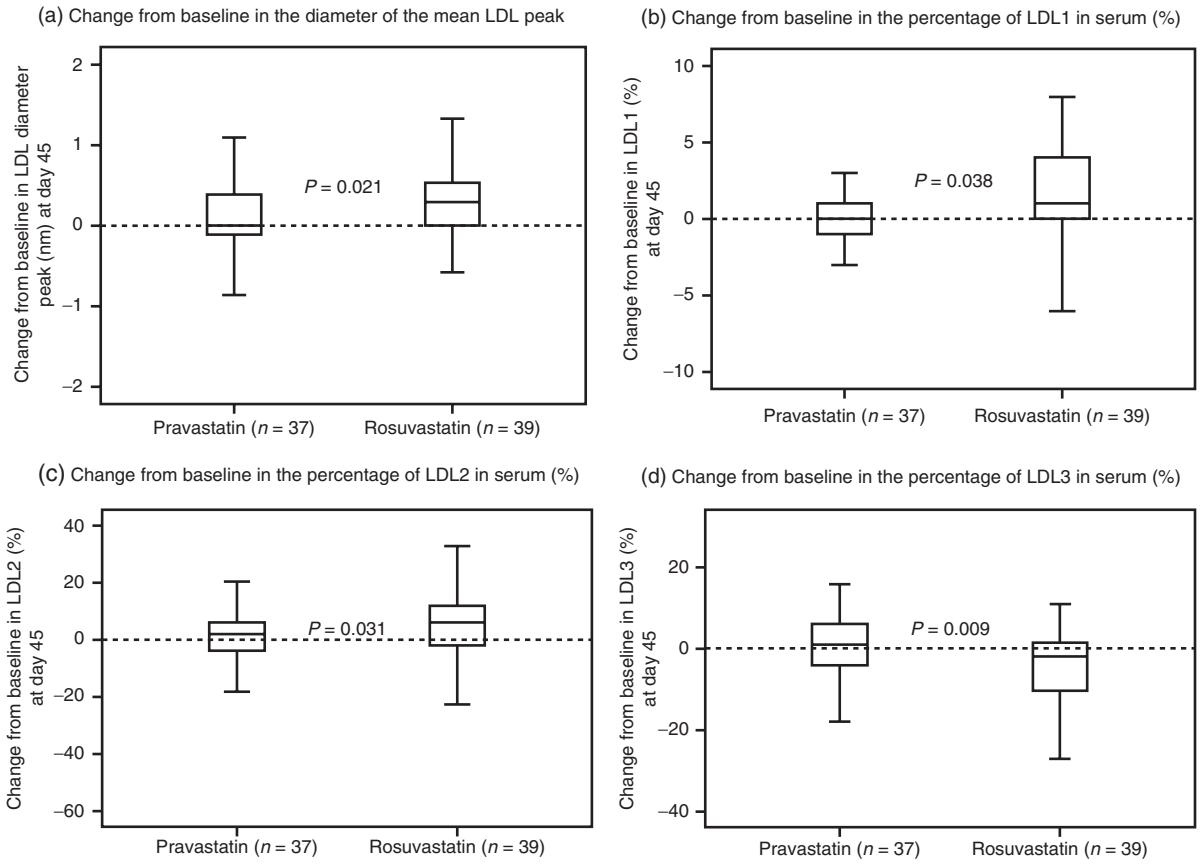

Fig. 1. Change from baseline in low-density lipoprotein diameter and subfractions according to the statin group. (a) Change from baseline in the diameter of the mean low-density lipoprotein (LDL) peak. (b) Change from baseline in the percentage of LDL1 in serum (%). (c) Change from baseline in the percentage of LDL2 in serum (%). (d) Change from baseline in the percentage of LDL3 in serum (%).

LDL levels receiving ritonavir-boosted protease inhibitors as part of combined antiretroviral treatment, we showed that pravastatin had a limited impact on the LDL size and distribution of LDL subfractions, whereas rosuvastatin led to an increased LDL size by increasing the proportion of LDL1 and LDL2 subfractions, with a significant difference between the two arms.

Our study is the first to evaluate in HIV-1-infected patients in a randomized study the effect of two widely used statins on the LDL size and on LDL subfractions. We could enrolled in the substudy 76 of the 83 patients (92%) who had frozen samples available, their characteristics were similar to those of the seven patients not enrolled.

Statins have an important role in the improvement of atherogenic lipoprotein phenotype (ALP) by decreasing sdLDL, resulting in a decrease in the cardiovascular risk [6,13,14]. In a substudy of the Pravastatin Limitation of Atherosclerosis in the Coronaries (PLAC-I) trial, LDL particle number and levels of small LDL were predictors of progression of coronary artery disease (CAD) [15], and pravastatin increased the LDL size compared with placebo in patients with high-risk coronary heart disease, whether they had small or large LDL particles [16]. Other reports have shown that pravastatin had a limited action in modifying LDL particle size and their subclasses in patients with either primary hypercholesterolaemia or type IIa dyslipidaemia or combined or familial combined hyperlipidaemia or with type 2 diabetes [17,18], and in children with familial hypercholesterolaemia [19]. The results of our study regarding pravastatin group were in accordance with these reports, with limited impact of pravastatin in the size and distribution of subfractions.

Until now, only one randomized, double-blind study ($n = 29$) investigated the efficiency of rosuvastatin versus placebo on LDL subfractions in hyperlipidaemia in the general population by reducing sdLDL and increasing LDL size [20]. We observed similar results in HIV-1-infected individuals.

In conclusion, rosuvastatin administered at 10 mg/day improved the ALP when compared with pravastatin, by modifying the LDL diameter from atherogenic sdLDL towards less atherogenic large LDL, and induced a redistribution of LDL subfractions in the HIV-1 patients under boosted protease inhibitors.

Acknowledgements

French National Agency for AIDS and Viral Hepatitis Research (ANRS) was involved in the study design. After approving the protocol, the sponsor had no involvement in the collection, analysis or interpretation of the data, writing of the report or the decision to submit the article for publication. This study was registered with clinicaltrials.gov, number NCT00117494.

Conception and design of the sub-study was done by E.A., R.K.-B., D.B.-R., D.C. and P.G. Provision of study materials or patients was done by E.A., R.K.-B., D.B.-R., C.C., M.C.F., O.K., M.A.V. Statistical analysis was performed by L.A. and D.C. Interpretation of the data was performed by E.A., L.A., R.K.-B., D.B.-R., D.C. and P.G. Drafting of the article was done by E.A., L.A., R.K.-B., D.B.-R., D.C. and P.G. Critical revision of the article for important intellectual content and final approval of the article was done by all the authors.

Conflicts of interest

L.A., D.B.-R. and O.K. have no conflicts of interest to declare. R.K.-B. has received travelling grants, payment of registration fees and lecture fees from Gilead Sciences and Solvay. P.G. has received lecture fees from Pfizer, AstraZeneca and Solvay and travel grants from Merck. E.A. has received travel grants from Sanofi Aventis, Pfizer and Bristol-Myers-Squibb. M.A.V. has received lecture fees, traveling expenses and payment of registration fees from Roche, Tibotec (Johnson and Johnson), Gilead, GlaxoSmithKline, Bristol-Meyers Squibb, MSD and Boehringer Ingelheim. D.C. has received travel grants, consultancy fees, honoraria or study grants from various pharmaceutical companies, including Abbott, Boehringer-Ingelheim, Bristol-Myers-Squibb, Gilead Sciences, Glaxo-Smith-Kline, Janssen, Merck, Roche and ViiV Healthcare.

The French National Agency for AIDS and Viral Hepatitis Research (ANRS) sponsored the trial. ANRS received a grant from Astra Zeneca for the study.

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