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Lack of effects of statins on high-density lipoprotein subfractions in HIV-1-infected patients receiving protease inhibitors



Absence d'effet des statines sur les sous-fractions des lipoprotéines de haute densité chez des patients infectés par le VIH-1 recevant des inhibiteurs de protéase

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ABSTRACT

Background. – We evaluated the effect of 45 days of rosuvastatin or pravastatin treatment on the distribution of HDL subfractions in HIV-1-infected individuals receiving boosted protease inhibitors (PIs) with cardiovascular risk.

Methods. – The distribution of HDL subclasses by gradient gel electrophoresis was blindly assessed in 74 HIV-1-infected individuals receiving boosted PIs at baseline and at day 45 of statin treatment, and compared with the distribution obtained in 63 healthy normolipidemic individuals taken as controls.

Results. – No significant modification appeared in HDL distribution between the two arms of statins for the HIV-1-infected individuals. Nevertheless, when compared to controls, HDL subfractions showed a significantly lower HDL2b proportion and significantly higher proportions of HDL2a and HDL3b ($P < 0.001$).

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Conclusion. – No difference was observed in HDL distribution between pravastatin and rosuvastatin after 45 days treatment, in HIV-1-infected individuals under PIs. Nevertheless, when compared to healthy normolipidemic subjects, HDL distribution is clearly different, with a distribution in HIV-infected individuals under PIs associated with an increased cardiovascular risk.

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R É S U M É

Mots clés :

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Contexte. – Nous avons évalué l'effet de 45 jours de traitement par la rosuvastatine ou la pravastatine sur la distribution des sous-fractions des HDL chez des personnes atteintes de VIH-1 recevant des inhibiteurs de la protéase boostées (IPs) et ayant un risque cardiovasculaire.

Méthodes. – La distribution des sous-classes d'HDL effectuée par une électrophorèse sur gel en gradient a été évaluée en aveugle sur 74 personnes infectées par le VIH-1 recevant des inhibiteurs de la protéase boostées (IPs), et ce au jour 0 et après 45 jours de traitement par les statines. Cette distribution a été ensuite comparée à celle obtenue chez 63 personnes en bonne santé, considérées comme témoins.

Résultats. – Aucune modification significative n'est apparue dans la distribution des HDL entre les deux bras des statines pour les individus infectés par le VIH-1. Néanmoins, comparativement aux témoins, les sous-fractions HDL présentaient une proportion significativement plus faible de HDL2b et des proportions significativement plus élevées de HDL2a et de HDL3b ($p < 0,001$).

Conclusion. – Aucune différence dans la modification de la distribution des HDL n'a été observée après 45 jours de traitement entre la pravastatine et la rosuvastatine chez les personnes infectées par le VIH-1 sous IPs. Cependant, la distribution des HDL chez des personnes infectées par le VIH sous IPs est clairement différente quand elle est comparée à celle observée chez les sujets sains, et est associée à un risque cardiovasculaire élevé.

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1. Abbreviations

cART	combination antiretroviral therapy
PI	protease inhibitor
sdLDL	small-dense low-density lipoprotein particles
LDL	low-density lipoprotein
SR-B1	scavenger receptor B1
ABCG1	ATP-binding cassette transporter A1
D0	baseline
D45	day 45

2. Introduction

HIV-1 infection and its treatment by protease inhibitors (PIs), especially when boosted with ritonavir, have been associated with increased cardiovascular risk [1–3]. This increased risk is partially related to metabolic disorders, including dyslipidemia as hypertriglyceridemia and low high-density lipoprotein (HDL) level [4], with a high prevalence of atherogenic lipoprotein phenotype and endothelial dysfunction [2,4]. Clinical studies suggested that cardioprotective properties were more associated with HDL2 than with HDL3, and low levels of HDL2b associated with elevated HDL3b were related to a high cardiovascular risk [5–7].

Many studies reported the role of the HIV-1 infection in addition to the role of PIs-containing combined antiretroviral therapy (cART) in accentuating the atherogenic profile in HIV-infected individuals, which is characterized by a low level of HDL-cholesterol and a high proportion of small-dense low-density lipoprotein particles (sdLDL) [8–10]. Mature HDL can be separated by gradient gel electrophoresis into five distinct subclasses of different sizes: HDL2b (12.9–9.71 nm), HDL2a (9.71–8.77 nm), HDL3a (8.77–8.17 nm), HDL3b (8.17–7.76 nm), and HDL3c (7.76–7.21 nm) [5,11,12]. The largest HDL2b and HDL2a subfractions displayed a superior capacity to mediate cellular free-cholesterol efflux via both scavenger receptor class B1 (SR-BI)- and ATP-binding cassette transporter G1 (ABCG1)-dependent pathways than smaller HDL3 subspecies [13]. HDL3c may provide potent protection of LDL from oxidative damage induced by oxygen reactive species in the arterial intima, resulting in the inhibition of the generation of pro-inflammatory oxidized lipids [14].

In a substudy of the randomized multicenter French National Agency for AIDS and Viral Hepatitis Research (ANRS) 126 VIHstatine trial, we evaluated the effect of 45 days of rosuvastatin (10 mg/day) vs. pravastatin (40 mg/day) on the LDL size and the distribution of LDL-subfractions [10]. We concluded that rosuvastatin was more effective than pravastatin in increasing the diameter of the LDL peak, in decreasing the percentage of

atherogenic small-dense LDL and increasing the percentage of less atherogenic large LDL [10].

In the current substudy, our aim was to evaluate the distribution of HDL subfractions at baseline in the same population of HIV-1-infected individuals receiving Pls [1,2,10], to appreciate the effect of a 45-day statin treatment on this distribution, and to compare with the distribution of HDL subfractions in a control population.

3. Materials and methods

Determination of lipid parameters by routine enzymatic methods was performed on fresh sera at baseline (D0) and day-45 (D45) in a central laboratory [1,2]. HDL subclasses were determined blindly of sampling time (D0 versus D45) or treatment arm (rosuvastatin versus pravastatin) in serum, as explained previously, after isolation of lipoproteins by sequential ultracentrifugation [10,15]. The lipoproteins from D0 and D45 of statin treatment were run simultaneously on the same gel, together with lipoproteins isolated from a normolipidemic subject taken as control [2,10].

Seventy-four patients of the 83 patients enrolled in the VIHstatine trial with available frozen samples at baseline and D45 were eligible for this substudy: 36 in the pravastatin arm and 38 in the rosuvastatin arm with the same demographic, immunovirological and lipid

parameters, particularly HDL-cholesterol [1,2,10]. Comparisons between the two statin arms or between the two statin arm combined versus 63 healthy normolipidemic controls were analysed using the non-parametric Mann–Whitney test, while comparisons between D45 and D0 were analysed using the paired Wilcoxon test.

4. Results

After 45 days, no significant difference was observed between the two arms of statin treatment regarding the distribution of different HDL subclasses percentage, respectively: HDL2a, HDL2b, HDL3a, and HDL3b.

Further analyses were thus performed considering a global statin group (Table 1). After 45 days of statin treatment, only HDL3b subfraction was decreased compared to baseline ($P = 0.003$). The D45 HDL subfractions in the HIV-infected individuals, compared to those in healthy controls, showed a significantly lower HDL2b proportion ($P < 0.001$) and significantly higher proportions of HDL2a and HDL3b, while no difference was evidenced for the HDL3a subfraction (Fig. 1).

5. Discussion

The low levels of HDL2b associated with high levels of HDL3b observed in our study are associated with cardiac

Table 1

Distribution of HDL subfraction proportions in the statin-treated HIV-infected patients ($n = 74$) and in healthy normolipidemic controls ($n = 63$).

%	Day 0 ($n = 74$)	Day 45 ($n = 74$)	Δ (D45 – D0)	P	Healthy controls $n = 63$	P^*
HDL 2a	26.90 \pm 5.51	27.30 \pm 5.45	0.49 \pm 3.74	0.159	23.87 \pm 3.99	< 0.001
HDL 2b	13.30 \pm 6.15	13.50 \pm 6.10	0.19 \pm 3.63	0.446	23.68 \pm 3.62	< 0.001
HDL 3a	31.50 \pm 3.70	32.20 \pm 4.35	0.69 \pm 3.22	0.070	33.14 \pm 4.22	0.164
HDL 3b	20.85 \pm 6.80	19.50 \pm 6.65	–1.36 \pm 4.75	0.003	15.05 \pm 3.40	< 0.001

The significant results are presented in bold. HDL: high-density lipoprotein; D45: day45; D0: baseline; P : comparison D0 vs. D45 in the pooled statin treatment group using the paired Wilcoxon test; P^* : comparison D45 vs. healthy subjects using the Mann–Whitney test.

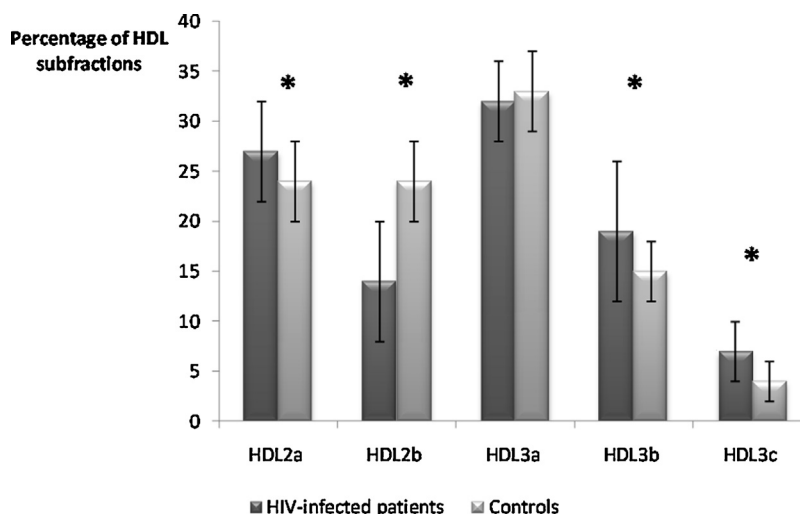


Fig. 1. Percentages of high-density lipoprotein (HDL) subfractions in the global statin-treated HIV-infected group of patients at D45 compared to healthy normolipidemic subjects. * $P < 0.001$.

heart disease in the general population [5,15]. Furthermore, HDL2b subclass is strongly inversely correlated with the coronary disease risk [16].

Dyslipidemia is a frequent side effect of cART, especially when boosted PIs are used [1]. The lipid disorders are more severe than before cART including PIs use, and usually consist of hypercholesterolemia, hypertriglyceridemia, elevated levels of small-dense LDL particles. Stein et al. [9] reported high proportion of small, less protective HDL particles associated with endothelial dysfunction in a group of 22 HIV-1 patients receiving ART under PIs with stable doses for more than 6 months. Use of PIs in HIV-1 population has been associated with endothelial dysfunction, an early step in atherosclerosis that predicts future adverse cardiovascular events [17] and cardiovascular disease remained increased when compared to the general population [18].

Statins can reduce total cholesterol and LDL-cholesterol in patients with HIV-1 infection under PIs, improve endothelial function and delay the progression of cardiovascular disease, while more side effects were observed during statin treatment [18]. Selection of statin treatment and the potential for drug interactions with antiretroviral therapy must be considered in this infected population [18,19].

Only a few studies compared the effect of statins on the distribution of HDL subfractions in dyslipidemic and in control subjects, and their results are contradictory. No data is currently available in HIV-1 patients. Schaefer et al. [16] found that atorvastatin increased the percentage of HDL2b by 25%, whereas those of medium and small HDL3 (i.e. HDL3b and HDL3c) did not significantly change. Harangi et al. [20] reported that atorvastatin significantly increased the proportion of HDL3, whereas HDL2a and HDL2b were decreased in patients receiving 20 mg per day during three months. Lower doses of atorvastatin used in this study could not restore the physiologic proportions of the HDL subclasses. The important anti-atherogenic functions of HDL subclasses include removal of excess cholesterol from peripheral tissues and reverse cholesterol transport to the liver for excretion into bile [5,14].

In conclusion, our study shows no difference in HDL distribution change between pravastatin and rosuvastatin after 45 days treatment, in HIV-1-infected individuals under PIs and only HDL3b subfraction decreased compared to baseline. Nevertheless, when compared to healthy subjects, HDL distribution is clearly different, with a distribution in HIV-1-infected individuals under PIs associated with an increased cardiovascular risk. Reducing atherogenic lipoprotein changes and endothelial dysfunction in HIV under PIs, by optimal statins and management of cardiovascular risk, is needed in this population. However, the lack of power of our study (respective power for each of the four tested variables i.e. HDL2a, HDL2b, HDL3a, and HDL3b: 20%, 7%, 45%, and 65%, respectively) could explain the negative results and constitutes a limitation. Interactions with cART should also be considered. Finally, these results need to be confirmed on a large number of HIV-1-infected patients.

Authors' contributions

Recruitment of patients was carried out by: E.A., M.-A.V., P.G., O.K., and the ANRS 126 study group.

Statistical analysis was performed by: L.A., D.C., P.G.

Measurement of lipid parameters, and HDL distribution was done by: M.-C.F., C.C., R.B., D.B-R.

Interpretation of biological data was performed by: R.B., D.B-R.

Drafting of the article was performed by: R.B., D.B-R., E.A., P.G., D.C.

Study design, analysis and interpretation of the data, and critical revision of the article for important intellectual content and final approval of the article was done by: R.B., D.B-R., E.A., P.G., D.C., L.A., M.-A.V., O.K.

Disclosure of interest

Lambert Assoumou, Dominique Bonnefont-Rousselot and Olga Kalmykova declare that they have no competing interest. Randa Bittar has received travelling grants, payment of registration fees and lecture fees from Gilead Sciences and Solvay. Philippe Giral has received lecture fees from Pfizer, AstraZeneca and Solvay and travel grants from Merck. Élisabeth Aslangul has received travel grants from Sanofi Aventis, Pfizer and Bristol-Myers-Squibb. Marc-Antoine Valantin has received lecture fees, traveling expenses and payment of registration fees from Roche, Tibotec (Johnson & Johnson), Gilead, Glaxo-Smith-Kline, Bristol-Meyers Squibb, MSD, and Boehringer-Ingelheim. Dominique Costagliola 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.

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References

- [1] É. Aslangul, K.L. Assoumou, R. Bittar, M.-A. Valantin, O. Kalmykova, G. Peytavin, M.-H. Fievet, F. Boccara, D. Bonnefont-Rousselot, J.-C. Melchior, P. Giral, D. Costagliola, Rosuvastatin versus pravastatin in dyslipidemic HIV-infected patients receiving protease inhibitors; the ANRS 126 randomized trial, *AIDS* 24 (2010) 77–83.
- [2] R. Bittar, P. Giral, É. Aslangul, L. Assoumou, M.-A. Valantin, O. Kalmykova, V. Fesél, D. Costagliola, D. Bonnefont-Rousselot, Determinants of low-density lipoprotein particle diameter during antiretroviral therapy including protease inhibitors in HIV-1-infected patients, *Antivir. Ther.* 17 (2012) 855–860.
- [3] S. Badiou, C. Merle De Boever, A.-M. Dupuy, V. Baillat, J.-P. Cristol, J. Reynes, Decrease in LDL size in HIV-positive adults before and after lopinavir/ritonavir-containing regimen: an index of atherogenicity? *Atherosclerosis* 168 (2003) 107–113.
- [4] H. Rose, J. Hoy, I. Woolley, U. Tchoua, M. Bukrinsky, A. Dart, D. Sviridov, HIV infection and high-density lipoprotein metabolism, *Atherosclerosis* 199 (2008) 79–86.

- [5] H. Watanabe, S. Söderlund, A. Soro-Paavonen, A. Hiukka, E. Leinonen, C. Alagona, R. Salonen, T.P. Tuomainen, C. Ehnholm, T. Jauhiainen, M.R. Taskinen, Decreased high-density lipoprotein (HDL) particle size, pre-beta-, and large HDL subspecies concentration in Finnish low-HDL families: relationship with intima-media thickness, *Arterioscler. Thromb. Vasc. Biol.* 26 (2006) 897–902.
- [6] P.T. Williams, R.M. Krauss, K.M. Vranizan, M.L. Stefanick, P.D. Wood, F.T. Lindgren, Associations of lipoproteins and apolipoproteins with gradient gel electrophoresis estimates of high-density lipoprotein subfractions in men and women, *Arterioscler. Thromb. Vasc. Biol.* 12 (1992) 332–340.
- [7] J. Johansson, L.A. Carlson, C. Landou, A. Hamsten, High-density lipoproteins and coronary atherosclerosis. A strong inverse relation with the largest particles is confined to normotriglyceridemic patients, *Arterioscler. Thromb. Vasc. Biol.* 11 (1991) 174–182.
- [8] P. El Khoury, M. Ghislain, E.F. Villard, W. Le Goff, C. Lascoux-Combe, P. Yeni, L. Meyer, C. Vigouroux, C. Goujard, M. Guerin, Plasma cholesterol efflux capacity from human THP-1 macrophages is reduced in HIV-infected patients: impact of HAART, *J. Lipid Res.* 56 (2015) 692–702.
- [9] J.H. Stein, M.A. Klein, J.L. Bellehumeur, P.E. McBride, D.A. Wiebe, J.D. Otvos, J.M. Sosman, Use of human immunodeficiency virus-1 protease inhibitors is associated with atherogenic lipoprotein changes and endothelial dysfunction, *Circulation* 104 (2001) 257–262.
- [10] R. Bittar, P. Giral, É. Aslangul, M.-A. Valantin, O. Kalmykova, M.-C. Federspiel, C. Cherfils, D. Costagliola, D. Bonnefont-Rousselot, French National Agency for AIDS and Viral Hepatitis Research (ANRS) 126 study group, Effects of Rosuvastatin versus pravastatin on low-density lipoprotein diameter in HIV-1-infected patients receiving protease inhibitors, *AIDS* 26 (2012) 1801–1805.
- [11] P.J. Blanche, E.L. Gong, T.M. Forte, A.V. Nichols, Characterization of human high-density lipoproteins by gradient gel electrophoresis, *Biochim. Biophys. Acta* 665 (1981) 408–419.
- [12] A.V. Nichols, R.M. Krauss, T.A. Musliner, Nondenaturing polyacrylamide gradient gel electrophoresis, *Meth. Enzymol.* 128 (1986) 417–431.
- [13] N. Bellanger, Z. Julia, E.F. Villard, E. Duchene, M.J. Chapman, N. Fournier, W. Le Goff, M. Guerin, Functionality of postprandial larger HDL2 particles is enhanced following CETP inhibition therapy, *Atherosclerosis* 221 (2012) 160–168.
- [14] A. Kontush, M.J. Chapman, Anti-atherogenic function of HDL particle subpopulations: focus on antioxidative activities, *Curr. Opin. Lipidol.* 21 (2010) 312–318.
- [15] R.S. Rosenson, H.B. Brewer Jr., M.J. Chapman, S. Fazio, M.M. Hussain, A. Kontush, R.M. Krauss, J.D. Otvos, A.T. Remaley, E.J. Schaefer, HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events, *Clin. Chem.* 57 (2011) 392–410.
- [16] E.J. Schaefer, J.R. McNamara, T. Tayler, J.A. Daly, J.A. Gleason, L.J. Seman, A. Ferrari, J.J. Rubenstein, Effects of atorvastatin on fasting and postprandial lipoprotein subclasses in coronary heart disease patients versus control subjects, *Am. J. Cardiol.* 90 (2002) 689–696.
- [17] J.H. Stein, Dyslipidemia in the era of HIV protease inhibitors, *Prog. Cardiovasc. Dis.* 45 (2003) 293–304.
- [18] J.M. Gilbert, K.V. Fitch, S.K. Grinspoon, HIV-related cardiovascular disease, statins, and the REPRIEVE Trial, *Top. Antivir. Med.* 23 (2015) 146–149.
- [19] J.H. Stein, Management of lipid levels and cardiovascular disease in HIV-infected individuals: just give them a statin? *Top Antivir Med.* 23 (2015) 169–173.
- [20] M. Harangi, H.Z. Mirdamadi, I. Seres, F. Sztanek, M. Molnár, A. Kassai, Z. Derdák, L. Illyés, G. Paragh, Atorvastatin effect on the distribution of high-density lipoprotein subfractions and human paraoxonase activity, *Transl. Res.* 153 (2009) 190–198.