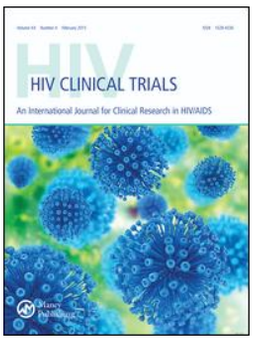

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Original Article

The effect of switching protease inhibitors to raltegravir on endothelial function, in HIV-infected patients

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Objective: Lipid management is one of the cornerstones of cardiovascular risk reduction. Treatment of HIV infection with protease inhibitors (PIs) may cause dyslipidaemia, whilst the integrase inhibitor raltegravir (RAL) has a relatively favorable effect on plasma lipids. We examined the effect of switching from PIs to RAL on endothelial function, and its effect on immunological and inflammatory parameters.

Methods: We performed a 16-week open-label prospective crossover study: 8 weeks intervention (switch PIs to RAL) and 8 weeks control (unchanged cART regimen). Flow-mediated dilatation (FMD), inflammatory plasma, and cellular markers of immune activation were measured at weeks 0, 8, and 16.

Results: Study participants (n = 22) with a median age of 50 years (IQR 42–60) and known HIV infection of 6.5 years (IQR 5.0–17.3) were on stable cART with undetectable HIV viral loads. After 8 weeks of RAL therapy, a reduction in FMD of –0.81% was seen, compared to +0.54% control (pairwise, p = 0.051), while fasting total cholesterol (–17% versus +10%; $p < 0.001$), LDL cholesterol (–21% versus –3%; p = 0.026), and triglycerides (–41% versus +18%; p = 0.001) significantly decreased during RAL therapy compared to the control.Furthermore, a relation between the change in percentage of B-1 cells and the change in FMD was found (β 0.40, 95%CI 0.16; 0.64, p = 0.005) during treatment with RAL. Finally, during RAL therapy, 27% of the patients experienced an increased ALT rise.

Conclusions: We present an overall negative study, where switching from PIs to RAL slightly reduced the endothelial function while decreasing plasma lipids, thus possibly decreasing the CVD risk in the long term. A transient elevation of ALT was seen upon switch to RAL.

Keywords: Cardiovascular disease, HIV, Raltegravir, Plasma lipids, Endothelial function, Immune activation, ALT

Background

Treatment of HIV infection with certain antiretroviral therapy, especially protease inhibitors (PIs), tends to cause dyslipidemia.[1,2] This is in contrast to the relatively favorable effect of the newer antiretroviral therapy, such as the integrase inhibitor raltegravir (RAL). When switching from a PI containing regimen,[3] a decrease of 12.6, 15 and 42.2% is seen, respectively, for total cholesterol, non-HDL cholesterol, and triglycerides.[3] PIs have also been associated with increased carotid intima media thickness (CIMT), increased risk for CVD events, and increased endothelial dysfunction.

Endothelial dysfunction can be measured by flow-mediated dilatation (FMD).[1,4–7] A low FMD of the brachial artery is related to an increased risk of cardiovascular events and is therefore recognized as a surrogate cardiovascular endpoint for evaluating pharmacological interventions.[8,9] In HIV-infected patients, on a PI-containing regimen, a lower FMD (2.6 ± 4.6%) was found compared to HIV patients on other combination antiretroviral therapy (cART)-regimens (8.1 ± 6.7%).[5] However, two

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other studies in healthy HIV-uninfected men showed no endothelial dysfunction after 4 weeks of lopinavir/ritonavir (LPV/r).[10,11]

A positive association has been see in HIV-infected patients between subclinical atherosclerosis and T cell and monocyte activation.[12,13] Furthermore, activated T cells, monocytes, and markers of monocyte activation are more prevalent in HIV-infected patients compared to HIV-negative patients.[14,15] In the recent ACTG 5260 study, the effect of starting on a PI-based regimen (ATV/r and DRV/r) or a RAL-based regimen was compared for inflammation and immune activation in cART-naïve patients. No consistent evidence was found that the reduction of inflammation and immune activation with cART initiation was different between RAL- and PI-based regimens.[16] In this study, the progression of CIMT was measured, reporting a slower progression in the ATV/r group compared to DRV/r, but an intermediate progression for RAL.[17] However, this study only compared the effect at baseline, not the difference during stable cART.

The effect of RAL on immune activation is still unclear; several intensification studies have addressed this question reporting conflicting outcomes. These studies hypothesized that by intensifying with RAL, the residual viral replication would decrease, impacting latent reservoirs and immune activation.[18–20] No effect on absolute CD4+ T cells count was seen after intensification of 48 weeks; however, a reduction in activated CD4+ and CD8+ T cells was found compared to randomized controls.[18] However, in a 24-week placebo-controlled study, no effect was found.[19] On note, none of these studies were switch studies.

Furthermore, a more favorable effect on immune activation was seen for elvitegravir, another integrase inhibitor, over efavirenz as measured by a decrease in high sensitivity CRP and a decrease in soluble CD14.[21] Possibly, this is a class effect and similar results can be found for raltegravir.

Therefore, we hypothesized that switching from PIs to RAL would positively impact endothelial function by decreasing inflammation, immune activation, and plasma lipids. To address this question, we evaluated the effect of this switch on FMD, in stable PI-treated HIV-infected patients, and evaluated the effect on plasma lipids, inflammatory cells, and inflammatory markers known to be associated with the process of atherosclerosis.

Methods

Patients

HIV-infected patients were recruited from the University Medical Centre Utrecht (UMCU) and the Onze Lieve Vrouwe Gasthuis (OLVG), Amsterdam for participation in the "RAltegravir Switch Study- effects on Endothelial Recovery" (RASSTER) study. Inclusion criteria were: age 18 years and older; chronic HIV-1 infection; treatment with LPV/r containing antiretroviral therapy for at least the last previous 3 months; undetectable plasma HIV RNA (50 copies/ml) for at least 6 months (one "blip" allowed, which was defined as a detectable plasma HIV RNA level between 50 and 400 copies/ml, preceded and followed by undetectable (<50 copies/ml) plasma HIV RNA measurements); no history of virological failure; results of previous resistance testing allowing switch of LPV/r to RAL to be made; a CD4+ T cell count > 200 cells/μL. Exclusion criteria were: pregnancy; breastfeeding; allergy for peanuts or soya (present in RAL); hypersensitivity to RAL; treatment of underlying malignancy; acute infection in the preceding 30 days; renal insufficiency requiring haemodialysis; acute or decompensated chronic hepatitis; modification of antiretroviral regimen in the previous 3 months. During the course of the study, an amendment was filed, since gradual switch from LPV/r to other PIs in the Dutch HIV population impaired inclusion of LPV/r-treated patients in the study. Besides patients on LPV/r, we also included patients on an ATV/r-containing regimen. Therefore, the main research question was changed to "the effect of switching PIs (instead of LPV/r) to RAL on endothelial recovery".

All patients provided written informed consent in accordance with the Declaration of Helsinki and the local Medical Ethics Committee approved the study. (ClinicalTrials.gov identifier: NCT01453933). After initial consent, 22 out of the 25 patients were included into the study (Figure 1).

Study design

The RASSTER study was a phase IV, randomized, open-label, crossover, intervention, study of 16 weeks: 8 weeks of intervention (switch PI to RAL) and 8 weeks as control (unchanged PI-containing cART regimen) for all patients (Figure 2(A)). The patients were randomized into two arms: arm A switched during the first 8 weeks (INT1) and returned to their normal PI-containing regimen in the final 8 weeks (C2); arm B stayed on their PI-containing regimen in the first 8 weeks (C1) and switched in the final 8 weeks (INT2). Randomization was performed by the pharmacy at the UMCU using Design Software, as per protocol.

Patients were seen for screening, at baseline and in weeks 2, 4, 8, 10, 12, and 16 thereafter. At screening physical examination, hematology, kidney and liver function, CD4+ T cell count, HIV RNA viral load, an electrocardiogram, and a pregnancy test (on indication) were performed. During all other study visits, venous blood was drawn for further laboratory measurements (see below), adverse events were reported, and physical examination

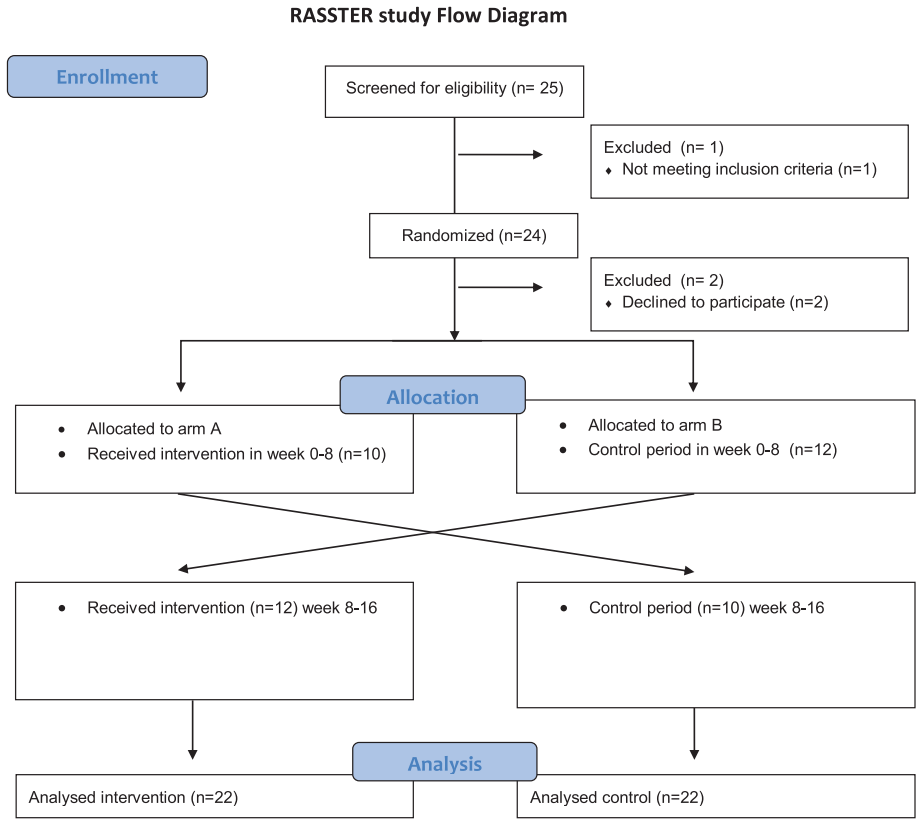

Figure 1 Flow diagram (CONSORT). Notes: The patients enrolled, randomized, and analyzed in the RASSTER study. The diagram shows the crossover design, pooling all intervention (left), and all control (right) to be compared.

(upon indication) was performed. FMD was performed at weeks 0, 8, and 16. RAL was dosed 400 mg twice daily, as specified in the package insert. Adherence to the study drug was assessed at every visit by self-reporting, and by pill count at week 8 or 16.

Laboratory measurements

The local site laboratory measured plasma total cholesterol (mmol/l), HDL cholesterol (mmol/l), triglycerides (mmol/l), creatinin (ml/min), alanine-aminotransferase (ALT U/l), high sensitive C-reactive protein (hsCRP mg/l), von Willebrand Factor antigen (vWF %), D-dimer (mg/l), insulin (mIU/l), and absolute $CD4^+$ T-cell counts (cells/mm^3) according to standard protocols. LDL cholesterol was calculated with the Friedewald formula.

The local site virology laboratory measured plasma HIV-RNA levels (COBAS® AmpliPrep/ COBAS® TaqMan®, Roche Diagnostics, Indianapolis, USA) at weeks 0, 4, 8, 12, and 16 from baseline using assays with a lower limit of detection of 50 copies/ml.

PBMC processing, staining, and flow cytometric analysis

Heparin blood was processed within 24 h. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque™ Plus (GE Healthcare) density gradient centrifugation and washed with RPMI 1640 culture media (Gibco®, life technologies™) containing 5% fetal calf serum (FCS) and penicillin-streptomycin before being cryopreserved with RPMI 20% FCS. Cryopreserved PBMCs were thawed with RPMI 20% FCS and subsequently used for flowcytometric analysis. Cells were washed using PBA (Sigma®, Life Science), stained with cocktails of monoclonal antibodies (Supplemental Table 1), and left to incubate for 20 min at 4 °C. Fluorescence minus one (FMO) controls were used to define positive gates for the expression of different proteins. Lymphocytes and monocytes were gated based on forward and side scatter using a FACS LSR Fortessa (BD Biosciences, Franklin Lakes, U.S.A.) and FACS Diva software version 8.0 (BD Biosciences, Franklin Lakes, U.S.A.).

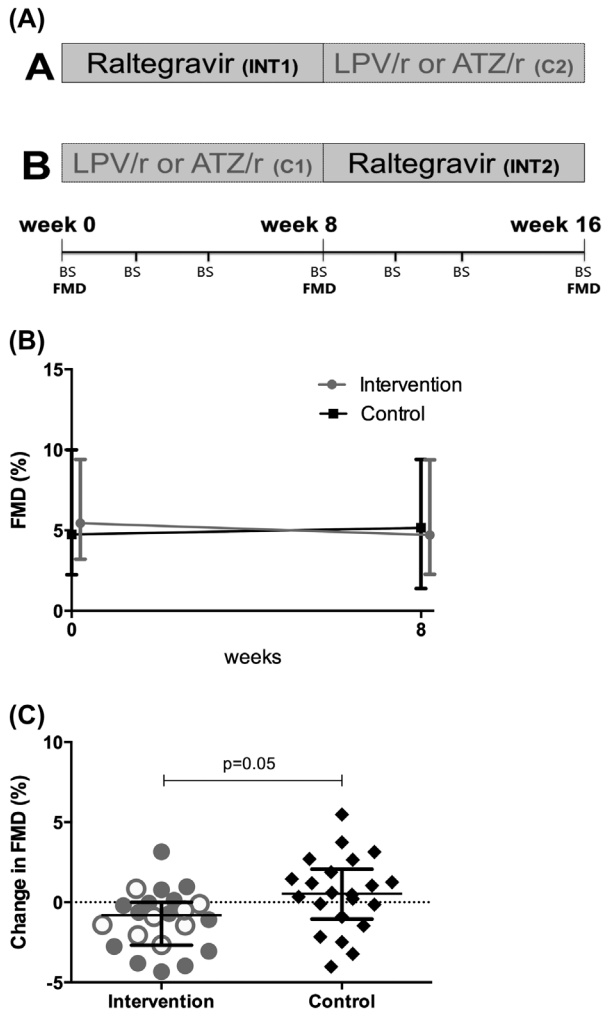

Figure 2 Study design. (A) Study design: Arm A: INT1 = intervention period 1, C2 = control period 2. Arm B: C1 = control period 1, INT2 = intervention period 2. LPV/r = Lopinavir/ritonavir, ATV/r = Atazanavir/ritonavir, FMD = Flow-mediated dilatation, BS = blood sampling. (B) FMD at start and end of intervention (grey) and control (black) periods. (C) Changes in the brachial artery FMD after switching to raltegravir (intervention) and in the control period. Open grey circles are patients previously on ATV/r; filled circles are patients previously on LPV/r. Horizontal lines represent the median with interquartile ranges.

Brachial artery flow-mediated dilatation

Given the known increased CVD risk due to PI treatment, we investigated the effect of switching from PIs to RAL on the cardiovascular system, by measuring the brachial artery FMD. FMD was performed at weeks 0, 8, and 16, as described previously.[22] In brief, patients were seen in a fasting state and not allowed to smoke prior to the scan. Baseline image was recorded for 1 min; hereafter, a blood pressure cuff was inflated to 250 mmHg; after a 5-min forearm occlusion, the cuff was released to produce reactive hyperaemia and the brachial artery was imaged for 3 min after cuff release. The images were saved in a Digital Imaging and Communications in Medicine (DICOM) clip and analyzed using Brachial Analyzer for Research (Medical Imaging Applications LLC, Coralville, Iowa, USA). The FMD was defined as (maximum-baseline diameter/baseline diameter) × 100%. All scans were coded with a random number and blindly assessed by two researchers (M.K. and F.Y.). If there was an inter-observer difference of more than 2% in the FMD result, the FMD analysis was repeated. After completion of all the FMD analyses, the scans were unblinded.

Table 1 Baseline characteristics

Characteristics	Median (IQR)
	n = 22
Male	91% (*n* = 20)
Age (years)	50 (42–60)
Smoking (current/previous)	18/36% (*n* = 4/8)
Pack years cigarettes (years)	30 (16–40)
Known CVD	9% (*n* = 2)
Statin use	23% (*n* = 5)
Antihypertensive treatment	23% (*n* = 5)
Aspirin use	14% (*n* = 3)
Systolic blood pressure (mmHg)	122 (114–134)
Diastolic blood pressure (mmHg)	83 (77–85)
BMI (kg/m^2)	24 (22–26)
Total Cholesterol (mmol/L)	5.3 (4.8–5.9)
HDL cholesterol (mmol/L)	1.24 (1.15–1.32)
LDL cholesterol (mmol/L)	3.4 (2.8–3.7)
Triglycerides (mmol/l)	1.8 (1.3–2.6)
Creatinin (µmol/l)	89 (82–98)
ALT (U/l)	29 (18–39)
hsCRP (mg/l)	1.5 (0.8–3.5)
D dimer (mg/ml)	0.22 (0.22–0.26)
vWF (%)	108 (100–146)
Known duration of HIV (years)	6.5 (5.0–17.3)
Duration of cART use (years)	6.1 (4.3–16.4)
CD4$^+$ T cell count (cells/mm^3)	650 (483–799)
Undetectable HIV-RNA viral load* (%)	100% (*n* = 22)
Current PI + NRTI use (%)	100% (*n* = 22)
PI use (years)	4.9 (3.5–6.9)
Flow-mediated dilatation (%)	5.1 (3.7–7.3)

Notes: BMI = body mass index, HDL = high-density lipoprotein, LDL = low-density lipoprotein, ALT = alanine aminotransferase, hsCRP = high-sensitive C-reactive protein, vWF = von Willebrand factor antigen, cART = combination antiretroviral therapy.
*<50 copies/ml.

Data analyses

The primary outcome of the study was the change in FMD between intervention and control periods. To detect a difference (two tailed) in change in FMD between intervention and control of 1.5%[23] with a power of 0.90 and alpha = 0.05, 21 study subjects were needed. Anticipating a dropout of 10%, we aim to include 24 patients. When comparing the intervention to the control period of the same patient, a pairwise test was used. A Mann–Whitney test was used to compare non-paired continuous variables.

Data were presented as percentages for categorical variables and as median with interquartile ranges (IQR) for continuous variables. Differences were considered statistically significant when $p < 0.05$. The absolute change

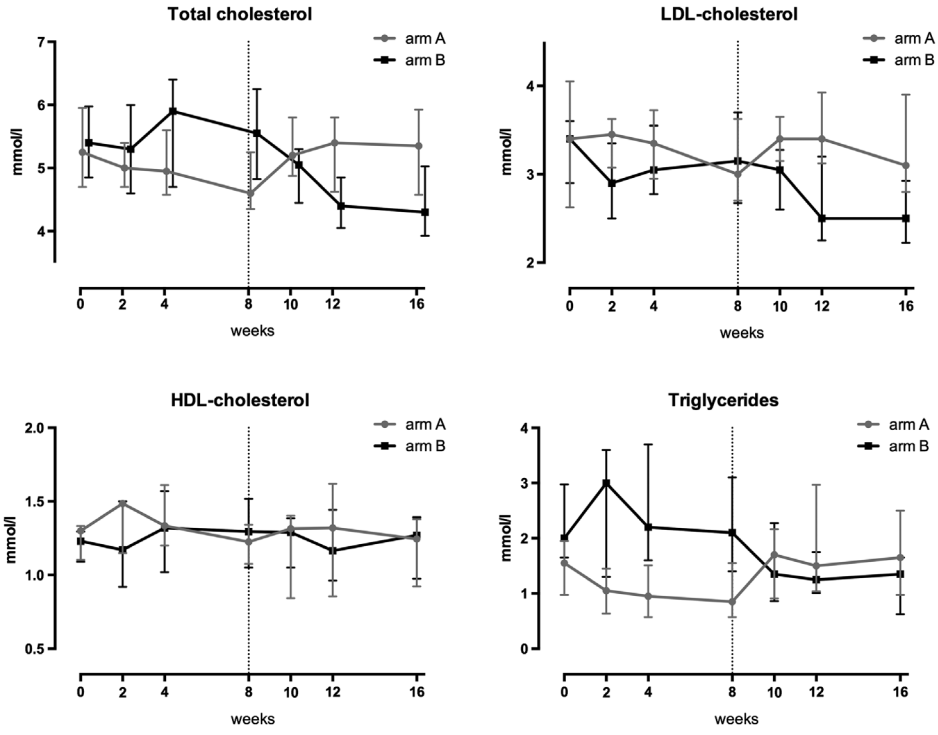

Figure 3 Changes in plasma lipids. Changes in total, LDL and HDL cholesterol, and triglycerides during the study. Arm A (grey) switched from PIs to RAL during weeks 0–8 and arm B (black) during weeks 8–16.
Notes: The medians with interquartile ranges are shown.

per variable was calculated as the difference between week 8 and baseline for C1 and INT 1 and the difference between week 16 and week 8 for C2 and INT2 (Figure 2(A)). Linear regression modeling was used to evaluate the relation between the change in FMD and change of the measured variables. A univariate model was used, as well as a multivariate model where each variable was tested individually and adjusted for age, gender, and duration of known HIV infection. Analyses were performed using SPSS version 21 (SPSS, Chicago, Illinois, USA).

Results

The study population consisted of mostly older males, with undetectable plasma HIV-RNA and adequate levels of CD4+ T cells (Table 1). Fourteen of the patients were on an LPV/r-containing regimen and eight on an ATV/r-containing regimen at the start of the study.

Effect of switching from PIs to RAL on endothelial function (FMD)

After 8 weeks of intervention with RAL, an absolute difference of 1.3% was found (Figure 2(B)) when comparing the intervention to the control period (pairwise, $p = 0.051$). In the intervention period, the FMD decreased with 0.81% ((IQR −2.69–0.01) (Figure 2(B)), with a FMD at start of 5.4% (IQR 4.0–8.1) and 4.7% (IQR 3.6–6.0) after 8 weeks). In the control period, an increase of 0.54% (IQR −1.04–2.08) was seen (FMD at start 4.7% (IQR 3.3–6.9) and 5.2% (IQR 3.8–8.1) after 8 weeks).

Effects of switching from PIs to RAL on plasma lipids

Next, we examined the effect of RAL on plasma lipid levels demonstrating significantly greater reductions in fasting total cholesterol (−17% versus +10%; $p < 0.001$), LDL cholesterol (−21% versus −3%; $p = 0.026$), and triglycerides (−41% versus +18%; $p = 0.001$) in the intervention compared to the control period (Figure 3). There was no difference in change in HDL cholesterol between groups (−4% versus + 3%; $p = 0.65$). The expression of the LDL-receptor on T cells at the start of the study was low with only 0.35% (IQR 0.17–0.57) of the CD4+ and 0.88% (IQR 0.47–1.88) of the CD8+ T cells expressing the receptor. No changes were seen in either of the study periods (control or intervention) for this marker. There was no relation between the change in FMD and change in plasma lipids (data not shown).

Inflammation, T cell activation, and B cells

We hypothesized that RAL could improve endothelial function through decreasing inflammation and immune activation. Therefore, we measured the effect of RAL (in the intervention group) on coagulation and inflammatory markers associated with the formation of atherosclerosis.

Table 2 The relation between the change in FMD and the change in inflammatory and coagulation markers, and immune activation (when using RAL)

	Adjusted for age, gender, and duration of HIV		
	(outcome change in FMD)		
Changes of following variables	β	95% CI	*p*-value
HsCRP	0.056	–0.22–0.33	0.672
D-dimer	3.195	–1.73–8.12	0.189
vWF	–0.014	–0.03–0.01	0.090
CD4:CD8 ratio	0.098	–0.75–0.94	0.810
T cell CD4+CD38+DR+	–0.043	0.86–0.78	0.914
T cell CD8+CD38+DR+	–0.023	–0.21–0.17	0.797
T cell CD4+CD57+	0.246	–0.42–0.91	0.444
T cell CD8+CD57+	0.048	–0.10–0.20	0.501
T cell CD4+CD95+	0.022	–0.10–0.14	0.707
T cell CD8+CD95+	0.049	–0.14–0.24	0.595
T cell CD4+CXCR3+	–0.034	–0.21–0.15	0.693
T cell CD8+CXCR3+	0.002	–0.12–0.12	0.968
T cell CD4+LDL+	–0.032	–2.36–2.30	0.977
T cell CD8+ LDL+	0.164	–0.68–1.01	0.679
B cell CD20+CD27+CD43+CD70-	0.400	0.16–0.64	**0.005**
Classical monocytes	0.025	–0.04–0.09	0.435
Non-classical monocytes	0.133	–0.52–0.78	0.665
Intermediate monocytes	0.024	–0.05–0.10	0.486
Monocytes CD36+	0.033	–0.13–0.20	0.650
Monocytes CD162+	–0.079	–0.34–0.18	0.524
Monocytes CD163+	–0.082	–0.17–0.01	0.071
Monocytes CD169+	0.014	–0.04–0.07	0.594

Notes: FMD = flow-mediated dilatation, hsCRP = high-sensitive C-reactive protein, vWF = von Willebrand factor antigen.
β = coefficient, CI = confidence interval, *P*-value significant <0.05. Bold is a statistically significant value.

No significant change was seen in the coagulation markers D-dimer (+0.0, IQR 0.0–0.1) and vWF (+2.0 IQR −9.5–21.5) after switching to RAL. In addition, hsCRP (+0.2, IQR −0.4–2.6) also remained unchanged irrespective of RAL treatment.

Next, we investigated the effect of switching to RAL on cellular immune activation. Baseline levels of the percentage of T cells expressing CD38+HLA-DR+ were 1.8% (IQR 1.4–2.6) for CD4+ and 5.3% (IQR 4.2–10.6) for CD8+ T cells, respectively. Treatment intervention had a minimal effect: percentages of activated CD4+ T cells increased 0.1% (IQR −0.3–0.7) for intervention and decreased 0.3% (IQR −0.7–0.1) for the controls ($p = 0.015$). CD8+ T cells decreased 0.1% (IQR −1.5–1.2) for the intervention and increased 0.4% for CD8+ T cells (IQR −2.3–0.6) for the controls ($p = 0.099$). Non-significant results were seen for changes in percentages of T cells expressing the activation and senescence markers CD95 and CD57, respectively (data not shown).

Trafficking of leukocytes to the vessel wall is mediated by CXCR3, 6.3% (IQR 2.5–15.5) of the CD4+ T cells expressed the receptor, and 34% (IQR 23–42; $p = 0.91$) of the CD8+ T cells before switch, with similar expressions during the control period. Changes in expression did not differ between intervention and control periods ($p = 0.11$).

In murine models, a protective effect on CVD is seen for certain B cells, namely B-1 cells. In our study, we measured the percentages of B cells containing the human homologue of these cells. The baseline expression of these CD20+CD27+CD43+CD70− B cells was 42% (IQR 16–47) for RAL and 44% (IQR 28–49; $p = 0.03$) for the control. During RAL, treatment a median change of +0.1% (IQR −3.2–0.8) was seen, which was −2.2% (IQR −5.8–1.0) for the control period ($p = 0.45$).

Furthermore, we analyzed the relation between the changes in inflammation, coagulation, and cellular immune activation and the change in FMD (when using RAL), adjusting for age, gender, and duration of HIV infection (Table 2). As can be seen in Table 2, a relation was only found for the percentage of CD20+CD27+CD43+CD70− B cells (β 0.40, 95%CI 0.16;0.64, $p = 0.005$).

Monocytes: their subpopulations and markers

Monocytes are abundantly present in atherosclerotic plaques and play an important role in the process of atherosclerosis. For this study, we measured the frequencies of monocytes subpopulations and their changes upon intervention in plasma. At baseline, subpopulations were as follows: classical (CD14++CD16−) 47% (IQR 23–56), non-classical (CD14+CD16++) 1.7% (IQR 0.9–3.0), and intermediate (CD14++CD16+) monocytes 28% (IQR 17–44). During intervention, a decrease of 7% (IQR −15–7) was seen for classical monocytes, whilst these were unchanged in the control period ($p = 0.23$). The intermediate monocytes also decrease with 5% (IQR −7–4), contrary to an increase of 4% (IQR −9–8) in the control group ($p = 0.74$). The non-classical monocytes were unchanged in both periods: 0.3% (IQR −1.0–0.9) and 0.4% (IQR −0.7–0.9), respectively ($p = 0.50$). Further analysis was done on monocyte markers (CD162, CD163, and CD169) associated with leukocyte migration and inflammation. These markers yielded no difference in

expression for intervention versus control on monocytes (data not shown). Finally, the CD36 receptor, which monocytes can use to uptake oxidized LDL, decreased in both intervention −0.1% (IQR −9.1–3.2) and control −0.9% ($p = 0.83$). No relation was found for the change in monocyte fractions and monocyte markers and the change in FMD.

Adverse events and safety

Throughout the study, all patients were virologically suppressed with a HIV viral load <50 copies/ml, no blips were seen. At baseline, a median $CD4^+$ T-cell count of 650 cells/mm^3 (IQR 483–799) was seen. No change was seen in the intervention (+2, IQR −67–66) and control groups (+36, IQR −62,5–89,5) throughout the study.

Surprisingly, nearly half of the patients (6 of 14; 43%) when switching from LPV/r to RAL experienced a marked increase in ALT in the first 4 weeks of treatment. In contrast, none of the patients on ATV/r experienced these ALT increases. At the peak of the increase (week 4), a median 3,5-fold higher ALT (122 U/l versus 35 U/l) was seen, compared to the other patients switching from LPV/r without an ALT increase. All patients with an ALT-increase were negative for acute (viral) infections (hepatitis B, hepatitis C, hepatitis E, and syphilis), denied recreational drug use, and reported no complains of jaundice or gastrointestinal problems.

Discussion

PIs are known for their unfavorable cardiovascular profile by causing dyslipidaemia, increasing CIMT, and therefore increasing the risk for CVD events. The availability of new classes of cART, like integrase inhibitors, with their much more favorable cardiovascular profile, has opened new possibilities to reduce CVD risk associated with cART. This study therefore investigated if switching from a PI-based regimen to RAL would improve endothelial function, as measured by an increased FMD. We used FMD as an endpoint since this is a well-known proxy of CVD risk.

The borderline significant decrease in FMD observed during RAL therapy, meaning slight decrease in endothelial function, can be interpreted in two ways. First, no difference in cells and markers for immune activation and inflammation was seen. On the contrary, a significant decrease in lipids was seen. Therefore, the question remains if the results found are a coincidence or not. More likely we are looking at negative results, with RAL not having an effect on endothelial function, as was also previously seen in the SPIRAL study.[24] Second, the question furthermore remains whether this decrease is of any clinical relevance. The median FMD of around 5% found at baseline in our study indicates moderate endothelial dysfunction and is comparable to a FMD reported in a HIV-negative population of the same age.[25] In addition, this moderate endothelial dysfunction at the start of the study might have reduced the window for possible potential benefits of RAL on the short term.[24] All our study participants were well-suppressed, relatively healthy patients with borderline dyslipidaemia at baseline. Despite the latter, intervention with RAL resulted in a significant decrease in total and LDL cholesterol and triglycerides, without changes in HDL cholesterol. This effect has been described previously; however, the exact mechanism is not known.[26] Lipids are known to play an important role in the risk for CVD. For example, for every 1 mmol/l decrease in LDL, a reduction of over 21% is seen for major cardiovascular events.[27] Decreasing lipids can be beneficial in the long term, by decreasing the risk of cardiovascular morbidity and mortality.[27,28]

One of our other hypotheses was that switching from PIs to RAL would decrease inflammation and immune activation, and as such would be beneficial to the prevention of atherosclerosis. To assess a possible effect on atherosclerotic plaque formation, we measured activated T cells, monocytes, and B cells' counts. Overall, we only found a significant correlation between the measured B1 cells and FMD; all other measured parameters did not show a correlation. This lack of change can probably be explained by the relatively low inflammatory state of our patients at the start of the study as all patients had an undetectable viral load with a CD4 count above 350cells/mm^3 throughout the study.

A subset of CD20+CD27+CD43+CD70− B cells was recently described as the human counterpart of B1 cells.[29] In murine models, B1 cells have been shown to be atheroprotective, by the production of IgM antibodies that bind oxLDL and apoptotic cells.[30] Despite the lack of human studies to date, our study demonstrates a significant correlation between the measured B1 cells and the change in FMD after switching to a RAL-containing regimen contributing to the hypothesis of atheroprotection.

In our study, when switching to RAL, an increase in ALT (grade 2) was observed in 27% (6/22) of our patients. Remarkably, this ALT increase was only found in those patients switching from LPV/r to RAL (in 43%). The ALT increase was transient and only seen in the first weeks after switching to RAL.

The strengths of this study are the comprehensive immunological analyses performed to assess the possible role of inflammation on endothelial function when switching from PIs to RAL. Also, only HIV-infected patients with a suppressed viral load were included to minimize the direct effect of HIV viremia on our immunological analyses. Unfortunately, the uniform homogeneity of our included patients was changed from only LPV/r to PIs

(LP/r and ATV/r) in general. Other study limitations are the relatively small sample size and the relatively short intervention period, although in a similar study with similar sample size we recently demonstrated a significant increase in FMD in the maraviroc therapy arm.[31] Finally, within our crossover design, we did not include a washout period; therefore, there could be a theoretical difference among the control groups.

Conclusions

Although switching from PIs to RAL in this short-term study resulted in a borderline significant reduction in endothelial function, significant decreases in lipids were seen, which suggest that longer duration of RAL treatment might reduce the risk of CVD in HIV-infected patients. In patients switching from LPV/r to RAL, an unexpected high incidence of ALT elevation was observed; fortunately, this elevation is transient.

Declarations

Contributors

M.K. coordinated the study, performed research, collected, analyzed and interpreted the data, and wrote the manuscript. K.T. contributed to the design of the study, the analysis and interpretation of the data, and writing of the manuscript. G.E.L.B. contributed to the collection of the data and writing of the manuscript. L.F. and S.O. contributed to the analysis and interpretation of the data. S.F.L.L. contributed to the design of the study and writing of the manuscript. F.L.J.V. contributed to the analyses and interpretation of the data, and the writing of the manuscript. A.I.M.H. contributed to the analyses and interpretation of the data, and the writing of the manuscript. J.E.A. was responsible for the overall supervision of the study and contributed to the study design, interpretation of the data, and the writing of the manuscript. All authors have read and approved the final version of the article.

Availability of data and materials

The data-sets used during the current study are available from the corresponding author on reasonable request.

Competing interests

None of the authors have a financial interest in or a financial conflict with the subject matter and materials discussed in this article. A.I.M.H. is a member of the advisory board for BMS, ViiV Healthcare, Janssen. and MSD. J.E.A. is a member of the advisory board for ViiV Healthcare, Abbvie, Janssen, Gilead, BMS and MSD. S.F.L.L. has received financial support for research, travel, speaking engagements. or consultancy from BMS, GSK, Janssen, Pfizer, Roche. and ViiV healthcare. M.K., L.F., K.T., G.E.L.B., S.A.O., and F.L.J.V. have received no financial support for this study.

Ethics approval and consent to participate

The local Medical Ethics Committee of the University Medical Centre Utrecht approved the study. The ClinicalTrials.gov identifier was NCT01453933. All patients provided written informed consent in accordance with the Declaration of Helsinki.

Trial registration

The RASSTER was registered at ClinicalTrials.gov (NCT01453933) before enrolment of the first participant.

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