The Effect of Rosuvastatin on Markers of Immune Activation in Treatment-Naive Human Immunodeficiency Virus Patients

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Background. Immune activation has been implicated in the excess mortality in human immunodeficiency virus (HIV)-infected patients, due to cardiovascular diseases and malignancies. Statins may modulate this immune activation. We assessed the capacity of rosuvastatin to mitigate immune activation in treatment-naive HIV-infected patients.

Methods. In a randomized double-blind placebo-controlled crossover study, we explored the effects of 8 weeks of rosuvastatin 20 mg in treatment-naive male HIV-infected patients (n = 28) on immune activation markers: neopterin, soluble Toll-like receptor (TLR)2, sTLR4, interleukin (IL)-6, IL-1Ra, IL-18, d-dimer, highly sensitive C-reactive protein, and CD38 and/or human leukocyte antigen-DR expression on T cells. Baseline data were compared with healthy male controls (n = 10). Furthermore, the effects of rosuvastatin on HIV-1 RNA, CD4/CD8 T-cell count, and low-density lipoprotein cholesterol were examined and side effects were registered.

Results. T-cell activation levels were higher in patients than in controls. Patients had higher levels of circulating IL-18, sTLR2, and neopterin (all \(P < .01\)). Twenty patients completed the study. Rosuvastatin increased the CD4/CD8 T-cell ratio (\(P = .02\)). No effect on other markers was found.

Conclusions. Patients infected with HIV had higher levels of circulating neopterin, IL-18, sTLR2, and T-cell activation markers. Rosuvastatin had a small but significant positive effect on CD4/CD8 T-cell ratio, but no influence on other markers of T-cell activation and innate immunity was identified (The Netherlands National Trial Register [NTR] NTR 2349, http://www.trialregister.nl/trialreg/index.asp).

Keywords. HIV-1; 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors; immune activation; statin.
a reduction of T-cell activation levels after 8 weeks of atorvastatin. Others demonstrated a similar decline in T-cell activation after 48 weeks of rosuvastatin in patients treated with ART [15].

In this study, we explored the baseline differences in soluble markers of immune activation and in T-cell activation status between HIV-infected subjects and controls, and we investigated the effects of rosuvastatin on these markers in HIV-infected subjects in a double-blind placebo-controlled crossover trial. Only ART-naive patients were selected, to exclude the effect of treatment. We postulated that the use of rosuvastatin decreases the concentrations of soluble markers of innate immunity with resulting modulation of T-cell activation. We chose to examine the effect of rosuvastatin, because this statin has been investigated in the JUPITER study [13] and is proven to be well tolerated in patients infected with HIV [16, 17].

METHODS

Study Population

We included ART-naive HIV-infected male patients from April 2011 until January 2013 in the Onze Lieve Vrouwe Gasthuis (OLVG) Hospital in Amsterdam. Male volunteers, confirmed HIV-negative, who underwent a wisdom tooth extraction for noninflammatory reasons more than 2 weeks before inclusion, were included as healthy controls. The inclusion criteria for HIV-patients were an age of 18 years or older, having CD4 cell counts on 2 occasions higher than 350 cells/mm³ and HIV-1 RNA higher than 1000 copies/mL. Patients were eligible if there was no evidence of acute HIV-infection, which was defined as the presence of a detectable HIV-1 viral RNA in the presence of a nonreactive HIV-1 or HIV-2 antibody assay or an indeterminate Western blot. Laboratory results at screening were aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 1.5 times the upper limit of normal (ULN), creatine kinase ≤ 3 times the ULN, absolute neutrophil count ≥ 1000/mm³, hemoglobin ≥ 7.5 mmol/L, platelet count ≥ 100 000/mm³, creatinine 2 times the ULN, amylase and lipase ≤ 1.25 times the ULN, and low-density lipoprotein (LDL) ≥ 1.0 mmol/L.

Exclusion criteria were active drug use or alcohol dependence, serious illness, including opportunistic infections or neoplasms, requiring systemic treatment and/or hospitalization 30 days before entry of the study.

To ensure rosuvastatin was well tolerated, participants were excluded if there was known hypersensitivity to this drug or its components, a history of myositis or rhabdomyolysis, the use of drugs that significantly interact with rosuvastatin, or the use of other lipid-lowering agents. To assure there was no confounding on the immune response, use of concurrent immune modulators or vaccinations within 6 weeks of study entry were exclusion criteria as well. All participants provided written informed consent.

Study Design

We completed a double-blind randomized placebo-controlled pilot study with a crossover design. The HIV-infected participants were assigned to 2 random groups: 1 received rosuvastatin 20 mg qd, and the other received placebo during 8 weeks. After this first cycle, both study groups discontinued all study-related medications for 4 weeks, referred to as the washout period. Next, subjects receiving placebo switched to rosuvastatin and vice versa for a period of 8 more weeks. Throughout the study, participants had scheduled visits at the clinic every 4 weeks. Blood was drawn at 4 weeks (screening) and 1 day (baseline) before start of medication and on week 4, 8, 12, 16, and 20 after the start of medication. Noninfected controls donated blood samples once for all the parameters.

The carryover effect is defined as the persistence of effect of a treatment applied in one period, in a subsequent period. To minimize this effect, a washout period of 4 times the action duration of a treatment has been recommended [18]. Because the half-life of rosuvastatin is 19 hours, we estimated a washout period of 4 weeks to be sufficient.

To ensure the double-blind character of the study, the treating physician was not informed on the results of blood tests during the study period, and the samples for the lipid panel were measured after completion of the study.

Because both immune activation and statin use may cause fatigue, a questionnaire estimating quality of life was filled out at baseline, week 8, and week 20. This contained the Medical Outcomes Study HIV Health Survey (MOS-HIV) questionnaire and a combined complaints list, which was derived from the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 and has been used in HIV-infected patients and statin users [19, 20].

Laboratory Methods

Blood was drawn in the morning, after the appliance of a light tourniquet, into vacutainer tubes using a 21-gauge butterfly needle (BD Safety Lok Blood Collection Set, Plymouth, UK). On the same day, in the Haematology and Clinical Chemistry Laboratory of the OLVG Hospital, CD3⁺, CD4⁺, and CD8⁺ counts and expression of the T-cell activation markers were measured using anti-CD8 fluorescein isothiocyanate (SK1, immunoglobulin [Ig]G1), anti-CD38 phycoerythrin (HB7, IgG1), anti-CD3 peridinin chlorophyll (SK7, IgG1), and anti-human leukocyte antigen (HLA)-DR allophycocyanin (L243, IgG2a) from BD’s MultiTest reagent kit on blood collected in tubes containing 7.2 mg of K2- ethylenediaminetetraacetic acid (EDTA) (BD, Plymouth, UK). Twenty microliters of monoclonal antibody mixture was added to a TruCOUNT FACS tube (BD) and mixed with 50 μL of EDTA whole blood. After an incubation period of 15 minutes, 1 mL lysing solution (BD) was added to lyse all erythrocytes. After another incubation period of 30 minutes, the samples were run on a FACS Calibur (BD). Gating was performed using CellQuestpro software (BD).
controls were assessed by a Mann–Whitney U test for con-
tinuous variables, or odds ratios (OR) for dichotomous
variables, geometric mean (GM) ratios for natural log-
transformed continuous variables, or odds ratios (OR) for dichot-
omous variables, and 95% confidential intervals were obtained.
All P values of <.05 were considered to be statistically significant.

RESULTS

Study Population

Of the 35 patients screened, 28 met the study criteria and were
randomized. During the first cycle, 4 subjects in the placebo
group dropped out due to start of ART or for personal reasons.
During the second cycle, 2 subjects in the statin group dropped
out due to subjective side effects or lost to follow-up. In the pla-
CEO group, 2 subjects dropped out due to start of ART. Twenty
participants completed both cycles (Figure 1).

Table 1 shows the baseline characteristics. Patients were signif-
ically older than controls (median 40 vs 27 years, P < .01). The
median duration of established HIV infection was 1.8 years, and
the median HIV-1 RNA was 30,750 copies/mL. Furthermore, at
baseline, patients had a significantly lower CD4 count (480 vs
1080 cells/μL, P < .01), a higher CD8 count (1010 vs 530 cells/μL,
P < .01), and lower CD4/CD8 T-cell ratio (0.5 vs 1.9, P < .01).
Cholesterol levels were similar between both groups.

Immune Activation Markers Between Human Immunodeficiency Virus
Patients and Controls

Patients had higher median T-cell activation levels than controls
(e.g., HLA-DR + CD38 - % of CD8 +; 33.4 vs 5.6, P < .01) (Table 2).
Only the CD38+ percentage of CD4+ cells was not significantly different. In addition, IL-18, sTLR2, and neopterin levels were significant higher in patients compared with controls (503.5 vs 322.5 pg/mL [\(P < .01\)], 3.4 vs 2.0 ng/mL [\(P < .01\)], and 19.5 vs 5.4 nmol/L [\(P < .01\)], respectively). The hsCRP levels were higher in patients, but this difference did not reach the level of statistical significance (1.3 vs 0.9 mg/L, \(P = .07\)). We did not observe a difference in leukocyte count, d-dimer, IL-1Ra, IL-6, and sTLR4 levels. In HIV-patients, age correlated negatively with neopterin (\(R = -0.33, P < .01\)), sTLR2 (\(R = -0.25, P < .01\)), HLA-DR+ % of CD8+ T cells (\(R = -0.26, P < .01\)), and HLA-DR+CD38+ % of CD8+ T cells (\(R = -0.24, P < .01\)). No correlation was found between age and IL-18 (\(R = -0.33, P < .01\)) and other investigated markers of T-cell activation (data not shown).

![Flow chart of study participants](image)

**Table 1. Characteristics of Participants at Baseline**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>Patients (n = 28)</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>27 (24–33)</td>
<td>49 (34–47)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td><strong>Race, Caucasian</strong></td>
<td>8 (80%)</td>
<td>23 (82%)</td>
<td>.65</td>
</tr>
<tr>
<td><strong>Lipid panel</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.1 (3.3–6.1)</td>
<td>4.3 (3.9–5.1)</td>
<td>.57</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.5 (1.5–3.8)</td>
<td>2.4 (2.1–3.1)</td>
<td>.51</td>
</tr>
<tr>
<td><strong>HIV parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of infection, years</td>
<td>–</td>
<td>1.8 (0.7–4.3)</td>
<td>–</td>
</tr>
<tr>
<td>CD4 count, cells/µL</td>
<td>1.080 (580–1,230)</td>
<td>480 (450–680)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>CD8 count, cells/µL</td>
<td>530 (400–670)</td>
<td>1.010 (780–1,150)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>CD4/CD8 T-cell ratio</td>
<td>1.9 (1.2–2.7)</td>
<td>0.5 (0.4–0.9)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>HIV-1 RNA, copies/µL</td>
<td>30,750 (13,350–91,375)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range; LDL, low-density lipoprotein.

\(a\) Data are presented as median (IQR) or as number (percentage). Differences between patients and controls were assessed by a Mann-Whitney U test for continuous variables and Fisher’s exact test for the dichotomous variable race (Caucasian vs other). Significant results (\(P < .05\)) are presented in bold type.

**Table 2. Markers of Immune Activation in Patients and Controls**

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>Patients (n = 28)</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-cell activation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD38+, % of CD4+</td>
<td>56.3 (51.5–62.0)</td>
<td>60.4 (54.7–71.2)</td>
<td>.11</td>
</tr>
<tr>
<td>HLA-DR+, % of CD4+</td>
<td>5.2 (3.2–6.4)</td>
<td>13.7 (11.1–20.6)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>HLA-DR+CD38+, % of CD4+</td>
<td>2.3 (1.6–3.8)</td>
<td>9.1 (6.6–14.8)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>CD38+, % of CD8+</td>
<td>36.8 (29.5–46.5)</td>
<td>71.4 (58.2–82.5)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>HLA-DR+, % of CD8+</td>
<td>8.7 (7.0–10.1)</td>
<td>39.0 (35.9–49.2)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>HLA-DR+CD38+, % of CD8+</td>
<td>5.6 (4.1–7.2)</td>
<td>33.4 (29.9–40.8)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td><strong>Immune markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes, (\times 10^9/\text{L})</td>
<td>6.2 (4.4–7.0)</td>
<td>4.8 (4.4–7.0)</td>
<td>.45</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>0.9 (0.7–1.4)</td>
<td>1.3 (1.1–2.0)</td>
<td>.07</td>
</tr>
<tr>
<td>d-dimer, mg/L</td>
<td>0.2 (0.2–0.4)</td>
<td>0.3 (0.2–0.6)</td>
<td>.13</td>
</tr>
<tr>
<td>IL-1Ra, pg/mL</td>
<td>9.8 (9.9–10.9)</td>
<td>9.8 (9.9–10.9)</td>
<td>.51</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>2.4 (2.4–3.4)</td>
<td>2.4 (2.4–3.4)</td>
<td>.22</td>
</tr>
<tr>
<td>IL-18, pg/mL</td>
<td>322.5 (159.0–390.0)</td>
<td>503.5 (382.0–859.5)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>sTLR2, ng/mL</td>
<td>2.0 (1.3–2.7)</td>
<td>3.4 (2.7–4.3)</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>sTLR4, ng/mL</td>
<td>1.3 (1.3–3.1)</td>
<td>1.3 (1.3–3.1)</td>
<td>.42</td>
</tr>
<tr>
<td>Neopterin, nmol/L</td>
<td>5.4 (2.9–6.9)</td>
<td>19.5 (11.7–27.8)</td>
<td>&lt; .01</td>
</tr>
</tbody>
</table>

Abbreviations: HLA, human leukocyte antigen; hsCRP, highly sensitive C-reactive protein; IL, interleukin; IQR, interquartile range; LDL, low-density lipoprotein; sTLR, soluble Toll-like receptor.

\(a\) Data are presented as median (IQR). Differences between patients and controls were assessed by a Mann-Whitney U test. Significant results (\(P < .05\)) are presented in bold type.
During rosuvastatin treatment, LDL cholesterol plasma level decreased significantly compared with placebo treatment ($B = -0.94$ mmol/L, $P < .01$) (Table 3). The CD4 counts increased during statin treatment, but this did not reach the level of significance ($B = 36$ cells/$\mu$L, $P = .08$). CD4/CD8 T-cell ratio showed a small but significant increase (GM ratio = 1.11, $P = .02$). No change in HIV-1 RNA (GM ratio = 0.97, $P = .77$) nor on CD8 count (GM ratio = 0.95, $P = .24$) was found. Moreover, we did not observe an effect on T-cell activation markers (eg, HLA-DR$^+$CD38$^+$ % of CD8$^+$; GM ratio = 1.00, $P = .95$).

Likewise, no treatment effect was found on levels of leukocytes (GM ratio = 1.04, $P = .39$), hsCRP (GM ratio = 0.02, $P = .69$), d-dimer (OR = 0.80, $P = .61$), sTLR2 (GM ratio = 0.89, $P = .25$), nor on neopterin ($B = 0.7$ nmol/L, $P = .75$).

During statin use, ALT and AST levels increased significantly (GM ratio = 1.49, $P < .01$ and GM ratio = 1.26, $P < .01$, respectively). Creatine kinase and creatinine did not show any change (GM ratio = 1.19, $P = .29$ and GM ratio = 1.01, $P = .37$, respectively).

### Quality of Life During the Trial
During rosuvastatin treatment, more flu-like symptoms were reported (~16 of 100 point, $P < .01$) (Table 4). There was no significant difference in other complaints because the MOS-HIV questionnaire did not reveal any significant differences between statin and placebo treatment.

### DISCUSSION
In this exploratory study in ART-naive HIV-patients, we investigated the effect of 20 mg of rosuvastatin on soluble markers of immune activation and on T-cell activation. As expected, the
patients infected with HIV displayed substantially increased T-cell activation compared with healthy controls. In contrast to other studies, we did not find increased concentrations of IL-6 or hsCRP (for the latter, the difference just failing statistical significance). This discrepancy may be due to the small sample size. However, other markers of monocyte/macrophage activation were increased in patients compared with controls, among which were neopterin, IL-18, and sTLR-2.

Increased concentrations of neopterin have been described before in patients infected with HIV and were associated with other markers of monocyte activation in these patients [22]. The increased concentrations of IL-18 in our patients is in accordance with the findings of other studies, in which IL-18 concentrations correlated positively with HIV viral load and negatively with CD4 cell counts [23, 24]. We are the first to describe elevated concentrations of sTLR-2 in patients infected with HIV. Soluble TLR-2 correlates positively with other inflammatory markers such as tumor necrosis factor-α, IL-6, and IL-1Ra [25]. Taken together, these findings confirm that our group of ART-naive HIV-patients showed substantial immune activation.

We did not find an effect of rosuvastatin on any of the soluble markers of inflammation measured. Very few other studies have investigated the effect of statins on soluble markers of monocyte activation or inflammation in patients infected with HIV. In an uncontrolled study, a reduction in CRP concentration was observed after 45 days of pravastatin or rosuvastatin in patients treated with ART [26]. Eckard et al [27], investigating patients treated with ART, did not find an effect on hsCRP concentrations after 24 weeks of 10 mg rosuvastatin, but the same group reported a decrease of soluble CD14 [28]. These effects seemed to be more pronounced after 48 weeks of rosuvastatin [15]. Thus, the lack of an effect on soluble markers of inflammation in our study may be due to the relatively short duration of treatment.

Although we found a significant activation of CD8 cells in our patients at baseline, we did not observe a reduction of T-cell activation after 8 weeks of rosuvastatin treatment. However, we did observe a small but significant increase in CD4/CD8 ratio, which may be regarded as an indirect sign of decreased T-cell activation. Our study design was similar to the study by Ganesan et al [14], who treated ART-naive HIV-patients with 8 weeks of 80 mg atorvastatin and found significant reductions in circulating proportions of CD4\(^+\)HLA-DR\(^+\), CD8\(^+\) HLA-DR\(^+\), and CD8\(^+\)HLA-DR\(^+\)CD38\(^+\) T cells. They did not report on changes of CD4/CD8 ratio. The differences between our findings and the study of Ganesan et al [14] remain speculative. Possible explanations may include the fact that rosuvastatin is a hydrophilic statin, whereas atorvastatin is lipophilic. Direct inhibition of T-cell activation in vitro has been reported with atorvastatin [29]. In vivo, the effects of statins on T-cell activation in non-HIV-patients have shown divergent results [30–32]. Of course, we cannot exclude the possibility that statins have only marginal effects on inflammatory markers in patients infected with HIV, and that small differences between studies are merely the result of biological variation between the patients in the various studies.

Rosuvastatin treatment resulted in well known side effects, such as flu-like symptoms and elevation of liver enzymes. Only 1 patient had to discontinue the study due to side effects.

Our study may have been underpowered due to a high number of patient withdrawal of an already small sample size. Therefore, we consider our study as being exploratory. In this respect, it is noteworthy that a substantial number of subjects withdrew from the study during the placebo phase because their CD4 cell count dropped and they had to start ART. As mentioned before, although the significant decline in LDL cholesterol suggests adequate rosuvastatin intake, 8 weeks may be too short a period for a detectable effect on immune activation markers. Future research may investigate the effects of statins on markers of inflammation for a longer time period and in a larger study population. Possible differential effects of hydrophilic versus lipophilic statins may also be of interest in future studies. The fact that we chose to study ART-naive HIV-infected subjects to exclude the effect of ART on immune activation has its limitations. In future studies, an additional effect of statins on immune activation in ART-treated patients may be harder to detect.

**CONCLUSIONS**

In conclusion, this small group of treatment-naive HIV-infected patients had higher levels of T-cell activation markers and higher circulating neopterin, IL-18, and sTLR2 levels compared with controls. Eight weeks of rosuvastatin had a small but significant positive effect on CD4/CD8 T-cell ratio, but no influence on other markers of T-cell activation and innate immunity was identified.

**Acknowledgments**

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**Table 4. Quality of Life During the Trial**

<table>
<thead>
<tr>
<th></th>
<th>Placebo (Mean)</th>
<th>Statin (Mean)</th>
<th>Statin Effect</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>10.0</td>
<td>18.7</td>
<td>–8.6</td>
<td>.09</td>
</tr>
<tr>
<td>Muscle pain</td>
<td>15.9</td>
<td>12.3</td>
<td>3.6</td>
<td>.38</td>
</tr>
<tr>
<td>Pain legs</td>
<td>10.0</td>
<td>10.7</td>
<td>–0.7</td>
<td>.84</td>
</tr>
<tr>
<td>Flu-like symptoms</td>
<td>1.8</td>
<td>17.9</td>
<td>–16.1</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>Tiredness</td>
<td>27.7</td>
<td>35.3</td>
<td>–7.4</td>
<td>.14</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; LMM, linear mixed modeling; MOS-HIV, Medical Outcomes Study HIV Health Survey.

* the validated MOS-HIV questionnaire and complaints list was used to assess the quality of life of the subjects, ie, the HIV-infected patients, during the trial, and analyzed by LMM (scale ranges from 1 to 100, whereas 100 is most negative). Significant results (P < .05) are presented in bold type.
Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References


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