

**In vivo and in vitro effects of simvastatin on inflammatory markers in pre-dialysis patients**

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**Abstract**

**Background.** The beneficial effects of statins in reducing cardiovascular events have been attributed predominantly to their lipid-lowering effects, recent studies suggest that these effects might be due to their anti-inflammatory properties. We here investigate the in vivo and in vitro effects of simvastatin on cytokine production in pre-dialysis chronic renal failure patients.

**Methods.** Our clinical study has been designed as a randomized double-blind placebo controlled study. A total of 55 chronic kidney disease (CKD) patients at stages 3 and 4 (mean creatinine clearance 45 ml/min, range 15–60) were randomly assigned to receive simvastatin 40 mg/day or placebo, added to their ongoing treatment, for 6 months. Blood samples were obtained at baseline, and after 3 and 6 months of observation for the determination of lipids, inflammatory markers and renal function. For the in vitro studies, the effect of increasing doses of simvastatin on cytokine production [namely interleukin (IL)-6 and IL-8] in human cultured monocytes from 10 healthy subjects (HS) and 15 CKD patients stimulated by lipopolysaccharide (LPS) was investigated.

**Results.** A significant reduction in total cholesterol from 221±44 mg/dl to 184±41 mg/dl (3 months) and to 186±39 mg/dl (6 months) (P < 0.02) and low-density lipoprotein cholesterol from 139±40 mg/dl to 104±29 mg/dl (3 months) and to 100±31 mg/dl (6 months) (P < 0.001) was observed in the 28 patients treated with simvastatin. In this group, C-reactive protein (CRP) levels significantly decreased from 2.6 mg/l [interquartile range (IQR 4.9)] to 2.0 mg/l (IQR 1.9) (P = 0.03) at 6 months (P < 0.05). A parallel reduction of IL-6 levels from 5.1 pg/ml (IQR 3.8) to 3.5 pg/ml (IQR 3.1) (P = 0.001) at 6 months was also observed. No significant reduction in inflammatory markers [CRP from 5.1 mg/l (IQR 1.9) to 5.4 mg/l (IQR 1.3) (P = NS) at 6 months] or plasma lipids [LDL-cholesterol from 127±32 mg/dl to 131±21 mg/dl (6 months)] was observed in the 27 patients of the placebo group. In the in vitro studies, the average value for cell-associated IL-6 and IL-8 was higher in CKD (155±95 pg/ml monocytes for IL-6 and 722±921 pg/ml monocytes for IL-8) vs HS (137±87 pg/ml monocytes and 186±125 pg/ml monocytes) (P < 0.01) and was not affected by simvastatin alone. LPS resulted in a significant increase in cytokine production (IL-6: 1954±321 pg/ml monocytes for CKD and 1451±237 pg/ml monocytes for HS; P < 0.001); the simultaneous addition of increasing doses of simvastatin to these cultures induced a dose-dependent inhibition of IL-6 and IL-8 production in stimulated peripheral blood mononuclear cells in all groups.

**Conclusions.** These results indicate that simvastatin in commonly used doses has an in vitro and in vivo anti-inflammatory effect in CKD patients, and may play an important role in counteracting the mechanisms involved on the pathogenesis of cardiovascular disease.

**Keywords:** chronic kidney disease; C-reactive protein; cytokines; simvastatin

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**Introduction**

Cardiovascular disease (CVD) in end-stage renal disease (ESRD) is responsible for the majority of...
morbidity and mortality in patients on haemodialysis and, despite all recent advances in prevention and treatment of CVD, the increased risk associated with ESRD persists [1]. The high cardiovascular mortality rate suggests that ESRD patients are subjected to a process of accelerated atherogenesis starting many years before dialysis. Although hypercholesterolaemia is a risk factor for CVD in the general population, the association between lipid abnormalities and CVD is less clear among ESRD patients. Increasing evidence suggests that the immune system plays an important role in the accelerated atherogenesis in these patients [2]. Plasma levels of inflammation markers have been shown to be predictors of coronary heart disease in chronic renal failure patients as well as in the general population, and the involvement of inflammatory cells such as monocytes or macrophages and subsets of T lymphocytes is critical to the progression of atherosclerosis [3]. In randomized, controlled trials, treatment of hypercholesterolaemia with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, in the form of statins, has been shown to reduce both total and cardiovascular mortality in the general population [4]. Moreover, in recent research, statins have been shown to exhibit a variety of pleiotropic effects [5] that include immune-modulating, antiproliferative and antithrombotic activities. Among these additional cholesterol-independent effects, further evidence suggested that statins may interfere with the biology of inflammation that plays a pivotal role in the pathogenesis of CVD, acting as anti-inflammatory agents. The possibility that statins may interfere with this low degree of inflammatory activity has been suggested from in vitro effects of statins on inflammatory cells in culture and from in vivo studies in humans. Thus, several studies have shown a reduction of C-reactive protein (CRP) by statins, and plasma levels of tumour necrosis factor-α (TNF-α) have been found to be reduced by HMG-CoA reductase inhibitors in vitro and in vivo [6]. Although substantial evidence suggests that treatment of dyslipidaemia with statins reduces mortality and morbidity associated with CVD, only a few studies have examined the anti-inflammatory efficacy of statins in patients with chronic renal failure (CRF) [7], and in particular in the pre-dialytic phase of renal insufficiency. Simvastatin is one of the most commonly prescribed HMG-CoA reductase inhibitors and has been used extensively in renal patients [8]. To this aim, we evaluated the effects of simvastatin on in vitro and in vivo cytokine production in patients affected by CRF in pre-dialysis.

Patients and methods

Clinical study

Patients and study protocol. This is a double-blind, placebo-controlled, simply randomized trial to evaluate the efficacy of simvastatin in reducing plasma levels of inflammatory markers in chronic kidney disease (CKD) patients.

Between January and June 2004, 55 patients (44 males and 11 females) with CRF and hypercholesterolaemia defined as low-density lipoprotein (LDL)-cholesterol >100 mg/dl were included in the study after informed consent. They were randomized to receive simvastatin 40 mg/day or placebo for 6 months added to their ongoing therapy. Patients, investigators and data analysers were blinded to the treatment groups.

The patients’ baseline characteristics and their laboratory parameters are shown in Table 1. Patients who were >75 years of age were not included; other exclusion criteria were the presence of nephrotic syndrome, diabetes, malignant diseases, treatment with immunosuppressant agents, connective tissue disease, any cardiovascular event in the previous 6 months, any acute disease, and hypersensitivity to statins. Patients did not receive corticosteroids, non-steroidal anti-inflammatory drugs or other drugs known to interfere with lipids or inflammation parameters throughout the study period. In particular, patients receiving sevelamer and aspirin were excluded from the study. The causes of CRF were chronic glomerulonephritis in 27 patients, polycystic kidney disease in seven patients, interstitial nephritis in five patients, nephrosclerosis in four patients, and other or unknown aetiologies in 12 patients. Mean systolic and diastolic blood pressure was 145±2 and 82±3 mmHg, respectively. Nearly half of the patients (53%) were on antihypertensive medications (β-blockers, n=5; calcium channel blockers, n=14; and angiotensin-converting enzyme inhibitors, n=16), as well as other commonly used drugs in CRF such as phosphate binders, calcium carbonate, diuretics, and vitamin B, C and D supplementation.

Table 1. Patients’ baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Simvastatin</th>
<th>Control</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>27</td>
<td>NS</td>
</tr>
<tr>
<td>Men</td>
<td>23</td>
<td>21</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60±10</td>
<td>55±13</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>25.2±2.8</td>
<td>24.6±2.2</td>
<td>NS</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>40±12</td>
<td>32±13</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>220.1±44</td>
<td>217.0±43</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>44.5±13</td>
<td>48±9</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>139±41</td>
<td>130±57</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>193±93</td>
<td>165±50</td>
<td>NS</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>2.6</td>
<td>5.1</td>
<td>NS</td>
</tr>
<tr>
<td>IQR</td>
<td>4.9</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>25th and 75th percentiles IL-6 (pg/ml)</td>
<td>1.1–6.1</td>
<td>3.9–5.9</td>
<td>NS</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>3.8</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>IQR</td>
<td>5.6</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>25th and 75th percentiles Serum albumin (g/dl)</td>
<td>4.8–10.4</td>
<td>4.7–10.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>51%</td>
<td>54%</td>
<td>NS</td>
</tr>
<tr>
<td>IMT</td>
<td>0.8±0.19</td>
<td>0.83±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>AP</td>
<td>21.4%</td>
<td>18.5%</td>
<td>NS</td>
</tr>
<tr>
<td>LVMi</td>
<td>121±18</td>
<td>116±14</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI = body mass index; GFR = glomerular filtrate rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; CRP = C-reactive protein; IQR = interquartile range; IMT = intima-media thickness; AP = prevalence of atherosclerotic plaques; LVMi = left ventricular mass indexed for body surface area.

There were no significant differences between groups according to the patients’ characteristics.
weight (kg)/height (m), was also determined at each visit. Since the first evaluation, all patients were recommended to perform standard physical activity and to keep to a cholesterol-lowering diet, standardized according to phase I of the National Cholesterol Education Program, Adult Treatment Panel II (NCEP II). A 3 month supply of the statin or placebo prescription was dispensed to the study patients in blister packs at each visit; adherence to the therapy was assessed by the count of the pills at the following visit. In all patients, peripheral venous blood samples were taken, after a 12 h overnight fast, at baseline, after 3 and 6 months of treatment and at the end of the study. Serum and plasma samples were frozen and stored at −70°C until assayed. For all analyses, samples were handled in a blinded fashion such that investigators had no knowledge of the randomization status.

**Biochemical methods.** Serum cholesterol and triglycerides were analysed by standard enzymatic procedures (Boehringer Mannheim, Mannheim, Germany). High-density lipoprotein-(HDL) cholesterol levels were determined after precipitation of apo B-containing lipoproteins by phosphotungstic acid. LDL-cholesterol levels were calculated using the Friedewald formula. Serum albumin was measured with a nephelometric technique (Dade, Berhing Diagnostics, Gmbh, Marburg, Germany) with an intra- and inter-assay variability of 4.3 and 4.4%, respectively. CRP was measured by a high sensitivity modified laser nephelometry technique (Berhing Diagnostics). The CRP assay was standardized according to the WHO First International Reference Standard and had a sensitivity of 0.1 μg/ml, with a standard reference range of between 0.1 and 0.4 mg/l. Plasma for CRP and cytokine analysis, collected using heparin as anticoagulant, had a sensitivity modified laser nephelometry technique (Berhing Diagnostics, Gmbh, Marburg, Germany) with an intra- and inter-assay variability of 4.3 and 4.4%, respectively. CRP was measured by a high sensitivity modified laser nephelometry technique (Berhing Diagnostics). The CRP assay was standardized according to the WHO First International Reference Standard and had a sensitivity of 0.1 μg/ml, with a standard reference range of between 0.1 and 0.4 mg/l. Plasma for CRP and cytokine analysis, collected using heparin as anticoagulant, was separated <30 min after drawing and stored at −40°C until analysis. Samples were assayed in duplicate and no cross-reactivity or interference was found with other factors related to or associated with interleukin IL-6 or IL-8. At the same time, in addition to the inflammatory and lipid profiles, the following variables were also determined: creatine phosphokinase, alanine aminotransferase and aspartate aminotransferase by enzymatic methods, and all standard laboratory analysis.

**Evaluation of renal function.** Plasma and urinary creatinine was measured with standard auto-analyser techniques (Boehringer Mannheim automated analysis for Hitachi 717/911). The glomerular filtration rate (GFR) was measured as the renal clearance of [99mTc]DTPA. Values of creatinine clearance and of GFR were reported in relation to the standard body surface area of 1.73 m².

**Carotid artery ultrasonography.** Carotid ultrasonography was performed, at baseline and after 6 months, using the Sonos 5500 (Philips Technologies, USA) phased-array echograph with M-mode, two-dimensional, and pulsed colour-flow Doppler capabilities. The imaging protocol involved obtaining a single longitudinal lateral view of the distal segment of the right and left common carotid arteries. The high-resolution images were analysed to calculate intima-media thickness (IMT) at each arterial site. We obtained the IMT in five consecutive regions of the far wall every 4–5 mm from the bifurcation of the common carotid artery with use of the 7.5 MHz linear-array probe. The value attributed to each subject represents the average value among 10 IMT measurements, five from the left and five from the right carotid artery. Intra- and inter-observer variability for IMT were 4.6 ± 0.4 and 5.2 ± 0.3%, respectively.

**Echocardiography.** The echocardiographic studies were performed, at baseline and after 6 months, using the Sonos 5500 ultrasound system. We measured the end-diastolic diameter of the left ventricle and the thicknesses of the interventricular septum and the posterior wall, according to the recommendations suggested by the American Society of Echocardiography. Left ventricular mass (LVM) was calculated according to the ‘Penn convention’ [9] and indexed (LVMi) for the body surface area.

**In vitro studies**

**Patients.** The study population consisted of 15 patients (nine male and six female) suffering from CRF with a mean creatinine clearance of 26 ± 8 ml/min (range 12–45), a mean age of 59 ± 11 years and a mean CRP value of 4.0 ± 1.7 mg/l. The clinical exclusion criteria were the same as previously described for patients enrolled in the clinical study. Ten sex- and age-matched healthy adult volunteers (HS) with normal renal function served as the controls. None of these subjects received statins, erythropoetin, vitamin D, iron or phosphate binders for at least 2 months before the study.

**Cell isolation.** Monocyte-enriched peripheral mononuclear blood cells (PBMCs) were isolated by Ficoll (Histopaque-1077, Sigma-Aldrich Co., Ltd, Poole, UK) gradient centrifugation from blood that was withdrawn into syringes containing 0.2 M EDTA as anticoagulant. Cells were washed in incomplete TTBSA (sodium chloride 0.8 g/l, potassium chloride 0.195 g/l, Tris 1.1 g/l, glucose 1.0 g/l, bovine serum albumin 2.5 g/l), counted and cultured in RPMI 1640 medium (Sigma-Aldrich Co.) supplemented with 10% heat-inactivated fetal calf serum, glutamine and antibiotics in 24-well plates (Costar, Cambridge, MA) at 37°C in an atmosphere containing 5% CO₂. RPMI contained no detectable amount of endotoxin as assessed by the Limulus amebocyte lysate test. Lymphomonocytes (non-adherent cells) were removed by aspiration with a Pasteur pipette and washing of the dishes with warm media [10]. The purity of the monocyte fraction was confirmed by fluorescence-activated cell sorting (Becton-Dickinson, San Jose, CA) analysis using anti-CD14 monoclonal antibody. Cells were incubated with lipopolysaccharide (LPS) from Escherichia coli and Pseudomonas maltophilia at a final concentration of 1 ng/ml and simultaneously with increasing doses of simvastatin (from 10⁻⁶ to 10⁻¹⁰ M) for 12 h. Monocytes were incubated in the following conditions: alone, with LPS alone, with LPS and 10⁻⁶ M simvastatin, with LPS and 10⁻³ M simvastatin, with LPS and 10⁻¹⁰ M simvastatin and with simvastatin alone at all the previous concentrations. The simvastatin was a gift from Merck Company (USA).

**Cytokine assays.** After the incubation, cells were lysed by three freezing (-40°C) and thawing (37°C) cycles. Intracellular and supernatant-associated cytokine concentrations were determined separately by enzyme immunoassay (IL-6, Biosource, Camarillo, CA, and IL-8, Bender MED-Systems, Vienna, Austria). The percentages of intra- and inter-assay variability were respectively 6.7 and 12.5% for...
IL-6, and 8 and 10.4% for IL-8. The sensitivity thresholds were < 5 pg/ml for IL-6 and < 6 pg/ml for IL-8. Samples were assayed in duplicate and results were normalized to the monocyte concentration of 1 x 10^6 monocytes and expressed in pg/ml.

Statistics
The intention to treat principle was followed for comparison of randomized groups. Continuous variables with a normal distribution are reported as means ± SD. CRP, IL-6 and IL-8 levels are reported as median, interquartile ranges (IQRs) and 25th and 75th percentiles. Results were evaluated by one-way analysis of variance and Student’s t-test for paired and unpaired data. Wilcoxon’s signed-rank test was used as a non-parametric test to compare CRP, IL-6 and IL-8 variations. The χ² test was used to compare differences in the percentages of co-morbidities and other dichotomous data. P-values < 0.05 were regarded as statistically significant.

Results
Clinical study
At baseline, no difference in total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, plasma inflammatory markers, renal function and incidence of co-morbidity was observed between the groups assigned to simvastatin or placebo (Table 1). A significant reduction of total serum cholesterol from 221 ± 44 mg/dl to 184 ± 41 mg/dl (3 months) and to 186 ± 39 (6 months) (P < 0.02) and of LDL-cholesterol from 139 ± 40 mg/dl to 104 ± 29 mg/dl (3 months) and to 100 ± 31 mg/dl (6 months) (P < 0.001) was observed in the simvastatin group. No significant variation was observed in HDL-cholesterol that varied from 44 ± 12 to 47 ± 13 mg/dl (P = NS) in the simvastatin-treated group and from 48 ± 14 to 40 ± 11 mg/dl in the placebo control group. Triglycerides were reduced from 193 ± 38 to 165 ± 31 mg/dl (P < 0.05) in the simvastatin group, whereas they remained unchanged in the placebo control group. In this group, CRP levels significantly decreased from 2.6 mg/l (IQR 4.9) to 2.0 mg/l (IQR 1.9) (P = 0.03) at 6 months. A parallel reduction of IL-6 levels from 5.1 pg/ml (IQR 3.8) to 3.5 pg/ml (IQR 3.1) (P = 0.001) (6 months) was also observed. IL-8 was significantly reduced from 8.4 pg/ml (IQR 5.6) to 6.7 pg/ml (IQR 6.1) (P = 0.03). No significant reduction in inflammatory markers [CRP from 5.1 mg/l (IQR 1.9) to 5.4 mg/l (IQR 1.3) (P = NS) (6 months)] or plasma lipids [LDL-cholesterol from 127 ± 32 mg/dl to 131 ± 21 mg/dl (6 months)] was observed in the placebo group. Simvastatin was discontinued in one patient for the occurrence of myopathy, and this patient was excluded from the study. The GFR at 6 months varied from 40 ± 12 to 41 ± 15 ml/min in the simvastatin-treated group and from 32 ± 13 to 30 ± 14 ml/min in the placebo control group (P = NS).

Carotid artery ultrasonography and echocardiography.
The vascular lesions noticed in our patients were small atherosclerotic plaques which were not haemodynamically significant; these plaques were present in 11 subjects (20%). In six of them (10%), the lesions were located in the carotid area of one side; in the other five patients the lesions were present in both carotid arteries. All the plaques presented a structure of hyperechogenicity (‘hard’ plaque), typical of old plaques, characterized by abundant fibrocalcified tissue. In the whole group of patients, the mean thickness of the common carotid wall was 0.82 ± 0.17 mm. The subgroup of treated and untreated patients showed a comparable IMT (0.80 ± 0.19 vs 0.83 ± 0.2 mm; P = NS). No significant variation was observed in the treated group and untreated group after 6 months (0.80 ± 0.19 vs 0.78 ± 0.14 mm; P = NS). LVM did not vary significantly during the follow-up time.

In vitro studies
The average value for cell-associated IL-6 and IL-8 was higher in CRF (155 ± 95 pg/ml monocytes for IL-6 and 722 ± 321 pg/ml monocytes for IL-8) vs HS (137 ± 87 pg/ml monocytes and 186 ± 125 pg/ml monocytes) (P > 0.01) and was not affected by simvastatin alone. LPS resulted in a significant increase of cytokine production (IL-6: 1954 ± 321 pg/ml monocytes >0.01) and was not affected by simvastatin alone. HS (137 ± 87 pg/ml monocytes and 186 ± 125 pg/ml monocytes) (P > 0.01); the simultaneous addition of increasing doses of simvastatin to these cultures induced dose-dependent inhibition of IL-6 and IL-8 production in stimulated PBMCs in both groups (Figures 1 and 2, and 3 and 4). However, the inhibitory effects of simvastatin seem to be nearly complete at the concentration of 10^-6 mol/l only in HS for IL-8, whereas complete inhibition was never observed in CRF at the same concentrations (P < 0.001).

Discussion
This study shows that simvastatin in commonly used doses in large clinical trials has an in vitro and in vivo anti-inflammatory effect in patients affected by pre-dialytic CRF, and may play an important role in counteracting the mechanisms involved in the pathogenesis of CVD. Data from several large clinical trials in the general population demonstrated that HMG-CoA reductase inhibitors are effective in CVD prevention with a relatively safe profile. Patients with CKD are at high risk for developing premature CVD, so the benefits of statin therapy might be expected to be substantial in this population. However, statin use in CKD patients has been associated with different efficacy in CVD prevention in several ongoing trials such as 4D (atorvastatin) [11] and AURORA (rosuvastatin) [12].
There is a growing body of evidence suggesting that chronic inflammation has a crucial role in the onset and progression of atherosclerosis in chronic renal patients as well as in the general population. Principal among these, due to its predictive power and clinical usefulness, is CRP. A high CRP level is associated with increased risk of peripheral vascular disease, myocardial infarction, stroke and sudden cardiovascular death. It has been reported that a single measurement of CRP is a stronger predictor of cardiovascular events than LDL-cholesterol, that it identifies at-risk individuals with low LDL-cholesterol levels, that it adds prognostic information to the currently used Framingham risk algorithm [13] and that it identifies high risk for cardiovascular events in individuals with the metabolic syndrome. Statin treatment has been found to reduce CRP levels in the general population [14]; this effect, which appears to be dose related, may be due to reduced LDL oxidization (as a consequence of lowered LDL particle number) and/or additional anti-inflammatory activities that statins exert independently of the decrease in LDL. Several other studies suggest that statins have a number of in vivo effects, in addition to their lipid-lowering capacity, including the reduction of different inflammatory mediators, such as CRP [3]. Moreover, recent research has shown that statins have other anti-inflammatory properties, including inhibition of leukocyte–endothelium interactions and the reduction of inflammatory cell numbers within atherosclerotic plaques [15]. The mechanism by which macrophages contribute to plaque rupture has not been completely clarified to date; however, release of pro-inflammatory cytokines may induce plaque instability and in turn favour its rupture. Regardless
of the mechanisms involved, it is apparent that statins can reduce the expression of adhesion molecules, such as intercellular adhesion molecule-1, which are involved in the recruitment of circulating monocytes [15]. Furthermore, published data suggest that statins effectively lower plasma CRP levels in hyperlipidaemic patients [16] and TNF-α and IL-6 concentrations in LPS-stimulated PBMCs [17]. In this study, we demonstrated a significant in vitro and in vivo anti-inflammatory effect of simvastatin in a population that has not been investigated previously. Little information, in fact, has been reported up to now in CRF patients. Simvastatin is a long-established HMG-CoA reductase inhibitor, first introduced in 1988, that can be used at the maximal recommended dose of 80 mg/day. This molecule has been studied in two large outcome trials, the Scandinavian Simvastatin Survival Study (4S) [4] and the Heart Protection Study (HPS), both of which demonstrated beneficial effects on a variety of cardiovascular outcomes, with minimal adverse effects. In this study, a significant decrease of CRP plasma levels was observed in the simvastatin-treated group of CRF patients at a dose of 40 mg/day. This is a potentially interesting observation because increasing data from prospective cohort studies have demonstrated a strong association between elevated CRP levels and all-cause and cardiovascular mortality in ESRD patients. Furthermore, in this study, we also decided to investigate simvastatin-induced IL-6 and IL-8 plasma level variation to gain insight into the peculiar role of these cytokines in the assessment of cardiovascular risk of the uraemic patient.
Recent studies have shown that plasma IL-6 rather than CRP may better predict outcome in ESRD patients. Pecoits-Filho et al. [18] demonstrated in 173 ESRD patients followed for 3.1 years that the strong predictive value of elevated IL-6 levels for poor outcome in ESRD patients is similar in both haemodialysis and peritoneal dialysis patients starting treatment, and we demonstrated that the independent prognostic value of IL-6 is superior to that of CRP in a retrospective study involving >200 chronic dialysis patients studied for 4 years [19]. Furthermore, in this study, the in vitro and in vivo production of IL-8 has been also evaluated according to the hypothesis that aberrant production of IL-8 has been shown to occur in various human inflammatory diseases, and IL-8 appears to be not only the plasma expression of the acute phase response but also a direct pathogenic mediator of the atherosclerotic process. Furthermore, IL-8 levels have been found to be elevated in PBMCs from hypercholesterolaeica patients, and serum IL-8 levels were associated with unstable angina pectoris and acute myocardial infarction [20].

The in vitro study provides support for the findings of the clinical study. In this study, we have used LPS stimulation—a well-characterized pathogenic factor of Gram-negative bacteria—capable of activating a wide variety of inflammatory functions in neutrophils and monocytes, including the induction of adhesion, the activation of the respiratory burst and cytokine secretion.

The dose of simvastatin given in this experimental study is around the usual pharmacological drug level (10⁻⁶ mol/l is equivalent to 60% of 5 mg/dl for humans). Preliminary time course studies have shown that IL-6 production increases very rapidly at 2 or 4 h of stimulation and therefore continues to rise at a slower rate, reaching a peak at 24 h [17]. Moreover, we demonstrated that increasing doses from 10⁻⁸ to 10⁻⁶ mol/l of simvastatin could inhibit the release of IL-6 and IL-8 in monocytes stimulated by LPS in a dose-dependent manner; however, regarding IL-8, the higher dose of simvastatin resulted in a nearly complete inhibition of cytokine release in HS, whereas at the same dose of 10⁻⁶ mol/l only a 42% inhibition was observed in CRF. Furthermore, a complete inhibition of cytokine production was achieved in HS only for IL-8, whereas a significant but not complete inhibition was observed for IL-6.

Finally, no significant variation was observed regarding IMT and LVM in the treated group and untreated group after 6 months of observation; this might be due to the short follow-up time of the study.

Conclusions

Statin therapy has been shown to reduce cardiovascular risk in a wide range of patients. The reduction of inflammatory components of atherosclerotic plaques may represent an additional mechanism accounting for the reduction of cardiovascular events after HMG-CoA reductase inhibitor treatment. Additional investigations are needed to define better the full range of benefits and potential uses of such therapy in CRF. This study shows that simvastatin has an anti-inflammatory property by inhibiting the monocyte expression of IL-6 and IL-8 and reducing the plasma levels of several inflammatory markers in a population of chronic pre-dialysis patients. However, simvastatin modulation of cytokine production in isolated PBMCs may be different in CRF patients compared with healthy subjects; this suggests the opportunity for further studies aimed at verifying a safe and effective dosage of HMG-CoA reductase inhibitors in this peculiar population.

Conflict of interest statement. None declared.

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