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Original article

Simvastatin and fluvastatin reduce interleukin-6 and interleukin-8 lipopolysaccharide (LPS) stimulated production by isolated human monocytes from chronic kidney disease patients

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Abstract

Background: Statins reduce lipid levels, inflammation and cardiovascular events in patients with coronary artery disease; CKD patients show increased risk of cardiovascular and increased plasma levels of IL-6 and IL-8.
Aim: To evaluate the in vitro effect of simvastatin (S) or fluvastatin (F) on the lipopolysaccharide (LPS) stimulated secretion of IL-6 and IL-8 from monocytes of chronic kidney disease patients (CKD) in K-DOQI stages 3-5.
Methods and subjects: Monocytes enriched peripheral blood (PBMC) from 28 CKD (15 in K-DOQI stages 3-4, Group I, and 13 in K-DOQI stage 5 on hemodialysis, Group II) and 10 healthy subjects (HS), were isolated by Ficoll-gradient centrifugation. Cells were incubated with LPS 100 ng/ml or with LPS plus increasing doses of statins (from 10^{-6} to 10^{-8} M) for 24 h. Surnatant IL-6 and IL-8 concentrations were determined by EIA.
Results: Basally the mean concentration of IL-6 and IL-8 was higher in patients than in HS and in Group II than in Group I (IL6: HS 285 ± 77 pg/ml, Group I 365 ± 178 pg/ml, Group II 520 ± 139 pg/ml- IL8 HS 180 ± 75 pg/ml, Group I 1722 ± 582 pg/ml, Group II 4400 ± 1935 pg/ml). After addition of LPS the mean concentration of IL-6 and IL-8 increased in all groups (IL6: HS 1740 ± 178 pg/ml, Group I 3754 ± 672 pg/ml, Group II 4800 ± 967 pg/ml; IL8: HS 450±132 pg/ml, Group I 9700±2837 pg/ml, Group II 11608 ± 2316 pg/ml). After the addition of LPS plus increasing doses of S or F from 10^{-10} to 10^{-6} M, a significantly lower cytokine concentration compared to the data after LPS alone was observed (IL6: HS 45%, Group I 75%, Group II 50%; IL8: HS 100%, Group I 65%, Group II 35%).
Conclusions: These data confirm that cytokine release is increased in CKD patients and that is highest in the most severe patients. Furthermore they suggest that fluvastatin or simvastatin can be used in order to reduce the high cardiovascular risk.

Keywords: Statin; Interleukin-6 (IL-6); Interleukin-8 (IL-8); Chronic kidney disease (CKD); PBMC

1. Introduction

Recent trials demonstrated that intensive lipid-lowering therapy with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, in the form of statins, reduced the progression of atherosclerosis [1] and improved clinical outcomes. The benefit of intensive statin therapy, as compared with moderate statin therapy, was attributed to reduction in the levels of atherogenic lipoproteins, particularly low-density lipoprotein (LDL) cholesterol [2]. However, statins have further effects, including anti-inflammatory action, a phenomenon commonly termed as ''pleiotropic effect'' [3,4].

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Simvastatin decreased macrophage content and vascular cell adhesion molecule-1, interleukin-1β (IL-1β) and tissue factor expression in abdominal aortas from cynomolgus monkeys independently on their effect of plasma cholesterol levels [5]. On the contrary lipophilic statin treatment resulted in pro-inflammatory cytokine secretion from freshly isolated monocytes and in enhanced leukocyte recruitment into mice peritoneal cavities after a thioglycollate challenge [6].

The high cardiovascular mortality rate of CKD patients is also due to a process of accelerated atherogenesis starting many years before dialysis [7]; this accelerated atherogenesis is tightly linked to the chronic inflammatory state of the uremic population [8–10].

Although treatment with statins reduced mortality and morbidity associated with cardiovascular disease CVD [11,12], only a few in vivo studies have examined the anti-inflammatory efficacy of statins in patients with CKD [12,13].

IL-6 and IL-8 are the most important pro-inflammatory cytokines produced by different cellular types in response to various stimuli. Simvastatin and fluvastatin are the most widely utilized statins in the clinical practice.

Our objective was to assess the in vitro the effect of simvastatin or fluvastatin on IL-6 and IL-8 secretion from human peripheral blood mononuclear cells of healthy subjects and patients with various stages of CKD.

2. Materials and methods

2.1. Study design

In this study we evaluated the secretion of IL-6 and IL-8 by cultured human PBMCs basally, after stimulation with LPS and after the addition of LPS plus simvastatin or fluvastatin.

2.2. Subjects

2.2.1. Patients, Group I

The first group of the study population consisted of fifteen patients (9 male and 6 female) (CKD) suffering from CKD in K-DOQI stages 3–4 [14] with a mean creatinine clearance of 28 ± 8 ml/min (range 16–42 ml/min) and a mean age of 59 ± 11 years. Patients who were more than 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 six months, any acute disease, and hypersensitivity to statins. The causes of CKD were chronic glomerulonephritis in 10 patients, polycystic kidney disease in 3 patients, interstitial nephritis and other or unknown aetiologies in 2 patients. Patients received antihypertensive medications (in 7 up to 15), as well as other commonly used drugs in CKD such as phosphate binders, calcium carbonate, diuretics, and vitamin B, C, and D supplementation. None of these subjects received statins or drugs known to influence the immune system for at least two months before the study.

2.2.2. Patients, Group II

This group consisted of 13 chronic dialytic patients (8M and 5F) with a mean age of 66 ± 9 years and a dialytic age of 45 ± 20 months. The exclusion criteria were the same as in Group I. CKD was caused by chronic glomerulonephritis in 7 patients, adult polycystic kidney disease in 3 patients and the remainder had renal failure of uncertain etiology. Ten pts had artero-venous fistula, 2 had a subcutaneous polytetrafluoroetylene graft (PTFE) and one had a semi-permanent transcutaneous access. All patients were receiving standard bicarbonate dialysis with a biocompatible membrane (Polysulphone or Polyamide). Patients were receiving phosphate binders, calcium carbonate, epoietin, iron and vitamin supplementation but none of them received statins or drugs known to influence the immune system for at least two months before the study.

2.2.3. Healthy subjects

Ten sex (6M and 4F) – and age (62 ± 8 years) – matched healthy adult volunteers (HS) with normal renal function (creatinine clearance 107 ± 11 ml/min) served as the controls.

2.3. Laboratory methods

2.3.1. Cell isolation

Monocytes enriched PBMC were isolated by Ficoll (Histopaque-1077, Sigma-Aldrich Co., Ltd., UK) gradient centrifugation from blood that was withdrawn into syringes containing EDTA 0.2 M as anticoagulant, as previously described [15]. Cells were washed in TTBSA inc. (sodium chloride 0.8 g/L, potassium chloride 0.195 g/L, Tris 1.1 g/L, glucose 1.0 g/L, BSA 2.5 g/L), counted and cultured in RPMI 1640 medium (Sigma-Aldrich Co., Ltd., UK) supplemented with 10% heat-inactivated fetal calf serum (FCS), glutamine and antibiotics in 24-well plates (Costar, Cambridge, MA) at 37 °C in an atmosphere containing 5% CO_2. RPMI contained no detectable amount of endotoxin as assessed by LAL test. Lymphomonocytes (non-adherent cells) were removed by aspiration with a Pasteur pipette an washing of the dishes with warm media [15]. Purity of monocytes fraction was confirmed by FACS (Becton-Dickinson, San Jose, CA) analysis using anti-CD14 monoclonal antibody. Cells were incubated with lipolysaccharide (LPS) from *Escherichia coli* and *Pseudomonas maltofilia* at a final concentration of 1 ng/ml and simultaneously with increasing doses of simvastatin or fluvastatin (from 10^{-6} M to 10^{-10} M) for 24 h. Monocytes were incubated in the following conditions: alone, with LPS alone, with LPS and S or F 1×10^{-6} M, with LPS and S or F 1×10^{-8} M, with LPS and S or F 1×10^{-10} M and with S or F alone at all the previous concentrations. Cell viability (>95%) was assessed by the gamma-eosin method.

2.3.2. Cytokine assays

After the incubation, cells were lysed by three freeze (40 °C) and thawing (37 °C) cycles. Surnatant-associated cytokine concentrations were separately determined by EIA (IL-6, Biosource, CA and IL-8, Bender MED-Systems, Vienna). The