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Effect of fluvastatin on serum prohepcidin levels in patients with end-stage renal disease

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Abstract

Objectives: Anemia, low-grade inflammation and/or alterations in lipid metabolism are common findings in individuals with end-stage renal disease (ESRD) despite advances in dialysis treatment. Hepcidin, a key regulator of iron metabolism, may play an important role in the interdependence of inflammation and anemia in ESRD patients. Statins may reduce cardiovascular events in dialysis patients and have pleiotropic effects in addition to lowering total and low-density lipoprotein (LDL)-cholesterol.

Design and methods: Because there is a paucity of data on the effect of statins on serum prohepcidin levels in dialysis patients, this 8-week study was conducted to test the effect of fluvastatin (80 mg/day, $n=22$) compared with placebo ($n=18$) on circulating serum prohepcidin, a prohormone of hepcidin, and high-sensitive C-reactive protein (hs-CRP) in dyslipidemic ESRD patients with renal anemia.

Results: Fluvastatin treatment decreased total cholesterol ($P<0.05$), LDL-cholesterol ($P<0.01$), hs-CRP ($P<0.05$) and serum prohepcidin levels ($P<0.05$) significantly.

Conclusion: Our pilot data suggest that short-term statin treatment may exert a beneficial effect on serum prohepcidin levels in ESRD patients. The potential clinical benefits of statins on renal anemia need to be confirmed and expanded with an appropriately powered long-term study.

Keywords: Anemia; Inflammation; End-stage renal disease; Prohepcidin; Statins

Introduction

Anemia, lipid alterations and/or systemic inflammation are common in end-stage renal disease (ESRD) patients on maintenance dialysis [1,2]. Such metabolic and clinical abnormalities may lead to an increased risk for cardiovascular disease. Notably, the presence of low-grade inflammation correlates with malnutrition and anemia in these patients [3].

The peptide hepcidin, which is produced by the liver, controls plasma iron levels by regulating the absorption of food iron from the intestine as well as the release of iron from macrophages [4,5]. Growing evidence has now accrued that low-grade inflammation as observed in ESRD causes an increase of production of hepcidin, which is in turn a potent mediator of anemia of chronic diseases [6]. It has been thus suggested that hepcidin may provide a link between inflammation and metabolism of iron, rendering more understandable the interdependence of inflammation and anemia in chronic kidney failure [7,8]. Although increased hepcidin levels may be a marker of cardiovascular risk in ESRD [8], thus far little is known about which factors may modulate its levels in this patient group.

Since statin therapy may exert significant antiinflammatory effects [9,10], in the present study we tested the effect of fluvastatin (80 mg/day) on high-sensitive C-reactive protein (hs-CRP) levels and circulating serum prohepcidin, a prohormone of hepcidin, in dyslipidemic subjects with ESRD and renal anemia, a population in whom the effect of statin monotherapy on prohepcidin has not been reported.

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Materials and methods

Study population

A total of 40 ESRD patients were included in this study. Patients receiving either hemodialysis ($n=21$) or peritoneal dialysis ($n=19$) were selected during 2007 from the patients attending the renal unit of the Uludag University Hospital, Bursa, Turkey. Subjects were eligible if they were aged 18 years or over. The criteria for inclusion into the study were duration of dialysis treatment of at least 6 months, and the presence of renal anemia and dyslipidemia. Exclusion criteria comprised pregnancy, malignancy, presence of acute inflammatory diseases, current drug use (statins, NSAIDs, immunosuppressors), liver or thyroid disease, and hemodynamic instability. A detailed medical history was taken in each subject. All patients were in good nutritional status according to the subjective global evaluation score. Diagnosis of diabetes mellitus was made according to the ADA criteria [11]. ESRD patients were considered hypertensive if the subject had either a systolic pressure >140 mmHg or a diastolic pressure >90 mmHg.

Patients were enrolled independently of the cause underlying ESRD. Overall, the causes were: diabetes mellitus ($n=1$), chronic glomerulonephritis ($n=6$), polycystic kidney ($n=3$), hypertension ($n=4$), chronic pyelonephritis ($n=5$), tubular necrosis ($n=1$), Alport syndrome ($n=5$), and unknown etiology ($n=15$). The mean duration of dialysis treatment was 86.08 ± 70.52 months (range: 7–252 months).

Hemodialysis was performed three times a week for 4–5 h on average, using F6 polysulphone capillary (Gambro Renal Products, Lakewood, CO, USA). A bicarbonate dialysis solution was used in hemodialysis patients. Peritoneal dialysis patients were either on automated peritoneal dialysis (APD) or standard continuous ambulatory peritoneal dialysis (CAPD).

This study was approved by the Institutional Review Board at Uludag University School of Medicine and all subjects gave informed consent.

Study design

Assuming a standard deviation of 30.0% in prohepcidin levels, a total of 40 subjects will provide a power of 75% to detect a mean changes of 20.0% or larger between the two treatment groups. After inclusion, patients were randomly assigned to receive either fluvastatin 40 mg twice daily (Lescol, Novartis Pharma AG, Basel, Switzerland) or matching placebo for a period of 8 weeks. We assessed patients at baseline and at the end of the 8-week period. The power calculation was performed by GraphPad StatMate 2 for Windows (GraphPad Inc., San Diego, CA, USA).

Table 1
General characteristics of ESRD patients randomized to either fluvastatin ($n=22$) or placebo ($n=18$).

	Fluvastatin ($n=22$)	Placebo ($n=18$)	*P*
Clinical and demographic characteristics			
Age (years)	48.7 (11.3)	43.6 (14.4)	0.21
Gender (male/female)	12/10	10/8	0.79
Dialysis type (hemodialysis/peritoneal dialysis)	11/11	8/10	0.97
Duration of dialysis (months)	103 (78)	83 (61)	0.27
Hypertension (yes/no)	9/13	8/10	0.92
Diabetes mellitus (yes/no)	0/22	2/16	0.38
EPO use (yes/no)	14/8	9/9	0.58
Routine blood count			
Red blood cells ($\times 10^9$/L)	3.7 (0.6)	3.9 (0.8)	0.30
White blood cells ($\times 10^9$/L)	7659 (1853)	8027 (2115)	0.56
Platelets ($\times 10^9$/L)	236277 (106952)	283545 (118872)	0.06
Hb (g/L)	113 (15)	116 (13)	0.37
Hematocrit (%)	32.4 (4.5)	33.3 (4.6)	0.18
MCV (fL)	88.1 (8.9)	88.7 (8.5)	0.72
MCH (pg)	29.4 (3.2)	29.6 (2.9)	0.83
MCHC (g/dL)	33.4 (1.2)	33.4 (1.4)	0.91
Biochemical characteristics			
hs-CRP (mg/L)	5.4 (3.8)	5.2 (3.7)	0.40
Prohepcidin (ng/mL)	332 (119)	318 (98)	0.32
Plasma glucose (mmol/L)	4.82 (0.61)	5.10 (0.88)	0.27
Serum creatinine (μmol/L)	901 (194)	1007 (274)	0.31
Ferritin (pmol/L)	1963 (1451)	1876 (1262)	0.77
Total iron binding capacity (μmol/L)	43 (10)	43 (13)	0.83
Serum albumin (g/L)	41 (3)	41 (4)	0.75
Total cholesterol (mmol/L)	6.16 (1.68)	5.67 (1.55)	0.44
LDL-cholesterol (mmol/L)	3.65 (1.32)	3.36 (1.45)	0.70
HDL-cholesterol (mmol/L)	1.11 (0.25)	1.19 (0.31)	0.52
Triglycerides (mmol/L)	2.26 (1.24)	2.42 (1.06)	0.64

Table 2
Effect of fluvastatin therapy on lipid variables, hs-CRP and prohepcidin levels in subjects with ESRD

Variable	Placebo ($n=18$)		Fluvastatin ($n=22$)	
	Baseline	Week 8	Baseline	Week 8
Total cholesterol (mmol/L)	5.67 (1.55)	5.82 (1.47)	6.16 (1.68)	5.10 (1.04)*
LDL-cholesterol (mmol/L)	3.36 (1.45)	3.54 (1.26)	3.65 (1.32)	3.00 (1.13)**
HDL-cholesterol (mmol/L)	1.19 (0.31)	1.11 (0.28)	1.11 (0.25)	1.16 (0.28)
Triglycerides (mmol/L)	2.42 (1.06)	2.33 (1.13)	2.26 (1.24)	2.46 (1.07)
hs-CRP (mg/L)	5.2 (3.7)	4.9 (4.0)	5.4 (3.8)	3.2 (2.2)*
Prohepcidin (ng/mL)	318 (98)	301 (99)	332 (119)	259 (88)*

Data are mean (S.D.) for all variables.
* $P<0.05$ compared to baseline and placebo.
** $P<0.01$ compared to baseline and placebo.

Laboratory methods

Samples of venous blood were collected in the morning and before that day's dialysis session. The serum was separated, and samples were kept frozen at −70 °C if not analyzed immediately. Routine blood counts and biochemical parameters, including serum total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and fasting triglyceride concentrations, were assessed at our central laboratory. RBC, hematocrit, hemoglobin, WBC and platelet counts were measured using an electronic counter. Serum ferritin concentrations were measured with the ADVIA Centaur Ferritin Assay Reagent and an ADVIA Centaur Analyzer (Bayer Diagnostics, Newburg, UK). The level of hs-CRP was measured on serum by nephelometry (Dade-Behring, Marburg, Germany). Serum prohepcidin concentration was measured by enzyme-linked immunoassay using a commercially available kit (DRG Diagnostics, Marburg, Germany). The detection limit was 4 ng/mL. The intra- and interassay coefficients of variation were <7%. Laboratory staff was unaware of the clinical characterization of the subjects. All measurements were performed in duplicate and in a random order.

Statistical analysis

All parameters were normally distributed according to the Kolmogorov–Smirnov test. Values were expressed as counts or means (S.D.). The baseline characteristics of patients randomized to fluvastatin or placebo were compared by the χ^2-test and the independent samples *t*-test. We used paired *t*-tests to assess mean changes between baseline and final assessment in all continuous outcome measures at week 8. Correlations were performed by the Spearman rank test. General linear regression analysis with adjustment for differences in baseline variates was performed to assess the effect of fluvastatin treatment. The statistical analysis was performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). A P value <0.05 (two-tailed) was considered to be statistically significant.

Results

Table 1 shows the baseline characteristics (clinical and biochemical) of the fluvastatin and placebo arms. There were no notable differences in baseline characteristics between the groups. Overall, patients randomly assigned to fluvastatin or placebo were well matched. In the entire study cohort, baseline levels of prohepcidin were significantly and positively correlated with hs-CRP ($r=0.338$, $P<0.05$) and ferritin ($r=0.423$, $P<0.01$).

Table 2 shows the changes in lipid concentrations, hs-CRP levels and serum prohepcidin concentrations with either fluvastatin or placebo. Fluvastatin produced a significant reduction in total cholesterol ($P<0.05$) and LDL-cholesterol ($P<0.01$) concentrations. No significant changes in either triglycerides or HDL-cholesterol concentrations attributable to fluvastatin were observed. During the observation period, serum hs-CRP decreased from 5.4 (3.8) to 3.2 (2.2) mg/L ($P<0.05$) in the fluvastatin arm. Similarly prohepcidin decreased from 332 (119) to 259 (88) ng/mL in patients treated with fluvastatin ($P<0.05$, Fig. 1). No significant change was observed in the placebo arm.

Linear regression analysis showed that fluvastatin treatment significantly and independently predicted the changes in total cholesterol, LDL-cholesterol, hs-CRP, and prohepcidin concentrations (all P values <0.05). There were no dropouts or adverse events during the 8-week study period.

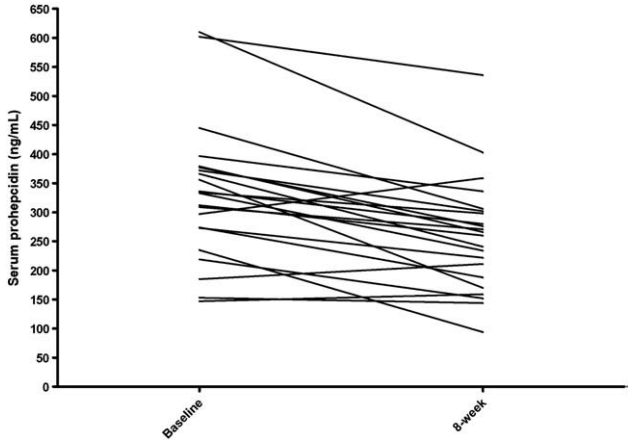

Fig. 1. Change in serum prohepcidin levels during the 8-week study period in patients ($n=22$) treated with fluvastatin 80 mg daily.

Discussion

Hepcidin has recently emerged as the key hormone in the regulation of iron balance and recycling [5,6]. In addition, a growing amount of evidence suggests that hepcidin may exert a proatherogenic activity [5,8]. It has been also suggested that the interplay between altered iron metabolism and inflammation could play a crucial role in atherogenesis among patients with ESRD [8]. The active hepcidin hormone is derived from an 84-amino-acid-long propeptide, termed preprohepcidin, after N-terminus cleavage of a 22-amino-acid signal peptide to give first rise to prohepcidin, and finally of a 37-amino-acid peptide [4]. Currently, only prohepcidin may be measured in serum using a commercially available immunoassay [7].

We planned this small, 8-week, placebo-controlled trial to explore fluvastatin's impact on plasma prohepcidin levels in ESRD patients with renal anemia. The main findings of our study were that basal prohepcidin levels in ESRD were positively correlated with hs-CRP and ferritin, and that short-term fluvastatin therapy in ESRD subjects reduces significantly plasma prohepcidin levels. To our knowledge, the latter finding has not previously been demonstrated.

The molecular mechanisms whereby fluvastatin affects prohepcidin are unclear. Previous clinical studies have shown that there are three main regulatory factors of hepcidin production, namely iron status, inflammation and anemia/hypoxia [12-14]. In keeping with our findings, previous data obtained in kidney transplant patients showed a positive association between serum prohepcidin and inflammatory parameters [15]. In this regard, low-grade inflammation, frequently found in ESRD patients, might also contribute to elevated prohepcidin concentration. Statins could reduce prohepcidin by at least two mechanisms. One possibility is by a decrease in the production and/or circulation of mediators of prohepcidin production, e.g., hs-CRP. Secondly, a direct effect of fluvastatin to reduce prohepcidin production by the liver is possible, but without precedent at present.

Two main limitations of this study merit consideration. Firstly, we included statin-näive ESRD patients in the present study. Although the inclusion criteria do not adhere to today's standards of clinical care, we think that the interpretations of the results are not hampered in a major way. Our inability to show changes in HDL-cholesterol may be due to the relatively low statin dose, the study duration, or the number of patients included. Second, as our data are limited to a study of Turkish patients with ESRD, they might not be applicable to other populations.

In summary, 8 weeks of fluvastatin treatment reduced circulating prohepcidin levels in ESRD patients with renal anemia. The reduction of prohepcidin seems to suggest a pleiotropic effect of fluvastatin per se, potentially decreasing the risk for cardiovascular events in the short term. On the basis of these findings, it is hypothesized that early lipid lowering treatment should be initiated for all ESRD patients with renal anemia. The potential clinical benefits of statins on renal anemia need to be confirmed and expanded with an appropriately powered long-term study.

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