

Atorvastatin reduces proteinuria in non-diabetic chronic kidney disease patients partly via lowering serum levels of advanced glycation end products (AGEs)

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There is accumulating evidence that advanced glycation end products (AGEs) play a role in the development and progression of chronic kidney disease (CKD). We have previously found that atorvastatin treatment significantly reduces serum levels of AGEs in type 2 diabetic patients and subjects with non-alcoholic steatohepatitis in a cholesterol lowering-independent manner. In this study, we examined whether atorvastatin could reduce proteinuria partly via reduction of serum levels of AGEs in non-diabetic CKD patients. Ten non-diabetic normotensive stage I or II CKD patients with dyslipidemia were enrolled. Patients were treated with atorvastatin (10 mg/day) for one year. All subjects underwent determination of blood chemistries, proteinuria and serum levels of AGEs at baseline and after one year. Atorvastatin treatment for one year significantly decreased circulating levels of total cholesterol, LDL cholesterol, triglycerides and AGEs, while it increased HDL cholesterol levels. Further, although atorvastatin treatment did not affect estimated glomerular filtration rate, it significantly reduced proteinuria. In univariate analyses, proteinuria levels were correlated with total cholesterol, LDL cholesterol, triglycerides, HDL cholesterol (inversely) and AGEs. Multiple stepwise regression analysis revealed that AGE level was a sole independent correlate of proteinuria. In this initial examination of the patients in this study, our present study suggests that atorvastatin could decrease proteinuria in non-diabetic CKD patients with dyslipidemia partly via reduction of serum levels of AGEs. Atorvastatin may have AGE-lowering effects in CKD patients as well that could contribute to renoprotective properties of this agent.

Introduction

Dyslipidemia not only contributes to cardiovascular disease (CVD), but also plays a role in the progression of chronic kidney disease (CKD).^{1,2} Indeed, experimental studies have demonstrated that lipid deposition elicits pro-inflammatory and pro-fibrotic reactions in the kidney, thus being involved in CKD.^{1,2} Further, several clinical studies have shown that dyslipidemia is associated with an increased risk for progressive decline of renal function and that statins ameliorate renal function and/or reduce proteinuria in patients with CKD.³⁻⁸ Since statins have pleiotropic effects *in vivo*,⁹ it is generally thought that statins could protect against the development and progression of CKD via both cholesterol lowering-dependent and -independent manners. However, which cholesterol lowering-independent variables could be involved in renoprotective properties of statins in CKD patients is not fully understood.

Glucose can react non-enzymatically with the amino groups of proteins to form reversible Schiff bases and then Amadori products.^{10,11} These early glycation products undergo further complex reactions and rearrangements to become irreversibly cross-linked fluorescent protein derivatives termed advanced glycation end products (AGEs).^{10,11} The formation and accumulation of AGEs have been known to progress in patients with CKD and/or diabetes.¹²⁻¹⁵ There is a growing body of evidence that AGEs evoke inflammatory and fibrogenic reactions, thereby contributing to the development and progression of CKD.¹⁶⁻¹⁸ Since we have previously shown that atorvastatin decreases serum levels of AGEs in type 2 diabetic patients in a cholesterol lowering-independent manner,¹³ it is conceivable that atorvastatin may exert renoprotective properties partly via AGE-lowering effects. Therefore, in this study, we examined whether atorvastatin could reduce proteinuria and serum levels of AGEs in non-diabetic stage I or II CKD patients.

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Table 1. Clinical variables before and after atorvastatin treatment

Characteristics	Before treatment	After treatment	p-value
Age (years)	36.0 ± 5.8	37.0 ± 5.8	
Number (male number)	10 (7)	10 (7)	
BMI (kg/m ²)	23.4 ± 2.0	n.d.	
FPG (mg/dl)	88.6 ± 0.2	n.d.	
HbA1c (%)	5.0 ± 0.8	n.d.	
SBP (mmHg)	126.2 ± 7.5	124.8 ± 5.3	0.132
DBP (mmHg)	76.6 ± 5.0	75.2 ± 3.3	0.153
T-chol (mg/dl)	224.0 ± 39.7	177.6 ± 15.2	0.012
TG (mg/dl)	166.6 ± 12.8	146.8 ± 7.8	<0.001
LDL-C (mg/dl)	172.0 ± 13.4	105.8 ± 15.4	<0.001
HDL-C (mg/dl)	39.0 ± 3.7	42.4 ± 2.6	<0.001
Creatinine (mg/dl)	0.77 ± 0.06	0.77 ± 0.06	0.343
eGFR (ml/min)	85.0 ± 9.1	84.7 ± 8.7	0.376
Proteinuria (g/day)	1.13 ± 0.20	0.79 ± 0.14	<0.001
AGEs (U/ml)	13.2 ± 1.4	10.5 ± 1.2	<0.001

Data are shown as mean ± SD. n.d., not determined.

Table 2. Univariate analyses for determinants of proteinuria

Characteristics	β	SE	p-value
Sex	0.065	0.121	0.786
SBP	0.109	0.009	0.647
DBP	-0.056	0.014	0.814
T-chol	0.639	0.001	0.002
TG	0.472	0.003	0.036
LDL-C	0.722	0.001	<0.001
HDL-C	-0.576	0.013	0.008
Creatinine	-0.025	1.007	0.915
eGFR	-0.269	0.006	0.251
AGEs	0.765	0.019	<0.001

Male, 0; Female, 1; β, regression coefficients; SE, standard error.

We further studied here whether AGE-lowering effects of atorvastatin are independently correlated to its proteinuria-reducing properties.

Results

Background of the patients is shown in Table 1. All patients were normotensive and dyslipidemic. Atorvastatin treatment (10 mg/day) for one year significantly decreased circulating levels of total cholesterol (T-chol), low-density lipoprotein-cholesterol (LDL-C), triglycerides (TG) and AGEs, while it increased high-density lipoprotein-cholesterol (HDL-C) levels. Further, atorvastatin treatment significantly reduced urinary protein excretion levels, although it did not affect systolic blood pressure (SBP), diastolic blood pressure (DPB), serum levels of creatinine or estimated glomerular filtration rate (eGFR). As shown in Table 2, in univariate analyses, proteinuria levels were correlated with T-chol,

LDL-C, TG, HDL-C (inversely) and AGEs. Because these parameters could be closely correlated with each other, to determine independent determinants of proteinuria levels, multiple stepwise regression analysis was performed. This analysis showed that only AGE value ($p < 0.001$) was independently correlated with urinary protein excretion levels ($R^2 = 0.585$).

Discussion

In this study, we demonstrated for the first time that atorvastatin treatment for one year significantly decreased proteinuria and serum levels of AGEs in non-diabetic early stage CKD patients with dyslipidemia. Since in multiple stepwise regression analysis, AGE level was a sole independent correlate of proteinuria in our subjects, the present study suggests that atorvastatin could exert renoprotective properties in stage I or II CKD patients partly via reducing serum levels of AGEs.

We have previously found that 10 mg atorvastatin treatment significantly reduces circulating levels of AGEs in both type 2 diabetic patients and non-alcoholic steatohepatitis subjects in cholesterol- and glucose-lowering-independent manners.^{13,19} So, the present findings have extended the previous observations; atorvastatin treatment could decrease serum levels of AGEs in non-diabetic CKD patients as well. In this study, we limited our analysis to non-diabetic CKD patients without anti-hypertensive drugs such as angiotensin II type 1 receptor blockers because (1) it is well known that serum levels of AGEs are elevated in diabetic patients and could be affected by cumulative hyperglycemic burden⁹⁻¹² and (2) treatments with inhibitors of renin-angiotensin system may reduce circulating levels of AGEs in humans.²⁰ Therefore, it is unlikely that these factors could confound our present results.

There is accumulating evidence that AGEs play a role in the development and progression of CKD in both animal models and humans.^{12-18,21-23} Indeed, an inhibitor of AGE formation, aminoguanidine treatment was reported to reduce urinary albumin excretion levels and prevent the development of mesangial expansion in streptozocin-induced diabetic rats.²¹ Furthermore, aminoguanidine treatment was found to prevent albuminuria in diabetic hypertensive rats without affecting blood pressure (BP) levels.²² In addition, a double-blinded, placebo-controlled, randomized clinical trial of aminoguanidine (Pimagedine®) revealed that Pimagedine® therapy reduced the 24 hour total proteinuria levels and prevented the decrease in eGFR in patients with type 1 diabetes.²³ These observations further support the concept that atorvastatin could exert beneficial effects in our CKD patients partly via reducing serum levels of AGEs.

Proteinuria is not merely a biomarker for the progression of CKD, but also a mediator of this devastating disorder.^{24,25} Further, proteinuria is a strong and independent indicator of CVD in CKD patients as well.²⁶ These observations suggest that AGEs and proteinuria were correlated with each other, thus being involved in CVD and CKD. Reduction of serum levels of AGEs and proteinuria with atorvastatin may be a novel therapeutic strategy for the prevention of CVD and CKD.

In this study, we did not know how atorvastatin decreased serum levels of AGEs in our subjects. However, oxidative stress participates in the formation of AGEs,⁹ and hydroxyl metabolites of atorvastatin have been shown to have anti-oxidative properties.²⁷ Atorvastatin may reduce serum AGE levels via its anti-oxidative property, which could partly explain the cardioprotective actions of this agent.^{28,29}

Limitations

Because the number of subjects in the present study is small, it could not have enough statistical power to totally exclude the possibility that atorvastatin decreased proteinuria and serum levels of AGEs partly via lipid-lowering properties. Further large clinical study is needed to elucidate whether reduction of circulating levels of AGEs by atorvastatin could be mechanistically related to renal protection in non-diabetic CKD patients. In addition, although all patients received salt restriction (<5 g/day NaCl) during the treatment period, differences in daily sodium intake during the treatment period may interfere with the present results because high salt intake is associated with oxidative stress generation in experimental animals.³⁰

Research Design and Methods

Subjects. Ten non-diabetic stage I or II CKD patients with dyslipidemia (T-chol) > 220 mg/dL, LDL-C > 140 mg/dL, 150 < TG < 400 mg/dL or HDL-C < 40 mg/dL (7 males and 3 females, IgA nephropathy n = 7, non-IgA type proliferative glomerulonephritis n = 3, mean age; 36.0 ± 5.8 years) were enrolled in the present study. All patients were normotensive (BP was less than 130/80 mmHg) and none of them received anti-hypertensive drugs such as angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers. We excluded any patients with chronic pulmonary diseases, liver diseases, neoplastic disorders, and those who had recent (<6 months) acute coronary syndromes, stroke and any acute infections. Patients whose age

was younger than 20 years old, whose serum creatinine level more than 1.5 mg/dL, whose proteinuria more than 3.0 g/day were also excluded. To control dyslipidemia, all patients received atorvastatin (10 mg once daily), but not other anti-dyslipidemic drugs, for 12 months. Further, all patients received salt restriction (<5 g/day NaCl). The study protocol was approved by the local ethical committee of Shinmatsudo Central General Hospital, and informed consent was obtained from all study participants. The study complied with the principles of the Helsinki Declaration.

Data collection. BP was measured in the sitting position twice after 2 minutes of rest using an upright standard sphygmomanometer. Mean value of blood pressures was used for analysis. Renal function was evaluated by serum creatinine levels and eGFR according to the Modification of Diet in Renal Disease (MDRD) equation modified for the Japanese population.¹⁶ Serum levels of T-chol, TG and HDL-C were measured enzymatically at Shinmatsudo Central General Hospital. LDL-C level was calculated using Friedewald's formula. Serum levels of AGEs were measured with an enzyme-linked immunosorbent assay as described previously.^{13,19} In this study, 1 U of AGEs corresponds to 1 µg of glyceraldehyde-derived AGE-bovine serum albumin as described previously.^{13,19}

Statistical methods. Data were expressed as mean ± standard deviation (SD). To compare the parameters, we used the Wilcoxon signed-rank test. A correlation between proteinuria and other clinical variables was determined by a linear regression analysis. To determine independent determinants of proteinuria, multiple stepwise regression analysis was performed. Statistical significance was defined as p < 0.05. All statistical analyses were performed with the use of the SPSS system (SPSS Japan Inc., IBM company, Tokyo, Japan).

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