Anti-inflammatory effects of simvastatin on adipokines in type 2 diabetic patients with carotid atherosclerosis

Yun Hu1, Guoyu Tong1, Wei Xu1, Jiajia Pan1, Kathy Ryan2, Rongze Yang2, Alan R. Shuldiner2, Da-Wei Gong2 and Dalong Zhu1

Abstract
Objective: Statins are extensively used for lowering LDL-cholesterol and reducing cardiovascular events. Recent studies have shown that statins have beneficial anti-inflammatory effects. We aimed to determine whether and how adipokines are regulated during statin treatment in type 2 diabetic patients.

Method: In this study, we investigated the changes of CRP and inflammation-related adipokines (SAA, IL-6, TNFα and adiponectin) in 23 type 2 diabetic patients with atherosclerosis who received statin therapy, and 20 diabetic patients with atherosclerosis and 14 diabetic patients without atherosclerosis who did not receive statin therapy for a period of three months.

Results: By the end of the simvastatin treatment (40 mg, daily), LDL-cholesterol was decreased by 16.7% and HDL-cholesterol was increased by 31.9%. SAA, CRP, TNFα and IL-6 levels were decreased by 31.8%, 66.2%, 53.9% and 14%, respectively, and adiponectin was increased by 59.6%, compared with the baseline levels. Interestingly, the decrease of SAA was positively correlated with that of LDL-cholesterol but negatively with HDL-cholesterol during statin treatment. Among the adipokines, the decrease of SAA was positively correlated with TNFα (r = 0.50, p = 0.016).

Conclusion: The results suggest that adipokines may be differentially regulated and independent of cholesterol changes and that adipokines may be a mediator, and the adipose tissue may be a target of statins’ anti-inflammatory effect.

Keywords: statin, anti-inflammation, adipokines, atherosclerosis, type 2 diabetes

Introduction
Recent studies indicate a close association between chronic inflammation and CVD in that inflammatory status is elevated in patients with CVD risk factors including obesity, insulin resistance, type 2 diabetes and dyslipidaemia, as evidenced by the increase of inflammation marker CRP1,2 and SAA.3 Furthermore, pharmacological interventions (e.g. statin, thiazolidinediones) that improve CVD risk factors, also decrease serum inflammatory markers.5-7 Thus, a decrease of systemic inflammatory status may be partly responsible for the reduction of CVD events by the treatment. However, questions remain about the source of the inflammation and the target tissue of the anti-inflammatory action of these drugs.

Statins are a class of chemicals that inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, a rate-limiting enzyme in cholesterol synthesis, and are extensively used for reduction of cardiovascular events through a well-known mechanism of lowering LDL-cholesterol. Recent studies have shown that statins have beneficial pleiotropic effects, decreasing inflammation.8-10 Many studies show elevated levels of systemic inflammatory markers such as CRP, matrix metalloproteinase-9, TNFα and IL-6 in patients with dyslipidaemia or atherosclerosis.10,12-15 Fewer studies have been carried out to examine the effect of statins on adipokines, a group of inflammatory and anti-inflammatory cytokines secreted by adipose tissue. Obesity, a risk factor for CVD, is associated with ‘inflamed’ adipose tissue, obesity, a risk factor for CVD, is associated with ‘inflamed’ adipose tissue, with decreased expression of the anti-inflammatory adipokine, adiponectin, and increased secretion of a variety of inflammatory cytokines, e.g. TNFα, IL-6 and SAA.17-22

1 Division of Endocrinology, The Affiliated Drum Tower Hospital of Nanjing University, Nanjing 210008, China
2 Division of Endocrinology, Diabetes and Nutrition Department of Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Corresponding author:
Dalong Zhu, Division of Endocrinology, The Affiliated Drum Tower Hospital of Nanjing University, Nanjing 200021, China.
Email: zhudldr@gmail.com
Data on the effect of statins on adipokines in type 2 diabetic patients are sparse. Thus, we investigated the effect of simvastatin on changes in the levels of adipokines in type 2 diabetes patients with atherosclerosis during three months of statin treatment.

**Methods**

**Study subjects**

All human studies were approved by the Ethical Committee of the Nanjing University Drum Tower Hospital. Human participants were enrolled in the Nanjing University Drum Tower Hospital between 2006 and 2007. All subjects had no history of heart, liver, kidney and lung diseases, and had no overt acute or chronic infection, trauma or surgery during the follow-up period. Type 2 diabetes was diagnosed based on diagnostic criteria of the American Diabetes Association. Diabetic patients were consecutively recruited and divided into groups of AS and non-AS and based on carotid atherosclerotic plaques and IMT (see below). The AS group was randomised to either statin or non statin therapy.

Anthropometric measurements including weight, height and waist measurements were obtained using standardised techniques. BMI was calculated using the following formula: weight (kg)/height (m²). Venous blood was drawn at pretreatment baseline and every month after a 12- to 14-hour overnight fast. HbA1c was measured from whole blood. Plasma was promptly separated by low-speed centrifugation at 4°C and immediately used for measurement of lipid, lipoprotein and glucose. Aliquots of the plasma specimens were also immediately frozen at -80°C for cytokine assays.

**Carotid IMT measurement**

The carotid ultrasound examination was carried out using a colour Doppler ultrasound system (HDl-5000, Philips, Eindhoven, Netherlands) with a scanning frequency of 5–12 MHz in B-mode, following the same protocol throughout, by a single trained operator blind to the study group. The flow divider between the internal and external carotid arteries was identified, and the common carotid arteries were explored starting 1 cm below the flow divider. Measurements were obtained by tracing the leading edge of the lumen-intima and the media-adventitia interfaces. Maximum right and left IMTs were averaged to obtain the carotid IMT measurement. Subjects with significant carotid plaques were defined as carotid IMT > 1.2 mm. IMT ≥ 0.9 mm with or without carotid plaques were defined as having AS.

**Statin intervention**

A total of 57 type 2 diabetic patients completed the clinical trial and were divided into two groups of non-AS (n = 14) and AS (n = 43) by their IMT thickness and plaque presence. The AS group was further randomly divided into the statin group of 23 subjects who received 40 mg simvastatin (Merck), daily for 12 weeks and the control group of 20 subjects without simvastatin treatment. All patients with diabetes received routine medication (e.g. insulin, metformin, sulfonylurea) for glucose control. During the three-month study period, dose adjustments for diabetes medication were allowed, but there were no changes in medication.

**Measurement of adipokines and inflammation markers**

Serum levels of human high-sensitive CRP were determined by a highly sensitive nephelometric assay (Kyowa, Tokyo, Japan). Serum levels of adiponectin, IL-6 and TNFα (eBioscience, San Diego, CA, USA) and human SAA (BiSource, Camarillo, CA, USA) were measured in duplicate with enzyme-linked immunosorbent assay kits according to instructions of the respective manufacturers. The intra-assay coefficient of variations of the CRP, adiponectin, IL-6, TNFα, and SAA measurements were 5.5%, 10%, 2%, 10% and 7.4%, respectively.

**Other laboratory analyses**

Lipids in plasma and fractionated lipoproteins were analysed by standard enzymatic methods. HbA1c levels were measured by high-performance liquid chromatography.

**Statistical analyses**

Results are expressed as the mean ± SEM. Non-normally distributed variables were ln-transformed prior to analysis, and were compared using the Student’s unpaired or paired t-test, or analysis of covariance, as appropriate. Differences were considered to be significant at p < 0.05. Spearman rank order correlation analyses were used to determine the relationships between the inflammatory factors and lipoprotein parameters. Statistical analysis was carried out using SPSS statistical software.

**Results**

**Basal clinical profile**

Study participants were recruited and divided into non-AS and AS groups; the AS group was further randomised to statin treatment and no statin treatment. Table 1 shows baseline clinical characteristics of the study participants. IMT was the main criterion for the diagnosis of AS and by definition, was higher in the AS group than the non-AS group. Prior to intervention, the pooled AS group (n = 43) had no statistically significant differences in age, sex or BMI from the non-AS control group (n = 14), except for larger waist conference (p = 0.03). As for lipids and adipokines, serum triglyceride level was higher and HDL was lower in the AS
group compared with the non-AS group (Table 1). Moreover, serum levels of inflammatory adipokines CRP, SAA and IL-6 were higher and the anti-inflammatory adipokine adiponectin level was lower in the AS compared with the non-AS group (Table 1). The differences in these parameters between AS and non-AS groups remained statistically significant after adjustment for age and sex, however these differences, except for adiponectin, were attenuated after adjustment for BMI (data not shown). After adjustment for age, sex and BMI, SAA and IL-6 levels were higher whereas adiponectin and HDL levels were lower in the AS group than in the non-AS group. There was no significant difference in IMT and adipokine levels between the AS group randomised to statin treatment (AS+statin) compared with the AS group that did not receive statin treatment (AS no statin), although the AS+statin group was slightly older and had higher TG and lower HDL levels (Table 1).

**Correlations among baseline variables**

When data before simvastatin treatment from all three groups (n = 57) were pooled, the result (shown in Table 2) provides insight into the relationships among anthropometric and biochemical parameters. As shown in Table 2, the ln of SAA, lnCRP and IL-6 were correlated well with BMI (r = 0.42, p < 0.01; r = 0.34, p < 0.01 and r = 0.46, p < 0.001, respectively) and with lnTG (r = 0.55, p < 0.001, r = 0.28, p < 0.05 and r = 0.37, p < 0.01, respectively). Significantly, lnSAA and IL-6 levels are negatively HDL levels (r = -0.58, p < 0.01 and r = -0.35, p < 0.01, respectively). Interestingly, lnCRP was correlated with BMI, lnTG and TNFα, but not with SAA; however, the correlation became significant after adjustment for BMI (r = -0.27, p < 0.05). No significant correlations were observed for the level of adiponectin with those of other adipokines and lipids.

**Changes of plasma lipids during simvastatin treatment**

During the treatment period, there were no significant changes in glucose levels and BMI in any of the groups (data not shown). We measured changes in cholesterol and adipokines monthly during the three-month study period. In the simvastatin group, LDL was decreased by 15.8% from 2.65 mmol/L to 2.22 mmol/L within the first month whereas HDL was increased by 28.4% from 0.92 mmol/L to 1.18 mmol/L in the same period. The LDL and HDL levels remained at similar levels thereafter. Interestingly, TG levels were increased by 10% in the first month but decreased significantly by 30% by the second month and continued to decrease by an additional 18% by the third month (Figure 1a). In comparison, no significant changes were observed in plasma lipids in the no-statin group during the same period (Figure 2a).

With respect to adipokines, SAA was significantly decreased by 20.0%, 10.1% and 5%, and CRP level was decreased by 45.4%, 25.1% and 17.2% each month in the statin treatment group. Likewise, TNFα and IL-6 levels were decreased in the same group. Conversely, adiponectin gradually increased during the treatment (Figure 1b). Overall, by the end of the three-month simvastatin treatment, SAA, CRP, TNFα and IL-6 levels were decreased by 31.8%, 66.2%, 53.9% and 14%, respectively, and adiponectin was
increased by 59.6%, compared with the baseline levels. In contrast, there were no significant changes in adipokine levels during the same period in the non-statin AS patients (Figure 2b) or in the non-AS controls (data not shown).

Correlations of changes among lipids and adipokines during simvastatin treatment

We next analysed the correlations of percentage changes among plasma lipids and adipokines during simvastatin treatment in the AS patients. After the first month of simvastatin treatment, positive correlations were observed between the increase of adiponectin and HDL ($r = 0.45$, $p = 0.031$). By the end of the second month of treatment, the decrease of SAA was correlated with that of LDL ($r = 0.53$, $p = 0.009$) and with the increase of HDL ($r = -0.54$, $p = 0.007$). At the end of the third month of statin treatment, the correlation of the changes of SAA was no longer correlated with LDL but remained negatively correlated with HDL. Among adipokines, the decrease of SAA was positively correlated with TNFα ($r = 0.50$, $p = 0.016$) but not with the other adipokines.

Discussion

Diabetic patients have at least a two-fold greater risk of developing CVDs than the general population. Dyslipidaemia and systemic inflammation in diabetes may contribute to the increased CDV risk, as atherosclerosis is considered an inflammatory vascular disease, characterised by...
by accumulation of lipids and infiltration of inflammatory cells. Recently, a large population study has shown that statin treatment can not only reduce CVD events but also CRP levels in individuals whose serum CRP levels are high but LDL-cholesterol levels are within the normal range, suggesting that lowering inflammation status may contribute to reducing the CVD events. However, questions remain regarding whether the reduction of CRP or other cytokines are the cause of the decreased CVD events. In the present study, we treated with simvastatin diabetic patients whose LDL-cholesterol level did not require pharmacological therapy based on current clinical guidelines to test the hypothesis that statin treatment might lower inflammatory status as well as LDL-cholesterol levels in diabetic patients at high CVD risk. As adipokines play a significant role in local and systemic inflammation and in the pathogenesis of insulin resistance and CVD, the main objective of this study was to examine how adipokines respond to simvastatin treatment in type 2 diabetic patients with carotid atherosclerosis. Consistent with published findings, inflammatory markers and adipokines were higher in diabetic patients with AS than those without, suggesting increased inflammation in AS patients. However, the significance was attenuated after adjustment for BMI (Table 1), indicating a major contribution of adiposity to inflammation. We found that serum levels of SAA, predominantly produced by adipose tissue and IL-6 were correlated with each other prior to the statin treatment, and were positively associated with BMI and negatively with HDL levels. Although the mechanistic link between IL-6 and cholesterol is largely unknown, the role of SAA in cholesterol metabolism has been studied. SAA is a component of HDL and is reported to facilitate HDL catabolism, which may explain the negative correlation between SAA and HDL before and during the simvastatin treatment when endogenous cholesterol synthesis is pharmacologically suppressed. SAA is a potent stimulus for the expression and release of IL-6, TNFα in neutrophils, endothelial cells and adipose stromal vascular cells. On the other hand, SAA can be induced by TNFα and IL-6 in hepatoma cells. Thus reciprocal regulation exists between SAA with IL-6 and TNFα.

Many studies have shown a reduction of inflammation markers or cytokines by statin treatment. However, those studies were not focused on adipokines. In this study, we monitored the changes of adipokines during simvastatin treatment. As expected, simvastatin lowered LDL-cholesterol by 16.7% while raising HDL-cholesterol by 31.9%. Pro-inflammatory mediators, SAA, CRP and TNFα, were decreased significantly within the first month of the treatment whereas IL-6 started to drop during the second month. By the end of the three-month simvastatin treatment, SAA, CRP, TNFα and IL-6 levels were decreased by 31.8%, 66.2%, 53.9% and 15%, respectively. Conversely, anti-inflammatory adiponectin gradually increased up to 59.6% by the end of the three-month treatment. The extent of pro-inflammatory cytokines reduction by statin was remarkable, which is probably attributed to the elevated inflammation status in the patients with both AS and diabetes and therefore, the anti-inflammatory effect of statin might be augmented. Notably, reduction of pro-inflammatory adipokines appeared independent of the improvement in the lipid profile suggesting that adipokines may be effectors of statins. Moreover, there appeared to be no significant correlation (except between SAA and TNFα) among the changes of adipokines during simvastatin treatment, suggesting that these cytokines may be differentially regulated with independent biological function and diagnostic value.
Abbreviations and acronyms

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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AS</td>
<td>carotid atherosclerosis</td>
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<tr>
<td>BMI</td>
<td>body mass index</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<td>HDL</td>
<td>high density lipoprotein</td>
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<td>IL</td>
<td>interleukin</td>
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<td>IMT</td>
<td>intima media thickness</td>
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<td>LDL</td>
<td>low density lipoprotein</td>
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<td>ln</td>
<td>natural logarithm</td>
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<td>SAA</td>
<td>serum amyloid A</td>
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<td>SEM</td>
<td>standard error of the mean</td>
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<td>TG</td>
<td>triglyceride</td>
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<td>TNFα</td>
<td>tumor necrosis factor alpha</td>
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Indeed, even CRP and SAA belong to the family of acute-phase proteins, and were independent predictors for CVD risk in a large population study.38 Whether the reduction of SAA or other inflammatory adipokines may contribute to a decrease in CVD events in diabetic or non-diabetic patients warrants further investigation.

In summary, we investigated the effect of statin therapy on systemic adipokine levels in diabetic patients. Three months of treatment with simvastatin significantly decreased serum concentrations of pro-inflammatory adipokines including SAA, TNFα and IL-6 as well as CRP, and increased adiponectin levels in type 2 diabetic patients with AS. These results support a beneficial anti-inflammatory effect of statins and suggest that adipokines may be effectors, and/or adipose tissue may be a target of statins’ anti-inflammatory effect, and that improved adipokine profile (decrease in pro-inflammatory adipokines and increase in anti-inflammatory adipokine) may partly contribute to the decrease of CVD events by statin treatment.

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References


