The Effects of Different Doses of Atorvastatin on Plasma Endothelin-1 Levels in Type 2 Diabetic Patients with Dyslipidemia

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We investigated the effects of three different daily doses (10 mg, 20 mg, and 40 mg) of atorvastatin, a relatively new and potent statin, on plasma endothelin (ET)-1 and highly sensitive C-reactive protein (CRP) levels in type 2 diabetic subjects. Twenty-nine type 2 diabetic patients with dyslipidemia were enrolled and randomly assigned to receive atorvastatin orally at 10 mg (A10; $n = 10$), 20 mg (A20; $n = 10$), or 40 mg (A40; $n = 9$) daily for 12 weeks. Levels of plasma total cholesterol and low-density lipoprotein (LDL)-cholesterol (C) in all three studied groups were significantly decreased after treatment with atorvastatin for 12 weeks (all groups, $P < 0.001$). However, the greatest LDL-C lowering effect and the highest percentage of subjects achieving the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) LDL-C goal were observed in the A20 group. All diabetic subjects had a higher plasma ET-1 concentration (A10, 1.02 ± 0.37 pg/ml, mean ± SD; A20, 1.17 ± 0.55 pg/ml; and A40, 0.87 ± 0.45 pg/ml) than that of age- and sex-matched normal control subjects (0.64 ± 0.15 pg/ml; all groups, $P < 0.001$). Plasma ET-1 levels showed a borderline significant decrease at the end of study, by 22% in diabetic subjects treated with 10 mg atorvastatin ($P = 0.05$ compared with baseline), and by 30% in subjects treated with 20 mg atorvastatin ($P = 0.06$, compared with baseline). Paradoxically, the 40-mg dose of atorvastatin provided an increase of 2% in plasma ET-1 levels at the end of study, which is significantly different ($P < 0.05$) and marginally significant ($P = 0.057$) from the levels of the 10- and 20-mg doses, respectively. Similarly, although insignificantly, plasma concentrations of CRP also tended to decrease by 12% and 48%, and paradoxically increased by 18% in diabetic patients treated with 10 mg, 20 mg, and 40 mg atorvastatin, respectively. The clinical significance of these biphasic lipid-independent statin effects is unknown and the present study suggests that 20 mg atorvastatin may have the best benefits in treating diabetic patients with dyslipidemia. Exp Biol Med 231:1010–1015, 2006

Key words: atorvastatin; CRP; dyslipidemia; endothelin-1; type 2 diabetes

Introduction

Type 2 diabetes is associated with a 2-fold to 4-fold increased risk of both cardiovascular morbidity and mortality (1). Besides hypertension, dyslipidemia is an important prevalent and modifiable cardiovascular risk factor in subjects with type 2 diabetes. Type 2 diabetic dyslipidemic subjects typically have increased serum triglycerides and decreased high-density lipoprotein (HDL)-cholesterol (C) levels. Although absolute concentrations of low-density lipoprotein (LDL)-C in type 2 diabetic subjects are usually not significantly different from individuals without diabetes, type 2 diabetic subjects usually have a greater proportion of small, dense, atherogenic LDL-C particles (2). It is well known that accelerated angiopathy is a major complication in diabetes mellitus. Endothelin (ET)-1, in addition to being the most potent vasoconstrictor, acts as a mitogen on the vascular smooth muscle cells. Increased levels of circulating ET-1 in diabetic subjects were first reported by Takahashi *et al.* (3). Because generalized endothelial cell damage is thought to occur in diabetic subjects, ET-1, being released from the damaged endothelial cells, is able to make contact with the underlying vascular smooth muscle cells and, thus,

This work was supported in part by research grants from the Kaohsiung Veterans General Hospital, Taiwan (VGHKS92–45), (VGHKS92CT-304) and Pfizer, Inc.

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Received September 2, 2005.
Accepted December 5, 2005.

1535-3702/06/2316-1010$15.00

could be one important cause of diabetic angiopathy (4, 5). LDL cholesterol–lowering strategies, with the use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins), have demonstrated benefits for patients with diabetes in both primary and secondary prevention trials (6–8). Although the majority of the effect could be ascribed to a beneficial effect on the lipid profile, the statins might have additional effects (pleiotropic effects) that could confer benefit. It has been shown that statins could reduce the expression of pre-pro-ET-1 messenger RNA (mRNA) and the synthesis of ET-1 *in vitro* (9). Only a few studies have reported that statins could reduce plasma ET-1 concentration in type 2 diabetic subjects (10, 11). Atorvastatin is a relatively new and potent statin that has been shown to be highly effective in reducing LDL-C levels and cardiovascular events in subjects with type 2 diabetes (7, 8). However, the effects of different doses of atorvastatin on plasma ET-1 levels in type 2 diabetic Taiwanese subjects with dyslipidemia are unknown. The aim of the present study was to examine the effects of three different daily doses (10 mg, 20 mg, and 40 mg) of atorvastatin on plasma ET-1 and highly sensitive C-reactive protein (CRP) levels in type 2 diabetic dyslipidemic subjects.

Materials and Methods

Patients. The study was designed as a randomized, 12-week, dose-ranging, open-labeled, parallel group clinical trial comparing the effects of three different starting doses of atorvastatin in type 2 diabetic dyslipidemic subjects. Study participants were men and women (postmenopausal, surgically sterilized, or using a reliable method of birth control) aged 18 to 80 years, recruited from outpatient clinics of the Kaohsiung Veterans General Hospital. The main inclusion criteria were a stable glycosylated hemoglobin (HbA1c) level $\leq$ 10%, LDL-C $\geq$ 130 mg/dl, and a fasting serum triglyceride $<$ 400 mg/dl. Exclusion criteria were type 1 diabetes, secondary causes of hyperlipoproteinemia, serious medical or psychologic conditions, and known hypersensitivity to statins. All patients were required to discontinue any lipid-modifying drug at the screening visit and no such agents, other than atorvastatin, were permitted for the duration of the study. The patients were also advised not to consume alcohol during the study period. Antidiabetic medication and other concomitant medications were maintained at a constant dose throughout the study period. The study protocol was approved by an institutional review board at the hospital, and, after giving written informed consent, participants were randomized to one of three active treatment groups: 10 mg/day, 20 mg/day, or 40 mg/day of atorvastatin. A physical examination including vital signs (systolic and diastolic blood pressure and heart rate) monitoring was performed at each visit.

Laboratory Analysis. Overnight fasted (12-hr) venous blood samples were obtained from the forearm of participants at screening, at randomization, and at the end of the study. Plasma samples were frozen and stored at –20°C until assayed for ET-1, CRP, lipid profiles, routine blood chemistry, and hematology testing. Plasma glucose, total cholesterol, HDL-C, triglyceride, whole blood counts, liver function tests, electrolytes, blood urea nitrogen, creatinine, and HbA1c levels were measured by standard laboratory methods. LDL-C was calculated from the formula of Friedewald *et al.* (12). Routine urinalysis was also performed and CRP was measured by chemiluminescent immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA).

ET Assay. Plasma ET-1 concentrations were measured after extraction by the quantitative sandwich enzyme-linked immunosorbent assay (ELISA) method using a commercially available kit (R&D Systems, Minneapolis, MN). The samples were processed according to the instructions of the manufacturer. Briefly, 500 µl plasma samples were thoroughly mixed with 750 µl of extraction solvent (acetone:1 *N* HCl:water [40:1:5]) and centrifuged at 12,293 *g* for 20 mins in a refrigerated centrifuge at 4°C. The supernatant was decanted and dried in a Savant vacuum centrifuge. The pellet was reconstituted in 0.25 ml sample diluent, vortexed for 30 secs, and the aliquot was assayed in duplicate. The samples, which included standards in buffer, reconstituted extracts of the quality-control samples and test samples, and an enzyme (horseradish peroxidase)-labeled second antibody, were sequentially added to a 96-well microplate precoated with an antibody against ET-1. After 1 hr of incubation at room temperature and removal of unbound materials, the amount of enzyme-conjugated tracer bound to the wells was detected through reaction with a substrate specific for the horseradish peroxidase enzyme. The reaction product was measured with a microplate reader (MRX; Dynex Technologies, Chantilly, VA) and the absorbance was read at 450 nm with a correction wavelength of 630 nm. A standard curve was determined with the use of the mean absorbance values of the included ET-1 standards, and the ET-1 concentrations in all unknown plasma samples were calculated with linear regression. The assay sensitivity detected $<$1.0 pg/ml of ET-1. The crossreactivity of ET-2, ET-3, and big ET were 45%, 14%, and $<$1%, respectively. The mean intraassay and interassay coefficient of variances in our laboratory were 4.5% and 5.5 %, respectively.

Statistical Analysis. Results are expressed as the mean $\pm$ SD. Mean differences between the study groups were analyzed using analysis of covariance (ANCOVA). Correlations between different variables were examined with linear regression analysis. Analyses were performed using SPSS for Windows (version 10). All tests were two-sided and used a fixed level of significance at $P < 0.05$.

Results

Twenty-nine type 2 diabetic subjects with dyslipidemia were enrolled and randomly assigned to receive atorvastatin

Table 1. Baseline Characteristics of the Studied Groups[a]

	Control	A10	A20	A40
Number of subjects	12	10	10	9
Male (%)	50	40	40	89
Age (years)	57.4 ± 10.2	59.3 ± 12.8	58.5 ± 13.0	62.2 ± 9.6
Retinopathy (%)	—	0	20	22
Hypertension (%)	30	30	40	33
Body mass index (kg/m^2)	24 ± 3	25 ± 3	26 ± 2	23 ± 3
Systolic blood pressure (mm Hg)	124 ± 12	126 ± 13	126 ± 13	128 ± 13
Diastolic blood pressure (mm Hg)	75 ± 11	71 ± 10	74 ± 14	70 ± 10
Smoking (%)	8	0	0	11
Fasting glucose (mg/dl)	84 ± 19	151 ± 41*	177 ± 42*	175 ± 34*
HbA1c (%)	5.1 ± 0.7	7.6 ± 1.2*	8.1 ± 1.0*	8.3 ± 1.0*
Plasma ET-1 (pg/ml)	0.64 ± 0.15	1.02 ± 0.37*	1.17 ± 0.55*	0.87 ± 0.45*

[a] Data are presented as numbers or means ± SD. A10, A20, and A40: Atorvastatin 10 mg, 20 mg, and 40 mg, respectively.
*$P < 0.001$ vs. control.

orally at 10 mg (A10; $n = 10$), 20 mg (A20; $n = 10$), or 40 mg (A40; $n = 9$) daily for 12 weeks. Demographic and baseline characteristics of the study participants are shown in Table 1. Overall, demographic characteristics were similar for each treatment group, except that there were more men in the A40 group. Treatment with 10 mg atorvastatin daily for 12 weeks significantly reduced the levels of plasma lipids (total cholesterol, LDL-C, and triglyceride) by 30% (from 249 ± 26 to 174 ± 34 mg/dl; $P < 0.001$), by 36% (from 169 ± 15 to 108 ± 28 mg/dl; $P < 0.001$), and by 37% (from 161 ± 88 to 102 ± 36 mg/dl; $P < 0.01$), respectively. Treatment with 20 mg atorvastatin daily for 12 weeks significantly reduced the levels of plasma total cholesterol by 43% (from 241 ± 24 to 138 ± 32 mg/dl; $P < 0.001$), LDL-C by 54% (from 165 ± 20 to 76 ± 25 mg/dl; $P < 0.001$), and triglyceride by 36% (from 174 ± 56 to 111 ± 47 mg/dl; $P < 0.05$), whereas daily treatment with 40 mg atorvastatin for 12 weeks significantly reduced plasma total cholesterol level by 42% (from 235 ± 25 to 137 ± 25 mg/dl; $P < 0.001$), LDL-C by 51% (from 164 ± 23 to 81 ± 24 mg/dl; $P < 0.001$), and triglyceride by 29% (from 131 ± 60 to 93 ± 41 mg/dl; $P < 0.05$; Table 2). No age or sex-related differences in the change from baseline in LDL-C were observed. Although 20 mg and 40 mg atorvastatin each produced significantly ($P < 0.005$ and $P < 0.05$, respectively) greater decreases in LDL-C when compared with the 10 mg dose, the greatest LDL-C lowering effect was noted in the A20 group. The proportions of participants who attained the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) LDL-C goal (<100 mg/dl) by the end of the study are shown in Figure 1 (40%, 90%, and 78% in A10, A20, and A40 groups, respectively). Obviously, the A20 group has a significant higher percentage of subjects attaining the NCEP-ATP III LDL-C goal than the A10 group (90% and 40%, respectively; $P < 0.05$). Although no significant changes in plasma HDL-C levels were evident among the three treatment groups, an increase (12%) of HDL-C was only seen in the A40 group. All diabetic subjects had higher plasma ET-1 concentrations (A10, 1.02 ± 0.37 pg/ml [mean ± SD]; A20, 1.17 ± 0.55 pg/ml; A40, 0.87 ± 0.45 pg/ml) than those of age- and sex-matched normal control subjects (0.64 ± 0.15 pg/ml; all $P < 0.001$). Plasma ET-1 levels showed a borderline significant decrease, by 22%, in diabetic subjects treated with 10 mg atorvastatin ($P = 0.05$), and by 30% in subjects treated with 20 mg atorvastatin ($P = 0.06$). Paradoxically, the 40-mg dose of atorvastatin provided an increase of 2% in plasma ET-1 levels at the end of study, which is significantly different ($P < 0.05$) and marginally significant ($P = 0.057$) from the levels of the 10- and 20-mg doses, respectively (Fig. 2). Similarly, although insignificantly, plasma concentrations of CRP also tended to decrease by 12% and 48%, and paradoxically increased by 18% in A10, A20, and A40, respectively (Fig. 2). All doses of atorvastatin were well tolerated. No cases of rhabdomyolysis or myopathy were reported during the study and no dose-response relationship in the overall incidence of adverse effects was observed across the atorvastatin dose range. At the end of study, a slight increase of HbA1c level, from 8.3% to 8.7% ($P = 0.21$), was noted in the A40 group, whereas a slight decrease (0.2%) of HbA1c level was observed in both the A10 and A20 groups ($P = 0.39$ and $P = 0.64$, respectively). Both systolic and diastolic blood pressures dropped slightly but insignificantly during the study in all treatment groups.

Discussion

The present study demonstrates that atorvastatin can effectively and safely reduce serum total cholesterol, LDL-C, and triglyceride levels in Taiwanese diabetic subjects with dyslipidemia without affecting glycemic control. The reductions in LDL-C reported in this study are consistent with previously published values (13). Although HDL-C elevations were not obvious, the negative dose–response effect of atorvastatin on HDL-C as reported by Wierzbicki *et al.* (14) was not observed in our study. This may be because

Table 2. Measured Parameters at Baseline and End of the Study[a]

Atorvastatin dose	10 mg	20 mg	40 mg
Total cholesterol (mg/dl)			
Baseline	249 ± 26	241 ± 24	235 ± 25
Week 12	174 ± 34*	138 ± 32*	137 ± 25*
Triglycerides (mg/dl)			
Baseline	161 ± 88	174 ± 56	131 ± 60
Week 12	102 ± 36**	111 ± 47***	93 ± 41****
HDL-C (mg/dl)			
Baseline	48 ± 7	41 ± 12	43 ± 10
Week 12	46 ± 7	40 ± 8	38 ± 11
LDL-C (mg/dl)			
Baseline	169 ± 15	165 ± 20	164 ± 23
Week 12	108 ± 28*	76 ± 25*	81 ± 24*
CRP (mg/dl)			
Baseline	0.16 ± 0.14	0.32 ± 0.43	0.38 ± 0.52
Week 12	0.14 ± 0.15	0.17 ± 0.23	0.45 ± 0.53
CK (mg/dl)			
Baseline	77 ± 37	99 ± 57	118 ± 43
Week 12	85 ± 26	90 ± 51	123 ± 40
ET-1 (pg/ml)			
Baseline	1.02 ± 0.37	1.17 ± 0.55	0.87 ± 0.45
Week 12	0.79 ± 0.39	0.82 ± 0.37	0.89 ± 0.38
Fasting glucose (mg/dl)			
Baseline	151 ± 41	177 ± 42	175 ± 34
Week 12	137 ± 23	159 ± 28	180 ± 52
HbA1c (%)			
Baseline	7.6 ± 1.2	8.1 ± 1.0	8.3 ± 1.0
Week 12	7.4 ± 1.0	8.0 ± 1.1	8.7 ± 1.4
Systolic blood pressure (mm Hg)			
Baseline	126 ± 13	126 ± 13	128 ± 13
Week 12	122 ± 10	117 ± 15	121 ± 11
Diastolic blood pressure (mm Hg)			
Baseline	71 ± 10	74 ± 14	70 ± 10
Week 12	69 ± 8	66 ± 8	68 ± 8

[a] Data are presented as mean ± SD. Statistical test for differences between baseline and week 12 among the three groups: *$P < 0.001$; **$P < 0.01$; ***$P < 0.005$; ****$P < 0.05$.

of differences in the ethnic populations studied. A dose of 40 mg atorvastatin induced a slight increase in HbA1c levels, whereas in subjects taking 10 or 20 mg atorvastatin, the HbA1c level decreased slightly. Although these inconsistent results of atorvastatin on glycemic control in diabetic subjects have also been reported by others (15), the overall changes in HbA1c levels in our studied groups are insignificant.

Increased levels of circulating ET-1 in diabetic subjects was first reported by Takahashi *et al.* (3). Our results also demonstrate that plasma ET-1 concentrations are significantly higher in Taiwanese diabetic subjects compared with those of controls. As reviewed by us previously (4, 5), the increase of plasma ET-1 levels in diabetic subjects could act at least as a marker or may even contribute to the development of diabetic vascular complications. In the present study, plasma ET-1 levels showed borderline significant decreases by 22% in diabetic subjects treated with 10 mg atorvastatin ($P = 0.05$) and by 30% in diabetic subjects treated with 20 mg atorvastatin ($P = 0.06$). Similarly, Economides *et al.* reported that diabetic subjects have higher plasma ET-1 levels that could be reduced significantly after 12 weeks of 20 mg atorvastatin therapy (11). Actually, previous *in vitro* studies have already shown that both atorvastatin and simvastatin reduce the expression of pre-pro-ET-1 mRNA and the synthesis of ET-1 in a concentration- and time-dependent manner in bovine aortic endothelial cells (9), and this effect was reversed by mevalonate but not by cholesterol, indicating that it is exerted by the inhibitory action of statins on products of the mevalonate metabolism other than cholesterol. Hence, we think that this marginal failure to reach statistical significance in the present study is likely to represent a type 2 statistical error and that the observed effect is real. Given the involvement of ET-1 in the development of endothelial dysfunction, as well as the growing evidence for a contribution of ET-1 to diabetic vascular complications (4, 5) and various cardiovascular disease states (16), our findings and Economides' findings suggest a possible role for statins in the therapy of disease states associated with elevated ET-1 levels.

CRP is an acute-phase reactant that serves both as a marker of vascular inflammation and plays a direct role in

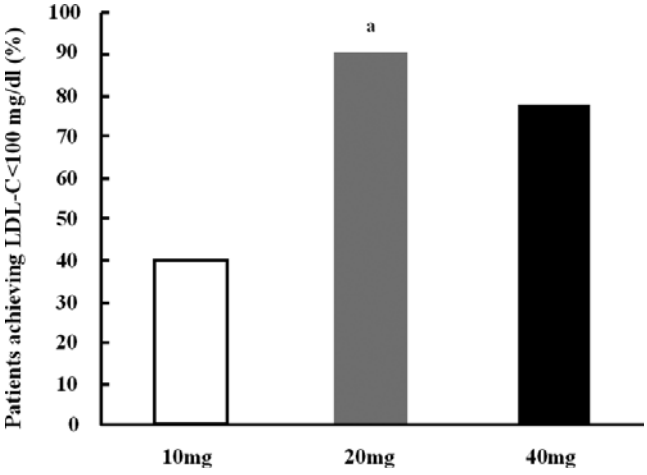

Figure 1. Proportions of participants who attained the NCEP-ATP III LDL-C goal (<100 mg/dl) by the end of the study among the 10-, 20-, and 40-mg atorvastatin-treated groups. $^{a}P < 0.05$ compared with the 10-mg dose.

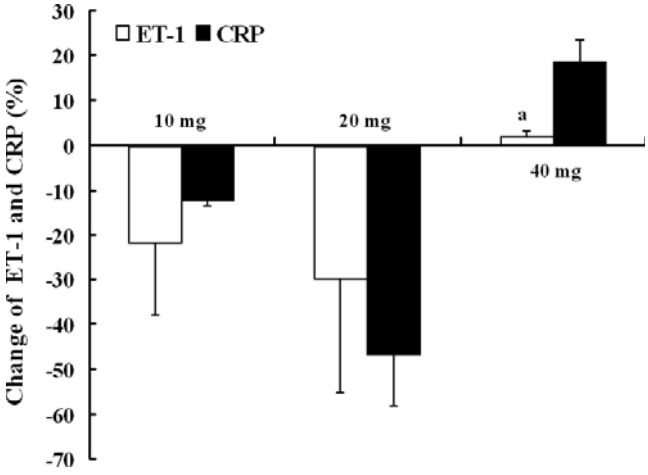

Figure 2. Percent change in plasma ET-1 and CRP from baseline to 12 weeks. $^{a}P < 0.05$ compared with the 10-mg atorvastatin dose.

the inflammatory process (17). Recently, it was reported that increased levels of CRP may predict the risk of developing myocardial infarction (18). Previous studies have shown that pravastatin can reduce CRP levels in diabetic subjects with dyslipidemia in a relatively short period of time (19). A reduction in plasma lipids by statins and a decreased exposure of tissue to modified lipoproteins could reduce the inflammation in the arterial wall and, thus, cause a lowering of CRP levels. On the other hand, the statin effect may be a direct effect on the liver because CRP is mainly liver-derived, and most statins are very liver selective in their action. Although insignificantly, plasma concentrations of CRP in our study tended to decrease, by 12% and 48%, in the A10 and A20 groups, respectively. Interestingly, although Economides *et al*. showed that 20 mg atorvastatin can significantly reduce the CRP levels by 50% in subjects at risk of developing diabetes, it has no effect on the CRP levels in diabetic subjects (11). A possible explanation for the discrepancy between these studies could be that the data obtained in these studies were from different ethnic populations and from a relatively small number of patients. Further studies will be required to explore the findings of the present study before solid conclusions can be reached.

Paradoxically, the 40-mg dose of atorvastatin provided an increase (2%) in plasma ET-1 levels, which is significant or marginally significant when compared with those of the 10- or 20-mg doses ($P < 0.05$ and $P < 0.01$, respectively; Fig. 2). Although nonsignificantly, plasma CRP levels also increased by 18% in diabetic subjects treated with 40 mg atorvastatin. The cause of these phenomena is unclear at the present moment. Although smoking has been shown to be associated with a higher salivary (20) and plasma (21) ET-1 level, as well as a higher plasma CRP concentration (22), the contribution of smoking may be minimal in the present study because only one subject (11%) in the 40-mg group smoked. Regardless, these biphasic lipid-independent atorvastatin effects may potentially counteract the beneficial effects of LDL-lowering by higher doses of atorvastatin in diabetic subjects. This may explain, at least partly, why aggressive lipid lowering by atorvastatin that resulted in substantial improvement of the lipid profile, could not reverse endothelial dysfunction in type 2 diabetic subjects with dyslipidemia (11, 23). Moreover, the present study also demonstrated that the greatest LDL-C lowering effect and the highest percentage of subjects achieving the NCEP-ATP III LDL-C goal were noted in diabetic subjects treated with 20 mg atorvastatin. Taken together, these results suggest that one should be careful with extrapolation of the pleiotropic effects of statins in nondiabetic subjects to subjects with diabetes, and that 20 mg atorvastatin may have the best benefit in treating diabetic subjects with dyslipidemia.

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