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ORIGINAL ARTICLE

Atorvastatin might improve ventricular electrostability and decelerate the deterioration of renal function in patients with heart failure and diabetes mellitus

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Summary
Background and purpose: Previous studies suggested that statins have pleiotropic effects, such as improvements in endothelial function, as well as anti-inflammatory, anti-proliferative, and anti-oxidative effects. These effects might benefit patients with heart failure. In those patients, statins relieved symptoms, decreased the frequency of hospitalization, suppressed neurohumoral activation, and improved cardiac function. However, it remains unknown how statins impact pathophysiology of heart failure with diabetes mellitus. The aim of this study was to investigate the effects of atorvastatin on pathophysiology of heart failure with diabetes mellitus.
Methods and results: We enrolled retrospectively 128 patients with heart failure with diabetes mellitus who were admitted from January 2003 to December 2005. Among these patients, 80 received atorvastatin (statin group) and the remaining patients served as controls (non-statin group). At study entry, there were no significant differences in the patient profiles between the two groups except for the low-density lipoprotein cholesterol level being higher in the statin group. After the follow-up period of two years, the frequency of re-hospitalization, brain natriuretic peptide, premature ventricular contractions, Lown grade, and deterioration of glomerular filtration rate were significantly less in the statin group.

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Conclusion: Atorvastatin might benefit patients with heart failure and diabetes mellitus by improving ventricular electrical stability and decelerating deterioration of renal function.

Introduction

Previous randomized clinical trials have shown that statins reduce cardiovascular events in patients with and/or without coronary artery disease. Statins have been shown to improve endothelial function [1], decrease plasma levels of proinflammatory cytokines [2], and exert antihypertrophic [3,4], antioxidant [5], and antifibrotic [6] effects on myocardium. Furthermore, other reports suggested that statins have beneficial effects on immune function, macrophage metabolism, and cell proliferation irrespective of changes in low-density lipoprotein (LDL) cholesterol concentrations [7].

Recent clinical trials suggested that statins might benefit patients with heart failure [8—13]. Short-term statin therapy improved cardiac function, neurohumoral imbalance, and symptoms in patients with idiopathic dilated cardiomyopathy [8]. Long-term atorvastatin therapy suppressed neurohumoral activation and improved cardiac function in mild to moderate heart failure [9]. Compared with a lower dose, high-dose treatment with atorvastatin in patients with stable coronary disease significantly reduced hospitalization for heart failure [10]. However, it remains unknown how statins impact pathophysiology of heart failure with diabetes mellitus, which is known to have deleterious effects on heart failure [14,15] and coronary artery disease [16].

The aim of this study was to investigate the effects of atorvastatin on pathophysiology of heart failure with diabetes mellitus. The results indicated that atorvastatin might benefit heart failure with diabetes mellitus by improving ventricular electrostability and decelerating the deterioration of renal function.

Subjects and methods

Patient populations

We retrospectively studied patients with symptomatic acute heart failure and diabetes mellitus who were admitted to Aso Iizuka Hospital from January 2003 to December 2005. The criteria for enrollment in the study were the clinical evidence of acute heart failure diagnosed by Framingham criteria [17], and diabetes mellitus diagnosed by the guideline of diabetes mellitus of the Japan Diabetes Society. In those patients, the New York Heart Association (NYHA) functional classification on admission ranged between II and IV. We excluded chronic obstructive pulmonary disease, right heart failure, and patients who had already taken atorvastatin or other statins. All patients were treated for acute heart failure, and were discharged after the improvement of heart failure. We enrolled 128 patients with heart failure and diabetes mellitus. Among them, 80 patients started to receive atorvastatin (10 mg) and the remaining 48 patients did not receive any statins (non-statin group). We followed up both groups for two years. As biochemical biomarkers, we measured plasma brain natriuretic peptide (BNP) and hemoglobin A1c (HbA1c). We calculated estimate of glomerular filtration rate (eGFR) from serum creatinine value and age using Japanese-coefficient-modified MDRD study [18]. As physiological biomarkers, we conducted echocardiography and 24-h Holter monitoring, and the severity of ventricular arrhythmias was evaluated in terms of Lown grade [grade 0: no premature ventricular contraction (PVC); grade I: <30 PVC/h, grade II: >30 PVC/h; grade III: multiform PVC; grade IVa: couplets; grade IVb; ventricular tachycardia runs] [19]. We acquired those biomarkers just before the statin therapy in the statin group and before discharge in the non-statin group, and one and two years after the discharge in both group. Hospitalizations due to worsening heart failure were diagnosed by the Framingham criteria, as described above.

Statistical analysis

Normally distributed variables were expressed as mean ± S.D. Unpaired *t* test or Mann—Whitney *U* test was used to compare the differences in normally distributed variables, respectively, between the statin and non-statin groups. The rate of re-hospitalization due to worsening heart failure between the statin and non-statin groups was compared by Kaplan—Meier analysis. All statistical tests were carried out against the baseline characteristics. Differences were considered significant at a p value of <0.05.

Table 1 Patient characteristics.

	Statin	Non-statin	*P*-value
n	80	48	NS
Male/female	54/26	34/14	NS
Age	65±7	61±5	NS
BMI	22±4	23±3	NS
Current smoker	18 (23%)	10 (20%)	<0.05
Causes of heart failure			
Coronary artery disease	22 (27%)	9 (18%)	NS
Dilated cardiomyopathy	25 (32%)	13 (28%)	NS
Hypertensive heart disease	14 (18%)	10 (21%)	NS
Valvular heart disease	15 (19%)	11 (23%)	NS
Systolic blood pressure (mmHg)	130±17	128±14	NS
Diastolic blood pressure (mmHg)	72±14	68±9	NS
Heart rate (bpm)	82±9	79±5	NS
Medications			
Diuretics	74 (92%)	44 (92%)	NS
β-Blockers	53 (66%)	34 (71%)	NS
ACE inhibitors	75 (94%)	46 (96%)	NS
Angiotensin receptor blocker	3 (4%)	2 (4%)	NS
Sulfonylurea	12 (15%)	8 (17%)	NS

Data are presented as number (%) or mean±S.D. BMI, body mass index; bpm, beats per minute; ACE, angiotensin-converting enzyme.

Results

Patient characteristics at baseline

The patient profiles at enrollment are summarized in Tables 1 and 2. As can be seen in Table 1, there were no significant differences in age, gender, or the prevalence of dilated cardiomyopathy, hypertensive heart disease, or valvular heart disease between the non-statin group and statin group just before statin therapy. Medications did not differ either. The frequency of patients taking

Table 2 Patient characteristics (2).

	Statin	Non-statin	*P*-value
Total cholesterol (mg/dl)	227±17	200±10	0.042
LDL cholesterol (mg/dl)	156±11	122±13	0.008
HDL cholesterol (mg/dl)	44±8	47±7	NS
Triglycerides (mg/dl)	129±7	132±11	NS
FBS (mg/dl)	112±7	119±5	NS
HbA1c (%)	6.6±0.7	6.3±0.4	NS
BNP (pg/ml)	128±27	142±36	NS
eGFR (ml/min/1.73 m^2)	62.4±7.9	66.8±4.4	NS
LVEF (%)	35±7	33±5	NS
LVEDD (mm)	56±5	57±7	NS
LVESD (mm)	40±4	42±7	NS
PVC per 24 h	1288±362	1194±443	NS
Lown grade			
I	28 (35%)	15 (31%)	NS
II	43 (54%)	26 (54%)	NS
III	8 (10%)	6 (13%)	NS
IVa/IVb	1 (1%)/0 (0%)	1 (2%)/0 (0%)	NS/NS

Data are presented as mean±S.D. LDL, low-density lipoprotein; HDL, high-density lipoprotein; FBS, fasting blood glucose; HbA1c, hemoglobin A1c; BNP, brain natriuretic peptide; eGFR, creatinine-based estimate of glomerular filtration rate; LVEF, left ventricular ejection fraction; LVDDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; PVC, premature ventricular contraction.

angiotensin receptor blockers or oral hypoglycemic agents also did not differ between the non-statin and statin groups. All patients with hypoglycemic agents took sulfonylurea. There is no patient with insulin-therapy for diabetes mellitus in the present study. As anticipated, the statin group had a higher prevalence of coronary artery disease than the non-statin group.

As shown in Table 2, fasting blood glucose (FBG), HbA1c, BNP, or eGFR did not differ between the two groups. As anticipated, however, total cholesterol and LDL cholesterol were higher in the statin group than in the non-statin group. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), the frequency of PVC and the frequency of patients who had Lown grade I, II, III, or IV did not differ between the two groups. There is no patient with Lown grade 0 in the present study.

Effects of atorvastatin on biochemical and physiological biomarkers

Effects of atorvastatin on biochemical biomarkers are summarized in Table 3. Atorvastatin markedly decreased total cholesterol and LDL cholesterol. As a result, LDL cholesterol values at the follow-up period of one year were comparable between the two groups and got lower at two years in the statin group than the non-statin group. BNP was lower at one year and got lower at two years in the statin group than the non-statin group. Systolic blood pressure, LVEF, LVEDD, LVESD, and HbA1c remained not different between the two groups.

As shown in Fig. 1, eGFR decreased more in the non-statin group than in the statin-group (-10.6 ± 2.8 ml/min/1.73 m^2 vs. -6.1 ± 3.3 ml/min/1.73

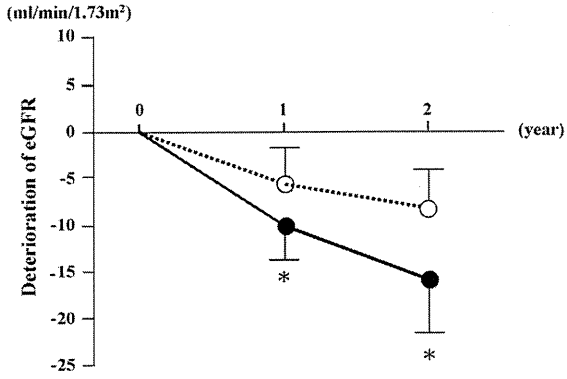

Figure 1 Changes in eGFR at entry, one and two years. Open circle and solid line indicate statin group. Closed circle and dotted line indicate non-statin group. *$p < 0.05$. eGFR, creatinine-based estimate of glomerular filtration rate.

Table 3 Changes in LDL-C, BNP, LVEF, LV dimension, systolic blood pressure, and HbA1c at one and two years.

	Statin	Non-statin	*P*-value
LDL-C (mg/dl)			
1 year	98 ± 16	116 ± 13	NS
2 years	94 ± 9	112 ± 11	<0.05
BNP (pg/ml)			
1 year	101 ± 12	136 ± 13	<0.05
2 years	76 ± 11	132 ± 13	<0.05
LVEF (%)			
1 year	38 ± 6	35 ± 6	NS
2 years	40 ± 7	38 ± 7	NS
LVEDD/LVESD (mm)			
1 year	57 ± 6/41 ± 4	59 ± 4/44 ± 5	NS/NS
2 years	58 ± 4/42 ± 4	60 ± 7/46 ± 7	NS/NS
Systolic blood pressure (mmHg)			
1 year	118 ± 13	122 ± 15	NS
2 years	116 ± 11	120 ± 13	NS
HbA1c (%)			
1 year	6.4 ± 0.3	6.5 ± 0.4	NS
2 years	6.1 ± 0.4	6.5 ± 0.3	NS

Data are presented as mean ± S.D. LDL-C, low-density lipoprotein cholesterol; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction; LVEDD, left ventricular end-diastolic dimension; LVESD, left ventricular end-systolic dimension; HbA1c, hemoglobin A1c.

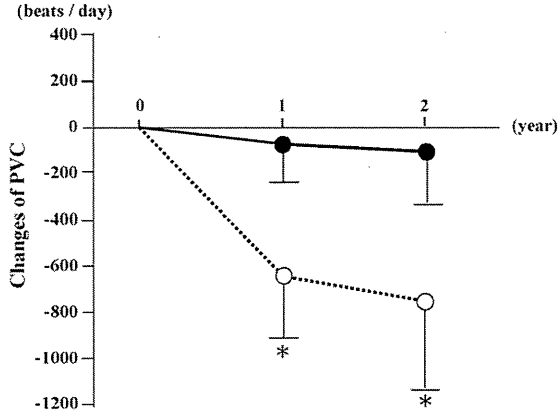

Figure 2 Changes in PVCs at entry, one, and two years. Open circle and solid line indicate statin group. Closed circle and dotted line indicate non-statin group. *$p < 0.05$. PVC, premature ventricular contraction.

m^2, $p < 0.05$) at one year. The difference increased at two years (-16.8 ± 4.1 ml/min/1.73 m^2 vs. -8.3 ± 5.4 ml/min/1.73 m^2, $p < 0.05$). Atorvastatin might have decelerated deterioration of renal function, and thereby might have a potent protective effect on renal function.

Shown in Fig. 2 is the effect of atorvastatin on the frequency of PVCs. Atorvastatin markedly decreased PVCs at one year and two years. There were no changes in PVCs in the non-statin group. Furthermore, the frequency of patients who had Lown grade ≥II was significantly lower in the statin group than in the non-statin group at one year and two years (Fig. 3).

Table 4 Cardiovascular events, hospitalization, and mortality at one and two years.

	Statin	Non-statin	*P*-value
Cardiovascular events			
1 year	5 (6%)	4 (8%)	NS
2 years	8 (10%)	6 (13%)	NS
CHF with hospitalization			
1 year	7 (9%)	8 (17%)	0.003
2 years	16 (20%)	15 (31%)	0.005
All-cause mortality			
1 year	0	0	NS
2 years	0	1	NS

Data are presented as number (%) or mean ± S.D. CHF, congestive heart failure.

Cardiovascular events and hospitalization

No patient died in the statin group, whereas one patient suddenly died in the non-statin group. Treatment with atorvastatin did not significantly reduce cardiovascular events, which was defined as nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, or cardiovascular death (Table 4). However, the frequency of re-hospitalization due to worsening heart failure was significantly reduced in the statin group than in the non-statin group, determined by Kaplan–Meier analysis (Fig. 4). For the patients with atorvastatin, the hazard ratio for re-hospitalization due to worsening heart failure was 0.68 (95% CI, 0.51–0.84) (Fig. 4).

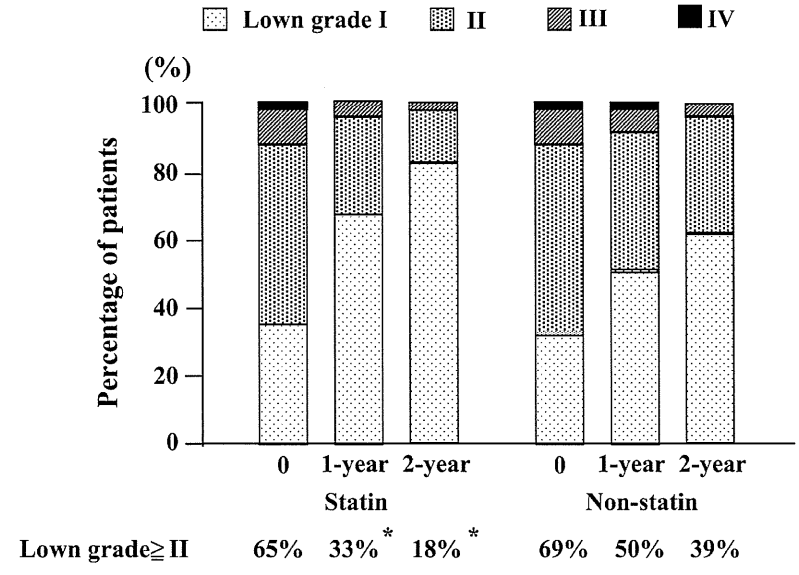

Figure 3 Kaplan–Meier analysis estimates for re-hospitalization due to heart failure. Solid line indicates non-statin group, and dotted line indicates statin-group. *$p < 0.05$.

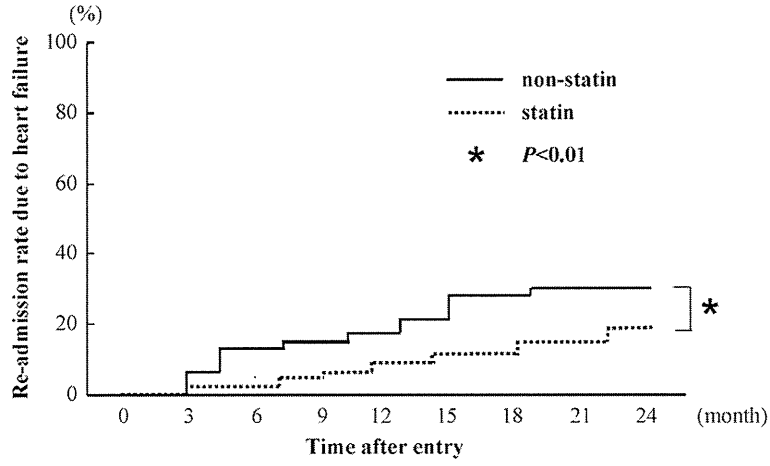

Figure 4 Lown grades at entry, one year, and two years after (grade 0: no PVC; grade I: <30 PVC/h, grade II: >30 PVC/h; grade III: multiform PVC; grade IV: couplets and/or ventricular tachycardia runs). *$p < 0.05$.

Discussion

In the present study, we demonstrated that atorvastatin reduced the frequency of re-hospitalization due to worsening heart failure, BNP, the frequency of PVCs, Lown grade and decelerated deterioration of GFR in the patients with heart failure and diabetes mellitus. These results suggested that atorvastatin might benefit patients with heart failure and diabetes mellitus by improving ventricular electrostability and decelerating deterioration of renal function.

Recent clinical trials and basic studies suggested that statins might benefit patients with heart failure [8—13,20—24]. In the present study, atorvastatin reduced the frequency of re-hospitalization due to worsening heart failure and BNP. These results are comparable to previous studies, which showed that statins might benefit patients with heart failure. Furthermore, the present study suggested that atorvastatin has beneficial effects on the pathophysiology of heart failure with diabetes mellitus, which is known to have deleterious effects on heart failure [14,15]. In the statin group, the LDL cholesterol level was significantly higher than in the non-statin group at entry. Atorvastatin markedly decreased the LDL cholesterol level, and the LDL cholesterol levels at the follow-up period of one year were comparable between the two groups. The LDL cholesterol levels were significantly lower at two years in the statin group than the non-statin group. The statin group had a higher prevalence of coronary atherosclerotic diseases than the non-statin group. These differences of backgrounds between the statin and non-statin groups might be responsible for the results in this study. However, these results indicate that the risks for atherosclerosis and ischemic heart diseases were higher in the statin group than in the non-statin group. Despite those with a background predisposed to coronary heart disease, atorvastatin significantly benefits the patients with heart failure. It is conceivable that atorvastatin might be a novel strategy of treatment for heart failure and diabetes mellitus.

The mechanism by which atorvastatin benefits patients with heart failure and diabetes mellitus remains unknown. We conjecture three mechanisms: anti-oxidant, anti-inflammatory, and improvement of the sympatho-vagal balance. In patients with heart failure, increased oxidative stress is associated with reduced LV function and severity of heart failure [25,26]. Previous studies suggested that statins inhibited vascular and myocardial oxidative stress by inhibiting rac-induced nicotinamide adenine dinucleotide phosphatase oxidase activity [27,28], and reducing oxidized LDL concentration [9], which is a marker of oxidative stress and a useful predictor of mortality in patients with heart failure [29]. Furthermore, diabetes mellitus increases the risk of heart failure and oxidative stress may contribute to the development of cardiac dysfunction [30]. With regard to the anti-inflammatory effects of atorvastatin, previous studies reported that statins suppressed the inflammatory process in patients with heart failure [8,21,22]. Heart failure is associated with increased levels of proinflammatory cytokines that exert negative inotropic effects and induce apoptosis in cardiac myocytes [31]. Yamada et al. reported that atorvastatin tended to decrease interleukin 6 and high sensitive C-reactive protein in patients with heart failure [9]. Finally, we consider that atorvastatin might improve the imbalance between

the sympathetic and parasympathetic nerve activity and this improvement resulted in the reduction of the frequency of PVCs. A previous study reported that statin therapy restored sympatho-vagal balance in experimental heart failure [23]. We have demonstrated that atorvastatin reduced the oxidative stress in the cardiovascular center of the brainstem [32], in which oxidative stress increased sympathetic nerve activity in hypertensive animal models [33]. These reports suggest that atorvastatin might reduce the oxidative stress in the cardiovascular center, which, in turn, decreases the sympathetic nerve activity, the frequency of PVCs, and Lown grade. Further investigations are needed to clarify the mechanisms.

Renal dysfunction has been known to worsen heart failure [34]. Campese and Park suggested that statin-mediated alterations in inflammatory responses and endothelial function reduced proteinuria and the rate of progression of kidney disease [35]. In the present study, atorvastatin protected the progressive worsening in renal function for two years in the patients with heart failure and diabetes mellitus. These results suggest that atorvastatin might prevent the worsening of heart failure through a renoprotective effect. Additional prospective and randomized trials in the Japanese population are needed to determine whether atorvastatin is truly renoprotective.

Limitations

There are several limitations to the present study. First, the study was retrospective, and observational. The number of patients enrolled is also limited. Second, we were not able to determine whether the beneficial effect of atorvastatin on heart failure with diabetes mellitus is a class effect or not. The results of the present study should be validated by large, prospective, well-controlled, and randomized clinical trials. Third, we did not measure the activity of sympathetic nerve activity, parasympathetic nerve activity, and sympatho-vagal balance using variability of R–R interval and blood pressure analysis. In the present study, we are not able to suggest that the data indicate the improvement of the imbalance between sympathetic and parasympathetic nerve activity by atorvastatin in heart failure with diabetes mellitus.

Conclusion

Atorvastatin might benefit patients with heart failure and diabetes by improving ventricular electrical stability and decelerating deterioration of renal function. Atorvastatin might be a novel strategy of treatment for heart failure and diabetes.

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References

[1] Treasure CB, Klein JL, Weintraub WS, Talley JD, Stillabower ME, Kosinski AS, Zhang J, Boccuzzi SJ, Cedarholm JC, Alexander RW. Beneficial effects of cholesterol-lowering therapy on the coronary endothelium in patients with coronary artery disease. N Engl J Med 1995;332:481–7.

[2] Ferro D, Parrotto S, Basili S, Alessandri C, Violi F. Simvastatin inhibits the monocyte expression of proinflammatory cytokines in patients with hypercholesterolemia. J Am Coll Cardiol 2000;36:427–31.

[3] Hayashidani S, Tsutsui H, Shiomi T, Suematsu N, Kinugawa S, Ide T, Wen J, Takeshita A. Fluvastatin, a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, attenuates left ventricular remodeling and failure after experimental myocardial infarction. Circulation 2002;105:868–73.

[4] Oi S, Haneda T, Osaki J, Kashiwagi Y, Nakamura Y, Kawabe J, Kikuchi K. Lovastatin prevents angiotensin-II-induced cardiac hypertrophy in cultured neonatal rat heart cells. Eur J Pharmacol 1999;376:139–48.

[5] Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. Circulation 1998;97:1129–35.

[6] Dechend R, Fiebeler A, Park JK, Muller DN, Theuer J, Mervaala E, Bieringer M, Gulba D, Dietz R, Luft FC, Haller H. Amelioration of angiotensin II-induced cardiac injury by a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor. Circulation 2001;104:576–81.

[7] Vaughan CJ, Murphy MB, Buckley BM. Statins do more than just lower cholesterol. Lancet 1996;348:1079–82.

[8] Node K, Fujita M, Kitakaze M, Hori M, Liao JK. Short-term statin therapy improves cardiac function and symptoms in patients with idiopathic dilated cardiomyopathy. Circulation 2003;108:839–43.

[9] Yamada T, Node K, Mine T, Morita T, Kioka H, Tsukamoto Y, Tamaki S, Masuda M, Okuda K, Fukunami M. Long-term effect of atorvastatin on neurohumoral activation and cardiac function in patients with chronic heart failure: a prospective randomized controlled study. Am Heart J 2007;153, 1055.e1–e8.

[10] Krum H, McMurray JJ. Statins and chronic heart failure: do we need a large-scale outcome trial? J Am Coll Cardiol 2002;39:1567–73.

[11] Horwich TB, Hamilton MA, Maclellan WR, Fonarow GC. Low serum total cholesterol is associated with marked increase in mortality in advanced heart failure. J Card Fail 2002;8:216–24.

[12] Khush KK, Waters DD, Bittner V, Deedwania PC, Kastelein JJP, Lewis SJ, Wenger NK. Effect of high-dose atorvastatin on hospitalization for heart failure. Circulation 2007;115:576–83.

[13] Kadota S, Matsuda M, Izuhara M, Baba O, Morikawa S, Shioji K, Takeuchi Y, Uegaito T. Long-term effects of early statin therapy for patients with acute myocardial infarc-

tion treated with stent implantation. J Cardiol 2008;51: 171—8.
[14] Cleland JG, Swedberg K, Follath F, Komajda M, Cohen-Solal A, Aguilar JC, Dietz R, Gavazzi A, Hobbs R, Korewicki J, Madeira HC, Moiseyev VS, Preda I, van Gilst WH, Widimsky J, et al. Study group on diagnosis of the working group on heart failure of the European society of cardiology: the Euroheart Failure survey programme-a survey on the quality of care among patients with heart failure in Europe. Eur Heart J 2003;24:442—63.
[15] Yusuf S, Teo KK, Pogue J, Dyal L, Copland L, Schumacher H, Dagenais G, Sleight P, Anderson C. Telmisartan, ramipril, or both in patients at high risk for vascular events. N Engl J Med 2008;358:1547—59.
[16] Sassa S, Shimada K, Yoshida K, Tanaka H, Jissho S, Yoshikawa J. Comparison of 64-slice multi-detector computed tomography coronary angiography between asymptomatic, type 2 diabetes mellitus and impaired glucose tolerance patients. J Cardiol 2008;52:133—9.
[17] Eriksson H, Svardsudd K, Larsson B, Ohlson LO, Tibblin G, Welin L, Wilhelmsen L. Risk factors for heart failure in the general population: the study of men born in 1913. Eur Heart J 1989;10:647—56.
[18] Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, Ura N, Kiyohara Y, Hirakata H, Watanabe T, Moriyama T, Ando Y, Inaguma D, Narita I, Iso H, et al. Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. Clin Exp Nephrol 2007;11:41—50.
[19] Lown B, Wolf M. Approaches to sudden death from coronary heart disease. Circulation 1971;44:130—42.
[20] Trochu JN, Mital S, Zhang X, Xu X, Ochoa M, Liao JK, Recchia FA, Hintze TH. Preservation of NO production by statins in the treatment of heart failure. Cardiovasc Res 2003;60:250—8.
[21] Mozaffarian D, Mina mi E, Letterer R, Lawler RL, McDonald GB, Levy WC. The effects of atorvastatin (10 mg) on systemic inflammation in heart failure. Am J Cardiol 2005;96:1699—704.
[22] Sola S, Mir MQ, Lerakis D, Tandon N, Khan BV. Atorvastatin improves left ventricular systolic function and serum markers of inflammation in nonischemic heart failure. J Am Coll Cardiol 2006;47:332—7.
[23] Pliquett RU, Cornish KG, Zucker IH. Statin therapy restores sympathovagal balance in experimental heart failure. J Appl Physiol 2003;95:700—4.
[24] Wojnicz R, Wilczek K, Nowalany-Kozielska E, Szygula-Jurkiewicz B, Nowak J, Polonski L, Dyrbuś K, Badziński A, Mercik G, Zembala M, Wodniecki J, Rozek MM. Usefulness of atorvastatin in patients with heart failure due to inflammatory dilated cardiomyopathy and elevated cholesterol levels. Am J Cardiol 2006;97:899—904.
[25] Mallat Z, Philip L, Lebret M, Chatel D, Maclouf J, Tedgui A. Elevated levels of 8-iso-prostaglandin F2alpha in pericardial fluid of patients with heart failure: a potential role for in vivo oxidant stress in ventricular dilation and progression to heart failure. Circulation 1998;97:1536—9.
[26] Dhalla AK, Hill MF, Singal PK. Role of oxidative stress in transition of hypertrophy to heart failure. J Am Coll Cardiol 1996;28:506—14.
[27] Li JM, Gall NP, Grieve DJ, Chen M, Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. Hypertension 2002;40:477—84.
[28] Maack C, Kartes T, Kilter H, Schafers HJ, Nickenig G, Bohm M, Laufs U. Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment. Circulation 2003;108:1567—74.
[29] Tsutsui T, Tsutamoto T, Wada A, Maeda K, Mabuchi N, Hayashi M, Ohnishi M, Kinoshita M. Plasma oxidized low-density lipoprotein as a prognostic predictor in patients with chronic congestive heart failure. J Am Coll Cardiol 2002;39:957—62.
[30] Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Circulation 2007;115:3213—23.
[31] Pinsky DJ, Cai B, Yang X, Rodriguez C, Sciacca RR, Cannon PJ. The lethal effects of cytokine-induced nitric oxide on cardiac myocytes are blocked by nitric oxide synthetase antagonism or transforming growth factor beta. J Clin Invest 1995;95:677—85.
[32] Kishi T, Hirooka Y, Shimokawa H, Takeshita A, Sunagawa K. Atorvastatin reduced oxidative stress in the rostral ventrolateral medulla of stroke-prone spontaneously hypertensive rats. Clin Exp Hypertens 2008;30:3—11.
[33] Kishi T, Hirooka Y, Kimura Y, Ito K, Shimokawa H, Takeshita A. Increased reactive oxygen species in rostral ventrolateral medulla to neural mechanisms of hypertension in stroke-prone spontaneously hypertensive rats. Circulation 2004;109:2357—62.
[34] Go AS, Chertow GM, Fan D, McCulloch CE, Hsu C. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004;351: 1296—305.
[35] Campese VM, Park J. HMG-CoA reductase inhibitors and the kidney. Kidney Int 2007;71:1215—22.

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Cilnidipine Inhibits the Sympathetic Nerve Activity and Improves Baroreflex Sensitivity in Patients with Hypertension

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N-type calcium channel blocker, cilnidipine, is reported not to increase the heart rate in spite of the strong depressor effect. However, it has not been determined whether cilnidipine has the sympatho-inhibitory effects or not. Moreover, the effect of cilnidipine on the baroreflex control has not been determined. The aim of this study was to determine the effect of cilnidipine on sympathetic and parasympathetic nerve activity, and baroreflex sensitivity. We studied five hypertensive patients treated with 10 mg cilnidipine (10-mg group) and five hypertensive patients treated with 20 mg cilnidipine (20-mg group). Before the treatment and 6 months after the treatment, we measured the blood pressure, spontaneous baroreflex sensitivity (BRS), heart rate variability (HRV), and blood pressure variability (BPV). After 6 months, systolic blood pressure (SBP) and the low-frequency component of systolic BPV expressed in normalized units (LFnuSBP), as the parameter of sympathetic nerve activity, was significantly decreased in both groups, and the suppressive effects were stronger in the 20-mg group than in the 10-mg group. The high-frequency component of HRV expressed in normalized units, as the parameter of parasympathetic nerve activity, and BRS were significantly increased in 20-mg group, but not significant in 10-mg group. These results suggest that 6 months treatment with cilnidipine for hypertension has the sympatho-inhibtory effect, and that high-dose cilnidipine improves the parasympathetic nerve activity and baroreflex control in patients with hypertension.

Keywords N-type calcium channels blocker, hypertension, sympathetic nerve activity, baroreflex sensitivity

Introduction

Hypertension is an established risk factor in the prognosis of cardiovascular diseases and organ damage. It may be feasible for patients with hypertension or at high cardiovascular risk to receive a blood pressure-lowering medication in order to achieve a

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reduction of stroke and cardiovascular complications (1). Ca channel blocker is widely used as the blood pressure-lowering agents. However, it has been reported that Ca channels blocker increases heart rate with lowering blood pressure. Among the Ca channels blockers, cilnidipine is known not to increase the heart rate and plasma norepinehrine concentrations in spite of the strong blood pressure lowering effects (2–4). Cilnidipine is a long-acting dihydropyridine calcium channel blocker by inhibiting L-type calcium channels directly associated with vascular tone, and N-type calcium channels related to sympathetic nervous activity (5–7). Whereas cilnidipine inhibits N-type calcium channels, it has not been well established whether cilnidipine decreases the sympathetic nerve activity and increases the parasympathetic nerve activity in the patients with hypertension.

Analysis of spontaneous heart rate and blood pressure variability offers insights into different features of autonomic control of circulation (8), including the arterial baroreflex regulation (9). In this context, heart rate spectral powers in the so-called high-frequency (HF; 0.15–0.40 Hz) and low-frequency (LF; 0.04–0.15 Hz) regions and blood pressure spectra powers in the LF regions have been repeatedly reported to provide relevant information (8, 10–12). The LF power of blood pressure was reported to be increased in parallel with the sympathetic nerve activation (13). Furthermore, the baroreflex control is one of the key mechanisms responsible for the short-term control of blood pressure. Impairment of this reflex has been found in a number of conditions, such as aging (14), heart failure (15), post-myocardial infarction (16), and the impairment of baroreflex sensitivity (BRS) is known as the predictive factor of mortality in hypertension (17). Baroreflex sensitivity was originally assessed by intra-arterial measurement of the change in pulse interval following a pharmacologically induced change in blood pressure. However, for some time now, noninvasive monitoring of blood pressure using finger plethysmography has been available (18), and is an accepted method for tracking beat-to-beat changes in blood pressure (19). Added to this, a further method for measuring BRS has been developed, which assesses spontaneous changes in blood pressure and pulse interval, and does not require pharmacological manipulation of blood pressure-spectral analysis (20,21). However, it has not been determined whether the cilnidipine improves the impaired BRS or not.

Therefore, the aim of the present study was to evaluate the effect of cilnidipine on the sympathetic nerve activity, parasympathetic nerve activity, and BRS in the patients with hypertension. We evaluated the sympathetic and parasympathetic nerve activity using the analysis of systolic blood pressure and heart rate variability, and BRS was measured by the spontaneous sequence method.

Materials and Methods

Subjects

The present study was conducted prospectively on 10 outpatients with hypertension (5 males and 5 females; mean age: 58.6 years; range 44–74 years) whose blood pressure was over 140/90 mmHg. No patients were currently receiving anti-hypertensive medication and all of them were newly diagnosed. Patients with the secondary hypertension were excluded. All studies were performed between 9 and 11 a.m., with each subject examined at the same time of day on each visit to reduce the possible influence of circadian variation in BRS. This study was performed in a quiet room, and every effort was made to keep stimuli to a minimum during the study period. Each subject gave informed

consent to the experimental procedures, which was approved by the ethics committee of our institution.

Measurement of Blood Pressure and Heart Rate

Subjects lay supine, and were rested for a minimum of 15 minutes prior to assessment. Each subject then underwent periods of blood pressure and heart rate monitoring. Blood pressure monitoring was performed using the TaskForce Monitor 3040i (CNSystems, Graz, Austria). The cuff was attached to a finger of the left hand and supported at heart level. Electrocardiogram electrodes were attached to the chest. After a minimum period of 5 minutes, and once a reading of blood pressure and heart rate had stabilized, three consecutive, 5-minute recordings were made of the blood pressure and electrocardiogram tracing. Noninvasive brachial blood pressure readings were taken with an appropriate-sized cuff.

Spectral Analysis for Systolic Blood Pressure and Heart Rate

Spectral analysis was performed using an adaptive auto-regressive model to provide power spectra for both systolic blood pressure (SBP) and R-R interval (RRI). Low Frequency power of SBP was computed by integrating the spectra between 0.04 and 0.15 Hz, and HF power of RRI was computed by integrating the spectra between 0.15–0.40 Hz. Parasympathetic nerve activity was represented by the normalized unit of HF component of RRI (HFnuRRI), and sympathetic nerve activity was represented by the normalized unit of LF component of SBP (LFnuSBP).

Measurement of Baroreflex Sensitivity by Spontaneous Sequence Method

Sequence analysis detected sequences of three or more beats in which there was either an increase in SBP and pulse interval (Up sequence) or a decrease in SBP and pulse interval (Down sequence). Baroreflex sensitivity was estimated as the mean slope of the up sequences (UP BRS), the down sequences (Down BRS), and also the mean slope of all sequences (Sequence BRS) (20,21). Previous reports showed that this protocol measures BRS accurately in animals compared to standard pharmacological techniques (20,21).

Administration of Cilnidipine

Cilnidipine was administered at a dosage of 10–20 mg (10-mg group and 20-mg group) once daily after breakfast according to the guidelines of the treatment with hypertension of the Japanese Society of Hypertension (JSH2004). All the patients were placed on monotherapy with cilnidipine.

Statistical Analysis

All values were expressed as the mean ± SEM. The student's paired *t*-test was used to analyze the changes of variables between pre- and post-treatment with cilnidipine. Differences in variables between the groups were analyzed by one-way ANOVA. A value of $p < 0.05$ was considered statistically significant.

Results

Patients Characteristics

Table 1 shows the baseline characteristics of the two groups. There were no significant differences in age, blood pressure, serum creatinine, and hemoglobin between 10-mg and 20-mg group of cilnidipine. None of the patients had the clinical side effects of cilnidipine.

Effects of Cilnidipine on Blood Pressure and Heart Rate

After the treatment with cilnidipine for 6 months, blood pressure was significantly reduced in all patients, and the effect of blood pressure lowering was significantly greater in 20-mg group than in 10-mg group (Tables 2, 3, and 4). Heart rate was not significantly decreased in both groups after the treatment with cilnidipine (Tables 2, 3, 4).

Effects of Cilnidipine on Sympathetic and Parasympathetic Nerve Activity

After the treatment with cilnidipine for 6 months, LFnuSBP were significantly decreased in both groups (Tables 2 and 3), and the suppressive effects were stronger in the 20-mg group than in the 10-mg group (Table 4). While HFnuRRI was not significantly changed in the 10-mg group (Table 2), it was significantly increased in the 20-mg group (Table 3).

Effects of Cilnidipine on Baroreflex Sensitivity

In the 10-mg group, BRS was not significantly changed between before and after the treatment with cilnidipine (Table 2). However, in the 20-mg group, BRS was significantly improved after the treatment with cilnidipine for 6 months (Table 3).

Table 1
Clinical profile of the patients in 10-mg and 20-mg group

	10-mg Group (n = 5)	20-mg Group (n = 5)	
Age (year)	56 ± 7	59 ± 8	NS
Systolic blood pressure (mmHg)	161 ± 13	157 ±12	NS
Diastolic blood pressure (mmHg)	100 ± 5	98 ± 8	NS
Heart rate (bpm)	80 ± 5	76 ± 6	NS
AST/ALT (IU/L)	26 ± 11/27 ± 13	26 ± 9/28 ± 6	NS
Cr (mg/dL)	0.7 ± 0.2	0.8 ± 0.2	NS
Total cholesterol (mg/dL)	182 ±19	178 ± 22	NS
Triglyceride (mg/dL)	92 ± 33	88 ± 36	NS
LDL cholesterol (mg/dL)	110 ± 14	108 ± 12	NS
HDL cholesterol (mg/dL)	46 ± 7	48 ± 6	NS
Glucose (mg/dL)	89 ± 11	93 ± 10	NS
HbA1c (%)	5.6 ± 0.4	5.5 ± 0.6	NS
BNP (pg/ml)	38 ± 14	32 ± 11	NS
Left ventricular ejection fraction (%)	70 ± 9	72 ± 8	NS
Cardio-thoracic ratio(%)	54 ± 8	51 ± 8	NS

Table 2
Changes in blood pressure, heart rate, and autonomic function in the patients with 10-mg group

	Pretreatment (n = 5)	Cilnidipine 10 mg (n = 5)	P
Systolic blood pressure (mmHg)	161 ± 13	137 ± 13	< 0.05
Diastolic blood pressure (mmHg)	100 ± 5	87 ± 4	< 0.05
Heart rate (bpm)	80 ± 5	76 ± 7	NS
HF-RR (ms2)	102 ± 63	106 ± 58	NS
HFnuRR (%)	39 ± 6	42 ± 6	NS
LF-SBP (mmHg2)	0.7 ± 0.3	0.5 ± 0.5	NS
LFnuSBP (%)	56 ± 5	49 ± 4	< 0.05
Baroreflex sensitivity (ms/mmHg)	14.2 ± 2.6	16.2 ± 4.8	NS

Table 3
Changes in blood pressure, heart rate, and autonomic function in the patients with 20-mg group

	Pretreatment (n = 5)	Cilnidipine 20 mg (n = 5)	P
Systolic blood pressure (mmHg)	157 ± 12	120 ± 13	< 0.05
Diastolic blood pressure (mmHg)	98 ± 8	81 ± 5	< 0.05
Heart rate (bpm)	76 ± 6	73 ± 6	NS
HF-RR (ms2)	92 ± 44	102 ± 66	NS
HFnuRR (%)	39 ± 3	44 ± 2	< 0.05
LF-SBP (mmHg2)	0.6 ± 0.4	0.4 ± 0.3	NS
LFnuSBP (%)	63 ± 6	50 ± 4	< 0.05
Baroreflex sensitivity (ms/mmHg)	13.6 ± 2.9	20.2 ± 2.1	< 0.05

Table 4
Degree of changes in blood pressure, heart rate and autonomic function in the patients with 10-mg and 20-mg group

	Cilnidipine 10 mg (n = 5)	Cilnidipine 20 mg (n = 5)	P
Systolic blood pressure	−15%	−24%	< 0.05
Diastolic blood pressure	−13%	−17%	< 0.05
Heart rate	−5%	−4%	NS
HFnuRR	+7%	+13%	< 0.05
LFnuSBP	−12%	−12%	< 0.05
Baroreflex sensitivity	+14%	+49%	< 0.05

Discussion

In the present study conducted among patients with essential hypertension, cilnidipine produced a significant reduction in blood pressure with the inhibition of sympathetic nerve activity and the improvement of impaired baroreflex control. This study was the first to report that cilnidipine treatment achieved the inhibition of sympathetic nerve activity and the improvement of the impaired baroreflex control in the patients with hypertension. These results suggest that cilnidipine is preferable for the treatment with hypertension among the Ca channel blockers.

Epidemiological studies have demonstrated that a higher heart rate is associated with a long-term risk of cardiovascular mortality, independent of other cardiac risk factors (22). Therefore, anti-hypertensive drugs that do not increase the heart rate would seem to be preferable. It has been reported that the treatment with short-acting Ca channel blockers may not prevent cardiovascular disease (23,24). Accordingly, long-lasting Ca channel blockers that exert less influence on the sympathetic nervous system are now recommended for the treatment of hypertension. Amlodipine and cilnidipine, which were known as long-acting Ca channel blockers, were reported not to increase heart rate. Eguchi et al. (27) reported that cilnidipine did not cause reflex tachycardia, and that cilnidipine, but not amlodipine, significantly decreased the ambulatory BP level without causing an increase in heart rate. In this study, cilnidipine did not increase heart rate, and caused a significant decrease in the LFnuSBP, as the marker of the sympathetic nerve activity. Our results of the sympatho-inhibitory effects of cilnidipine were similar to the previous reports which calculated the sympathetic nerve activity by other methods. From these results, cilnidipine is considered to be the preferable drug with the sympatho-inhibtory effect among the Ca channel blockers.

In this study, BRS was improved in the patients with hypertension treated with high-dose cilnidipine. A previous study reported that BRS values calculated by sequence analysis had reasonable reproducibility when up and down sequences were combined (25), and we measured the BRS by sequence analysis. It has been reported that BRS is impaired in the patients with hypertension (17,26–29), and that BRS is the predictive factor of mortality and cardiovascular events (17). The results of this study suggest that cilnidipine is preferable for the treatment of hypertension among the Ca channel blockers. Previous studies suggested BRS measured by the sequence method was impaired in the patients with hypertension (5–12 ms/mmHg) (27–29), and BRS obtained in this study was considered to be higher compared to that in those previous studies. This difference may be due to the patients' characteristics in this study. The patients in this study had no complications and their hypertension was in early stages.

The mechanisms in which cilnidipine inhibits the sympathetic nerve activity may be due to suppressing the release of catecholamines from sympathetic nerve endings by blocking the N-type calcium channels distributed widely in sympathetic nerves (30). Recent studies have demonstrated the beneficial effect of cilnidipine on cardiac sympathetic nerve activity and cardiovascular morbidity (31–33). Sakata, Yoshida, and Obayashi reported that cilnidipine suppressed cardiac sympathetic overactivity while amlodipine had little suppressive effect (32). The effect of cilnidipine on heart rate might be due to not only long-acting effects but also to a reduction in sympathetic nerve activity. The mechanisms in which cilnidipine improved the BRS has not been determined in this study. Our previous study in animal models indicated that sympatho-inhibition causes the improvement of BRS in hypertensive model rats (26). Further clinical studies are necessary.

There are several limitations in this study. First, this study was a small-size, nonrandomized study. To establish the sympatho-inhibitory effect and improvement of BRS of cilnidipine, a randomized study is required. Second, the ages of the patients in this study were relatively young, and none of them had organ damage due to hypertension. Whether the treatment with cilnidipine causes the beneficial effects in this study in the older and complicated patients with hypertension has not been determined. Third, we determined the effects of cilnidipine on autonomic function for only 6 months, and only at two points, before and after 6 months. The blood pressure-lowering effect of cilnidipine is determined in several days after the initiation of administration (3). In the present study, we examined the effects of cilnidipine on the autonomic function at only two points pre—administration and after 6 months. From the results of the present study, we have not determined whether the mechanisms of the action of cilnidipine is similar between several days and 6 months after the initiation of cilnidipine. Furthermore, the effects of cilnidipine on the autonomic function for longer periods must be determined.

Conclusion

The treatment with cilnidipine for essential hypertension produced a significant reduction in blood pressure with the inhibition of sympathetic nerve activity and the improvement of impaired baroreflex control. These results suggest that cilnidipine is preferable for the treatment of hypertension among the Ca channel blockers.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Staessen JA, Wang JG, Thijs L. Cardiovascular prevention and blood pressure reduction: a quantitative overview updated until March 2003. *J Hypertens.* 2003;21:1055–1076.
2. Yoshimoto R, Hashiguchi Y, Dohmoto H, Hosono M, Iida H, Fujiyoshi T, Ikeda K, Hayashi Y. Effects of a new dihydropyridine derivative, FRC-8653, on blood pressure in conscious spontaneously hypertensive rats. *J Pharmacobio Dyn* 1992;15:25–32.
3. Tominaga M, Phya Y, Tsukashima A, Kobayashi K, Takata Y, Koga T, Yamashita Y, Fujishima Y, Fujishima M. Ambulatory blood pressure monitoring in patients with essential hypertension treated with a new calcium antagonist, cilnidipine. *Cardiovasc Drugs Ther* 1997;11:43–48.
4. Hosono M, Fujii S, Hiruma T, Watanabe K, Hayashi Y, Ohnishi H, Takata Y, Kato H. Inhibitory effect of cilnidipine on vascular sympathetic neurotransmission and subsequent vasoconstriction in spontaneously hypertensive rats. *Jpn J Pharmacol* 1995;69:127–134.

5. Oike M, Inoue Y, Kitamura K, Kuriyama H. Dual action of FRC8653, a novel dihydropyridine derivative, on the Ba2+ current recorded from the rabbit artery. *Circ Res* 1990;57:993–1006.
6. Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. Effect of cilnidipine, a new dihidropyrydine Ca++ channel antagonist, on N-type Ca++ channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* 1997;280:1184–1191.
7. Uneyama H, Takahara A, Dohmoto H, Yoshimoto R, Inoue K, Akaike N. Blockade of N-type Ca++ current by cilnidipine (FRC-8653) in acutely dissociated rat sympathetic neurons. *Br J Pharmacol* 1997;122:37–42.
8. Mancia G, Parati G, Castiglioni P, Di Rienzo M. Effect of sinoaortic deervation on frequency-domain estimates of baroreflex sensitivity in conscious cats. *Am J Physiol* 1999;276:H1987–H1993.
9. Laude D, Elghozi JL, Girard A, Bellard E, Bouhaddi M, Castiglioni P, Cerutti C, Cividjian A, Di Rienzo M, Fortrat JO, Janssen B, Karemaker JM, Leftheriotis G, Parati G, Persson PB, Porta A, Quintin L, Regnard J, Rudiger H, Stauss HM. Comparison of various techniques used to estimate spontaneous baroreflex sensitivity. *Am J Physiol* 2004;286:R226–R231.
10. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation, and clinical ise. *Eur Heart J* 1996;17:354–381.
11. Lucini D, Meta GS, Malliani A, Pagani M. Impairment in cardiac autonomic regulation preceding arterial hypertension in humans: insights from spectral analysis of beat-by-beat cardiovascular variability. *Circulation* 2002;106:2673–2679.
12. Radaelli A, Perlangeli S, Cerutti MC, Mircoli L, Mori I, Boselli K, Bonaita M, Terzoli L, Candotti G, SIgnorini G, Ferrari AU. Altered blood pressure variability in patients with congestive heart failure. *J Hypertens* 1999;17:1905–1910.
13. Castiglioni P, Di Rienzo M, Veicsteinas A, Parati G, Merati G. Mechanisms of blood pressure and heart rate variability: an insight from low-level paraplegia. *Am J Physiol* 2007;292: R1502–R1509.
14. Latinen T, Hartikainen J, Vanninen E, Niskanen L, Geelen G, Lansimies E. Age and gender dependency of baroreflex sensitivity in healthy subjects. *J Appl Physiol* 1998;84:576–583.
15. Mortara A, La Rovere MT, Pinna GD, Prpa A, Maestri R, Fedo O, Pozzoli M, Opasich C, Tavazzi L. Arterial baroreflex modulation of heart rate in chronic heart failure: clinical and hemodynamic correlates and prognostic implications. *Circulation* 1997;96:3450–3458.
16. Farrell TG, Odemuyiwa O, Bashir Y, Gripps TR, Malik M, Ward DE, Camm AJ. Prognostic value of baroreflex sensitivity testing after acute myocardial infarction. *Br Heart J* 1992;67: 129–137.
17. Hesse C, Charkoudian N, Liu Z, Joyner MJ, Eisenach JH. Baroreflex sensitivity inversely correlates with ambulatory blood pressure in healthy normotensive humans. *Hypertension* 2007;50: 41–46.
18. Imholz BP, Wielong W, Langewouters GJ, van Montfrans GA. Continuous finger arterial pressure: utility in the cardiovascular laboratory. *Clin Auton Res* 1991;l1:43–53.
19. Imholz BP, Wieling W, van Montfrans GA, Wesseling KH. Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. *Cardiovasc Res* 1998;38:605–616.
20. Waki H, Kasparov S, Wong LF, Murphy D, Shimizu T, Paton JFR. Chronic inhibition of eNOS activity in nucleus tractus solitarii enhances baroreceptor reflex in conscious rats. *J Physiol* 2003;546:233–242.
21. Waki H, Katahira K, Polson JW, Kasparov S, Murphy D, Paton JFR. Automation of analysis of cardiovascular autonomic function from chronic measurements of arterial pressure in conscious rats. *Exp Physiol.* 2006;91:201–213.
22. Gillman MW, Kannel WB, Belanger A, D'Agostuno RB. Influence of heart rate on mortality among patients with hypertension: the Framingham Study. *Am Heart J* 1993;125:1148–1154.
23. Furberg CD, Psaty BM, Meyer JV. Nifedipine: Dose-related increase in mortality in patients with coronary heart disease. *Circulation* 1995;92:1326–1331.
24. Psaty BM, Heckbert SR, Koepsell TD. The risk of myocardial infarction associated with antihypertensive drug therapies. *JAMA* 1995;274:620–625.

25. Johnson P, Shore A, Potter J, Panerai R, James M. Baroreflex sensitivity measured by spectral and sequence analysis in cerebrovascular disease. *Clin Auton Res* 2006;16:270–275.
26. Kishi T, Hirooka Y, Kimura Y, Sakai K, Ito K, Shimokawa H, Takeshita A. Overexpression of eNOS in RVLM improves impaired baroreflex control of heart rate in SHRSP. *Hypertension* 2003;41:255–260.
27. Eguchi K, Tomizawa H, Ishikawa J, Hoshide S, Pickering TG, Shimada K, Kario K. Factors associated with baroreflex sensitivity: associated with morning blood pressure. *Hypertens Res* 2007;30:723–728.
28. Milan A, Caserta MA, Del Colle S, Dematteis A, Morello F, Rabbia F, Mulatero P, Pandian NG, Veglio F. Baroreflex sensitivity correlates with left ventricular morphology and diastolic function in essential hypertension. *J Hypertens* 2007;25:1655–1664.
29. Eguchi K, Tomizawa H, Ishikawa J, Hoshide S, Fukuda T, Numao T, Shimada K, Kario K. Effects of new calcium channel blocker, azelnidipine, and amlodipine on baroreflex sensitivity and ambulatory blood pressure. *J Cardiovasc Pharmacol* 2007;49:394–400.
30. Yamagishi T. Beneficial effect of cilnidipine on morning hypertension and white-coat effect in patients with essential hypertension. *Hypertens Res* 2006;29:339–344.
31. Sakaki T, Naruse H, Masai M. Cilnidipine as an agent to lower blood pressure without sympathetic nervous activation as demonstrated by iodine-123 metaiodobenzyl guanidine imaging in rat heart. *Ann Nucl Med* 2003;17:321–326.
32. Sakata K, Yoshida H, Obayashi K. Comparative effect of cilnidipine and quinapril on left ventricular mass in mild essential hypertension. *Drugs Exp Clin Res* 2003;29:117–123.
33. Nagai H, Minatoguchi S, Chen XH. Cilnidipine, an N+L-type dihydropyridine Ca channel blocker, suppresses the occurrence of ischemia/reperfusion arrhythmia in a rabbit model of myocardial infarction. *Hypertens Res* 2005;28:361–368.

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Therapeutic Neovascularization by Nanotechnology-Mediated Cell-Selective Delivery of Pitavastatin Into the Vascular Endothelium

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Therapeutic Neovascularization by Nanotechnology-Mediated Cell-Selective Delivery of Pitavastatin Into the Vascular Endothelium

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Objective—Recent clinical studies of therapeutic neovascularization using angiogenic growth factors demonstrated smaller therapeutic effects than those reported in animal experiments. We hypothesized that nanoparticle (NP)-mediated cell-selective delivery of statins to vascular endothelium would more effectively and integratively induce therapeutic neovascularization.

Methods and Results—In a murine hindlimb ischemia model, intramuscular injection of biodegradable polymeric NP resulted in cell-selective delivery of NP into the capillary and arteriolar endothelium of ischemic muscles for up to 2 weeks postinjection. NP-mediated statin delivery significantly enhanced recovery of blood perfusion to the ischemic limb, increased angiogenesis and arteriogenesis, and promoted expression of the protein kinase Akt, endothelial nitric oxide synthase (eNOS), and angiogenic growth factors. These effects were blocked in mice administered a nitric oxide synthase inhibitor, or in eNOS-deficient mice.

Conclusions—NP-mediated cell-selective statin delivery may be a more effective and integrative strategy for therapeutic neovascularization in patients with severe organ ischemia. (***Arterioscler Thromb Vasc Biol.* 2009;29:796-801.)**

Key Words: nanotechnology ■ drug delivery system ■ statin ■ therapeutic neovascularization

Restoration of tissue perfusion in patients with critical ischemia attributable to coronary artery disease and peripheral artery disease is a major therapeutic goal. Recently, double-blind placebo-controlled clinical trials designed to induce neovascularization by administering exogenous angiogenic growth factors failed to demonstrate a clinical benefit and produced some undesired side effects.[1,2] These nonoptimal clinical results were in contrast to the results obtained in animal experiments and small open-label clinical trials.[3,4] The disappointing results of the clinical trials of therapeutic angiogenesis may be attributable in part to less effective transfection of the genetic materials or the rapid washout of proteins. In addition, because the involvement of multiple endogenous angiogenic growth factors is required for the development of functional collaterals,[5,6] the strategy of simple intramuscular injection of an exogenous angiogenic growth factor is limited. A high local concentration of angiogenic growth factors increases the risks of edema,[3,7] angioma-like capillary formation,[7–9] atherosclerosis after vascular injury,[10–13] and tumor-angiogenesis.[7,8] A controlled drug delivery system (DDS) for an integrative approach to therapeutic neovascularization would be more favorable.

To address this challenge, we developed a novel nanoparticle (NP)-mediated DDS, formulated from the bioabsorbable polylactide/glycolide copolymer (PLGA).[14] The PLGA NP offers the advantages of safety, delivery of encapsulated drugs into the cellular cytoplasm, and slow cytoplasmic drug release.[14,15] PLGA NP are effectively and rapidly taken up by vascular endothelial cells in vitro.[16] To our knowledge, however, no prior studies have examined whether PLGA NPs are useful as an endothelial cell-selective DDS in vivo.

We hypothesized that HMG-CoA reductase inhibitors, so-called statins, are appropriate candidate drugs for this integrative approach, because statins have a variety of pleiotropic vasculoprotective effects that are independent of their lipid-lowering activity.[17] Statins increase the angiogenic activity of mature endothelial cells as well as that of endothelial progenitor cells (EPCs)[18,19] and augment collateral growth in ischemic heart and limb in experimental animals.[20,21] In addition, statins attenuate atherosclerosis formation[22,23] and have little potential risk of tumor angiogenesis in contrast to angiogenic growth factors.[24] Most of these beneficial effects of statin on therapeutic neovascularization, however, were observed after daily administration of high doses,[18–21] which

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may lead to serious adverse side effects in a clinical setting. Because vascular endothelium plays a primary role in the pathogenesis of ischemia-induced neovascularization, we hypothesized that NP-mediated cell-selective delivery of statins to the vascular endothelium would more effectively and integratively induce therapeutic neovascularization.

The major aim of this study was to test the hypothesis that selective NP-mediated delivery of statins to endothelial cells can be an integrative approach to enhance therapeutic neovascularization. We used a murine model of hindlimb ischemia to examine, (1) whether PLGA NPs are delivered selectively to vascular endothelial cells in ischemic tissues; and (2) whether NP-mediated delivery of statin is useful for increasing therapeutic neovascularization.

Materials and Methods

Preparation of PLGA NPs

Anionic PLGA NPs encapsulated with fluorescein isothiocyanate (FITC) or pitavastatin were prepared by a previously reported emulsion solvent diffusion method in purified water. The diameter of the PLGA NPs was 196±29 nm. The PLGA NPs had a negative surface charge (-15±10 mV). The FITC- and pitavastatin-loaded PLGA NPs contained 5% (wt/vol) FITC and 5% (wt/vol) pitavastatin, respectively. Additional details are provided in the supplemental information (please see http://atvb.ahajournals.org.).

Intracellular Uptake and Intracellular Distribution of NPs

Human umbilical vein endothelial cells (HUVECs) were obtained and cultured in EGM-2. Human skeletal muscle cells (SkMCs) were obtained and cultured in SkGM. Additional details can be found in the supplemental information.

Angiogenesis Assay of Human Endothelial Cells

Angiogenesis assay of human endothelial cells was tested using a 2-dimensional Matrigel assay. Additional details are provided in the supplemental information.

Animal Preparation and Experimental Protocol

Male 8-week-old C57BL/6J wild-type mice were used. After anesthesia, we induced unilateral hindlimb ischemia in the mice as previously described.[25] Immediately after the induction of ischemia, animals were randomly divided into 4 groups; a control no treatment group and the remaining groups received intramuscular injections of FITC-NPs (NP group), pitavastatin at 0.4 mg/kg (statin only group), or pitavastatin-NPs containing 0.4 mg/kg pitavastatin (statin-NP group) into the left femoral and thigh muscles. Biochemical parameters listed in supplemental Table I were measured 3, 7, and 14 days after treatment. Additional details are provided in the supplemental information.

Limb blood flow measurements were performed using a laser Doppler perfusion imaging (LDPI) analyzer (Moor Instruments). The LDPI index was expressed as the ratio of the LDPI signal in the ischemic limb compared to that in the normal limb.[25]

Histological and Immunohistochemical Analyses

Histological and immunohistochemical evaluation was performed. To determine capillary and arteriolar density, cross sections were stained with anti-mouse platelet endothelial cell adhesion molecule (PECAM)-1 antibody (CD31) and α-smooth muscle actin (α-SMA), respectively. Additional details are provided in the supplemental information.

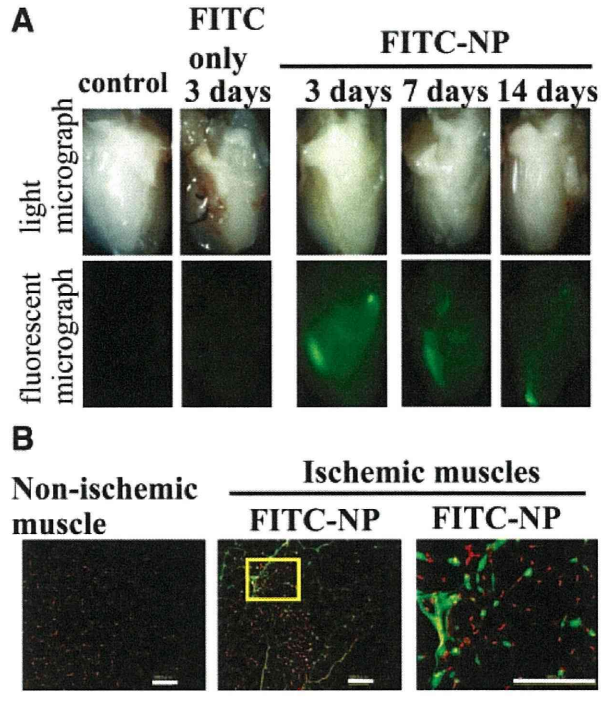

Figure 1. A, Representative light and fluorescent stereomicrographs of gastrocnemius muscles from control nonischemic hindlimb and from ischemic hindlimb. B, Fluorescent micrographs of cross-sections from nonischemic muscle with no injection, ischemic muscles 14 days after the injection of FITC-NP, and expanded view of boxed area of middle panel. Scale bars: 100 μm.

Western Blotting

Protein expression of Akt, eNOS, VEGF, FGF-2, and MCP-1 was examined 7 days after the induction of hindlimb ischemia. Additional details are provided in the supplemental information.

Flow Cytometric Analyses of EPC Mobilization

Peripheral blood was obtained from mice 7 and 14 days after hindlimb ischemia and analyzed with a FACS Caliber flow cytometer (Becton Dickinson). Additional details are provided in the supplemental Information.

Measurements of Statin Concentration in Serum and Muscle Tissue

Statin concentration in serum and muscle were measured at predetermined time points using a column-switching high performance liquid chromatography system. Additional details are provided in the supplemental information.

Statistical Analysis

Data are expressed as means±SEM. The statistical analysis was assessed using analysis of variance and multiple comparison tests. Probability values less than 0.05 were considered to be statistically significant.

Results

Cell-Selective Delivery of NPs In Vivo

Cellular distribution of FITC was examined 3, 7, and 14 days after intramuscular injection of FITC-NP or FITC only. On day 3 postinjection, strong FITC signals were detected only in FITC-NP injected ischemic muscle, whereas no FITC signals were observed in control nonischemic muscle or in ischemic muscle injected with FITC only (Figure 1A). The FITC