Fluvastatin Reduces Pulmonary Vein Spontaneous Activity Through Nitric Oxide Pathway

YU-FENG HU, M.D.,* YAO-CHANG CHEN, M.Sc.,† CHEN-CHUAN CHENG, M.D.,‡ SATOSHI HIGA, M.D., PH.D.,§ YI-JEN CHEN, M.D., PH.D.,¶ and SHIH-ANN CHEN, M.D.*

From the *Division of Cardiology and Cardiovascular Research Center, Veterans General Hospital-Taipei; School of Medicine, National Yang-Ming University, Taipei, Taiwan; †Department of Biomedical Engineering, National Defense Medical Center, Taipei, Taiwan; ‡Division of Cardiology, Chi-mei Medical Center, Tainan, Taiwan; §Second Department of Internal Medicine, University of the Ryukyus, Japan; ¶Division of Cardiovascular Medicine, Taipei Medical University-Wan Fang Hospital; Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan

HMG-CoA Reductase Inhibitors on Pulmonary Veins. ***Introduction:*** **Pulmonary veins (PVs) are the most important focus for the generation of atrial fibrillation. The HMG-CoA reductase inhibitors (statins) can reduce the occurrence of atrial fibrillation. The purposes of this study were to evaluate whether statins may inhibit the PV arrhythmogenic activity to prevent atrial arrhythmias from PVs and to investigate the link between fluvastatin, nitric oxide synthase (NOS) activity, mechanical activity, and electrical activity.**

Methods: **Conventional microelectrodes and Western blot were used to record the electrical activity, diastolic tension, contractility and expression of Akt, endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and phosphorylated Akt and eNOS before and after the administration of fluvastatin in rabbit PVs or atria.**

Results: **Fluvastatin decreased the PV spontaneous activity, diastolic tension, and contractility, but did not change the action potential duration or resting membrane potential. The effects of fluvastatin on the PV firing rate and diastolic tension were attenuated in the presence of L-NAME (100 μM), wortmannin (100 nM), and ODQ (3 μM). Fluvastatin (1 μM) increased the phosphorylated Akt and eNOS, but did not change the total Akt or eNOS in the PVs and atria. In contrast, fluvastatin (1 μM) decreased the total nNOS in the PVs and atria.**

Conclusions and implications: **Fluvastatin produced nitric oxide through the PI3kinase/Akt pathway, thus reducing the PV vascular diastolic tension and PV spontaneous activity. These results may contribute to the beneficial effects of statins.** *(J Cardiovasc Electrophysiol, Vol. 20, pp. 200-206, February 2009)*

atrial fibrillation, statins, nitric oxide, pulmonary veins, arrythmogenesis

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia observed in clinical practice and induces cardiac dysfunction and strokes. The use of statins (3-hydroxyl-3-methyl coenzyme A [HMG-CoA] reductase inhibitors) has been found to reduce the occurrence of AF.[1] However, the mechanisms underlying the antiarrhythmic effects of statins are not clear. Previous studies have suggested that statins may prevent AF through the antioxidation or anti-inflammation effects.[2-4] Moreover, statins may have electrophysiological effects on cardiomyocytes. Statins could alter Kv channel activity of cardiomyocyte,[5] or change the expression of Na^+/Ca^{2+} exchanger.[6] All of these findings suggest that statins may reduce AF through the effects beyond the antioxidation and anti-inflammation effects.

The pulmonary veins (PVs) have been demonstrated to be an important source of the initiation of AF and also to play a role in the maintenance of AF.[7,8] The PVs have been known to contain cardiomyocytes with pacemaker activity and have a arrhythmogenic potential for inducing atrial arrhythmias due to spontaneous activity.[9] It was reported that dissociated pulmonary vein activity that was suggestive of automatic mechanism might be responsible for AF initiation and maintenance.[10,11] Since the PVs play a critical role in the pathophysiology of AF, statins may alter the PV electrical activity and thus reduce the AF. Previous studies have shown that mechanoelectrical feedback (mechanical stretch on the atrial wall activating the ion channels and modifying current flow) plays an important role in the PV arrhythmogenic activity.[12-15] Statins can enhance the nitric oxide production[16,17] and reduce the vascular tension in the PVs with a decrease in the PV arrhythmogenesis due to mechanoelectrical feedback. Moreover, it has been reported that a nitric oxide donor, *S*-nitroso-*N*-acetylpenicillamine, suppressed PV electrical activity, and the nitric oxide synthesis inhibitor (Nomega-nitro-L-arginine) increased phasic electrical activity and tone,[18] which further supports the possible antiarrythmic effect of statins through nitric oxide. The

The present work was supported by the Center of Excellence for Clinical Trial and Research in Wan Fang Hospital (DOH-TD-B-111-002) and grants NSC 95-2314-B-016-015, NSC 95-2314-B-038-026, NSC 96-2628-B-038-012-MY3, NSC 96-2314-B-010-006, V95 A-008, and 96CM-TMU-07 from Chi-Mei Medical Center.

Address for correspondence: Yi-Jen Chen, M.D., Ph.D., Division of Cardiovascular Medicine, Taipei Medical University-Wan Fang Hospital, 111, Hsin-Lung Road, Sec. 3, Taipei, Taiwan. Fax: +886-2-29339378, +886-2-28735656; E-mail: a9900112@ms15.hinet.net

Manuscript received 15 March 2008; Revised manuscript received 10 June 2008; Accepted for publication 1 July 2008.

doi: 10.1111/j.1540-8167.2008.01281.x

production of nitric oxide can be due to the activation of different isoforms of nitric oxide synthase such as endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS).[19,20] Therefore, the purpose of this study was to investigate whether fluvastatin may alter the PV arrhythmogenic activity and to evaluate the underlying mechanisms.

Methods

Rabbit PV Tissue Preparations

The investigation conformed to the institutional Guide for the Care and Use of Laboratory Animals. Rabbits (weight, 1–2 kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed and the heart with the lungs was removed. For dissection of the PVs, the left atrium was opened by an incision along the mitral valve annulus extending from the coronary sinus to the septum in the Tyrode's solution with a composition (in mM) of 137 NaCl, 4 KCl, 15 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $mgCl_2$, 2.7 $CaCl_2$, and 11 dextrose. The PVs were separated from the atrium at the left atrium–PV junction and separated from the lungs at the ending of the PV myocardial sleeves. One end of the preparation, consisting of the PVs and atrium–PV junction, was pinned with needles to the bottom of a tissue bath. The other end was connected to a Grass FT03 C force transducer with a silk thread. The adventitia of the PVs faced upward. The tissue was superfused at a constant rate (3 mL/min) with the Tyrode's solution that was saturated with a 97% O_2–3% CO_2 gas mixture. The temperature was maintained constant at 37°C and the preparations were allowed to equilibrate for 1 hour before the electrophysiological study.

Electrophysiological and Pharmacological Studies

The transmembrane action potentials (AP) of the PVs and left atrial appendage were recorded by means of machine-pulled glass capillary microelectrodes filled with 3 M of KCl, and the PV preparation was connected to a WPI model FD223 electrometer under tension with 150 mg. The electrical and mechanical events (contractility and diastolic tension) were displayed and recorded simultaneously on a Gould 4072 oscilloscope and Gould TA11 recorder. The signals were recorded with DC coupling and a 10 kHz low-pass filter cut-off frequency using a data acquisition system. Signals were recorded digitally with a 16-bit accuracy at a rate of 125 kHz. Electrical stimuli with a 10-ms duration and suprathreshold strength (30% above the threshold) were provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. The PV and atrial preparations were treated with and without (control group) fluvastatin (0.1 and 1.0 μM) for at least 2 hours. Moreover, we evaluated whether fluvastatin could produce nitric oxide and cGMP through the induction of the phosphorylation of the eNOS via the PI3 K/Akt pathway. Fluvastatin was administrated in the presence of a nitric oxide synthase inhibitor (L-NAME, 100 μM), phosphatidylinositol 3-kinase (PI3 K) inhibitor (wortmannin, 100 nM), or guanylate cyclase inhibitor (ODQ, 3 μM) for 2 hours, respectively. The 90% and 50% AP durations (APD_{90}, APD_{50}), membranous diastolic potential and contractile force were measured during 2 Hz electrical stimuli before and after the drug administration.

Western Blot Analysis

The left atrial appendage and PV tissue samples were incubated in the oxygenated Tyrode's solution with or without fluvastatin (1 μM) for 2 hours at 37 °C. Tissue samples were washed in ice-cold saline and rapidly frozen in liquid nitrogen and homogenized in a lysis buffer containing 1 M Tris–HCl (pH 7.4), 0.25 M sucrose, 0.5 M EDTA (pH 8.0), 100 mM dithiothreitol, 100 mM phenylmethysulfonyl fluoride, apotinin (2.2 mg/mL), and leupeptin (5 mg/mL). The homogenate was centrifuged at 12,000 *g* for 30 min at 4°C. The protein extracts were applied to 10% SDS–PAGE and blotted on a polyvinylidene diflouride membrane. The blotted polyvinylidene diflouride membrane was incubated with a phosphate-buffered saline/Tween-20 (0.05%) solution containing 2% skim milk for blocking nonspecific antigens and incubated with monoclonal antibodies, respectively, at room temperature for 1 hour as below: goat eNOS antibody (R&D Systems, Minneapolis, MN, USA), mouse phospho-eNOS antibody (Ser1177) (BIOMOL International, Philadelphia, PA, USA), mouse Akt antibody (BIOMOL International), mouse phosphor-Akt (Ser 473) (BD Pharmingen™, San Jose, CA, USA), and goat nNOS antibody (R&D Systems). After the primary antibody reaction, the membranes were incubated with a secondary antibody conjugated with horseradish peroxidase, detected by the ECL detection system (SantaCruz Biotechnology), and exposed to film (BioMax MR; Kodak). Targeted bands were normalized to cardiac α-sarcomeric actin (Sigma-Aldrich Corp.) to confirm an equal protein loading.

Statistical Analysis

All quantitative data are expressed as the mean ± SEM. The paired Student's *t*-test was used to compare the differences before and after the drug administration. An unpaired *t*-test was used to compare the differences between the preparations with and without incubation with statins. Inverse regression was used to evaluate the correlation between the diastolic tension and PV firing rates. A P-value lower than 0.05 was considered statistically significant.

Results

Effects of Fluvastatin on the PV Electrophysiology and Contractility

In the PVs with spontaneous activity, fluvastatin (1 μM) administration for 30 minutes decreased the PV firing rates ($P < 0.05$, Fig. 1A,B), but fluvastatin at 0.1 μM did not change the PV firing rates ($P > 0.05$, Fig. 1A). Fluvastatin (1 μM, not 0.1 μM) administration for 1 hour decreased the diastolic tension (Fig. 1C). Those effects became significant after an administration for 30 minutes and reached a steady state after an administration for 1 hour. As the results show in Figure 1D, the diastolic tension correlated well with the PV firing rates in an inverse regression.

In the PVs without spontaneous activity, fluvastatin (0.1 μM and 1 μM) administration for 30 minutes decreased the PV contractile force ($P < 0.05$, Fig. 2A). However, the effects were similar for the fluvastatin at concentrations of 0.1 μM, and 1 μM ($P > 0.05$, Fig. 2A,B). Figure 2C shows the tracings of the AP before and after the administration of fluvaststin. Fluvastatin did not change the APD_{50}

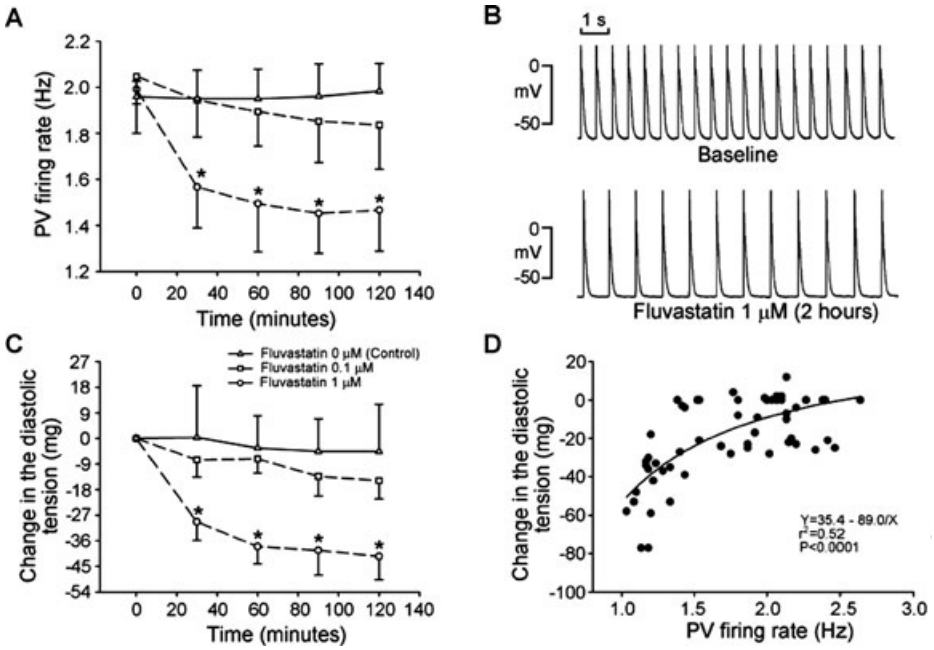

Figure 1. *Effects of fluvastatin on the PV electrical activity in the PVs with spontaneous activity. Panel (A) shows the PV spontaneous activity from the fluvastatin (0.1 μM and 1.0 μM, n = 5) treatment and control group (fluvastatin 0 μM, n = 3). Panel (B) shows the tracings of PV spontaneous activity before and after fluvastatin (1.0 μM) administration. Panel (C) shows the effects of fluvastatin (0.1 μM and 1.0 μM, n = 5) and control group (fluvastatin 0 μM, n = 3) on the vascular diastolic tension. Panel (D) shows the inverse regression of the PV firing rates and vascular diastolic tension (n = 10). *P < 0.05 versus before the fluvastatin administration.*

at concentrations of 0.1 μM (from 27 $\pm$ 3 ms to 27 $\pm$ 3 ms, P $>$ 0.05) or 1 μM (from 29 $\pm$ 2 ms to 28 $\pm$ 5 ms, P $>$ 0.05). Fluvastatin also did not change the APD_{90} at concentrations of 0.1 μM (from 96 $\pm$ 8 ms to 92 $\pm$ 5 ms, P $>$ 0.05) and 1 μM (from 97 $\pm$ 8 ms to 93 $\pm$ 7 ms, P $>$ 0.05). Additionally, fluvastatin did not change the resting membrane potential at concentrations of 0.1 μM (from $-$73 $\pm$ 2 mV to $-$73 $\pm$ 3 mV, P $>$ 0.05) and 1 μM (from $-$75 $\pm$ 1 mV to $-$74 $\pm$ 2 mV, P $>$ 0.05).

Effects of Fluvastatin on the Left Atrial Electrical Activity

In the left atrium, fluvastatin (0.1 μM) decreased the atrial contractility (P $<$ 0.05, Fig. 3A,B). Similar to that in the PVs, fluvstatin did not change the APD_{50} (24 $\pm$ 4 ms versus 24 $\pm$ 2 ms, P $>$ 0.05), APD_{90} (80 $\pm$ 4 ms versus 84 $\pm$ 3 ms, P $>$ 0.05), or the resting membrane potential ($-$79 $\pm$ 3 mV, versus $-$78 $\pm$ 1 mV, P $>$ 0.05, Fig. 3C).

Interactions of L-NAME, Wortmannin, ODQ, and Fluvastatin

In the presence of L-NAME (100 μM), fluvastatin (1 μM) did not change the PV firing rates (Fig. 4A), or diastolic tension (Fig. 4C). As the example shows in Figure 4B, in the presence of wortmannin (100 nM), fluvastatin (1 μM) did not change the PV firing rates or diastolic tension (Fig. 4A,C). Similarly, in the presence of ODQ (3 μM), fluvastatin (1 μM) did not change the PV firing rates or diastolic tension (Fig. 4A,C).

Effects of Fluvastatin on the Expression of nNOS and eNOS

The baseline value of the total protein in the PVs and atrium showed no significant differences between the protein level of the Akt, phosphor-Akt, eNOS, and phospho-eNOS. Only the protein level of the nNOS was higher in the atrium than in the PVs. An incubation of fluvastatin (1 μM) for 2 hours increased the phosphorylation of the Akt and eNOS both in the PVs and atrium (Fig. 5A,B). However, the expressions of the total Akt and eNOS between the PVs or atrium with and without incubation with fluvstatin were similar. Moreover, the incubation with fluvastatin (1 μM) for 2 hours decreased the protein level of the nNOS.

Discussion

Statins Regulate the PV Arrhythmogenesis Through Nitric Oxide-Related Mechanoelectrical Feedback

In this study, for the first time, we demonstrated that fluvastatin may decrease the PV firing rates and diastolic tension or contractility in the PVs and atrium. However, the present study showed that statins have little effect on the AP duration and resting membrane potential in the PVs and atrium. Those findings indicated that statins may regulate the PV electrical activity through a mechanoelectrical feedback. Moreover, the considerable correlation between the PV firing rates and diastolic tension also suggested that the statins decreased the PV firing rates through a mechanoelectrical feedback. It has been reported that increasing atrial pressure leads to a stretch of the atrium and PVs, and increases the vulnerability for AF and PV arrythmogenesis.[12-15] Therefore, through the effects of vasodilation, statins may relieve the stretch, which could decrease the PV firing rates. Since statins can cause vasodilation by enhancing the endothelium-derived nitric oxide and elevating the cGMP levels,[16,17] we evaluated whether statins could increase the nitric oxide level to regulate the PV electrical activity. The presence of L-NAME may attenuate the effects of statins on the PVs, which confirms that nitric oxide modulates the mechanoelectrical effects of statins in the PVs. These results may also account for the reduction in the contractility of the PVs and atrium because nitric oxide has been found to reduce the contractility in vessels and the myocardium, too.[17,20,21] Different from the insignificant effect of statins on the diastolic tension, fluvastatin at 0.1 μM can decrease contractility of PVs and atrium. These differences suggest that cardiomyocytes and vascular smooth muscle might have different sensitivity to fluvastatin. Furthermore, ODQ and wortmannin also reduced the effects of the statins,

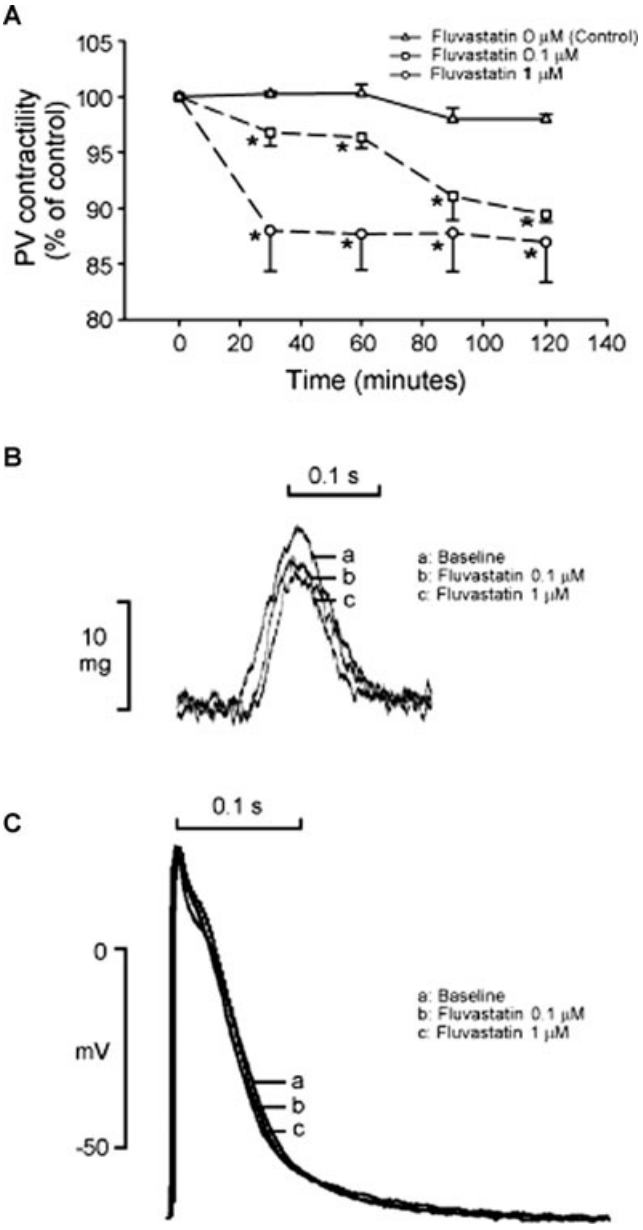

Figure 2. *Effects of fluvastatin on the PVs without spontaneous activity. Panel (A) shows the PV contractility from the fluvastatin (0.1 μM and 1.0 μM, n = 5) treatment and control group (fluvastain 0 μM, n = 3). Panel (B) shows the superimposed tracings of the PV contractility before and after the administration of fluvastatin. Panel (C) shows the superimposed tracings of the AP configuration before and after the administration of fluvastatin.* $^{*}P < 0.05$ *versus before the fluvastatin administration.*

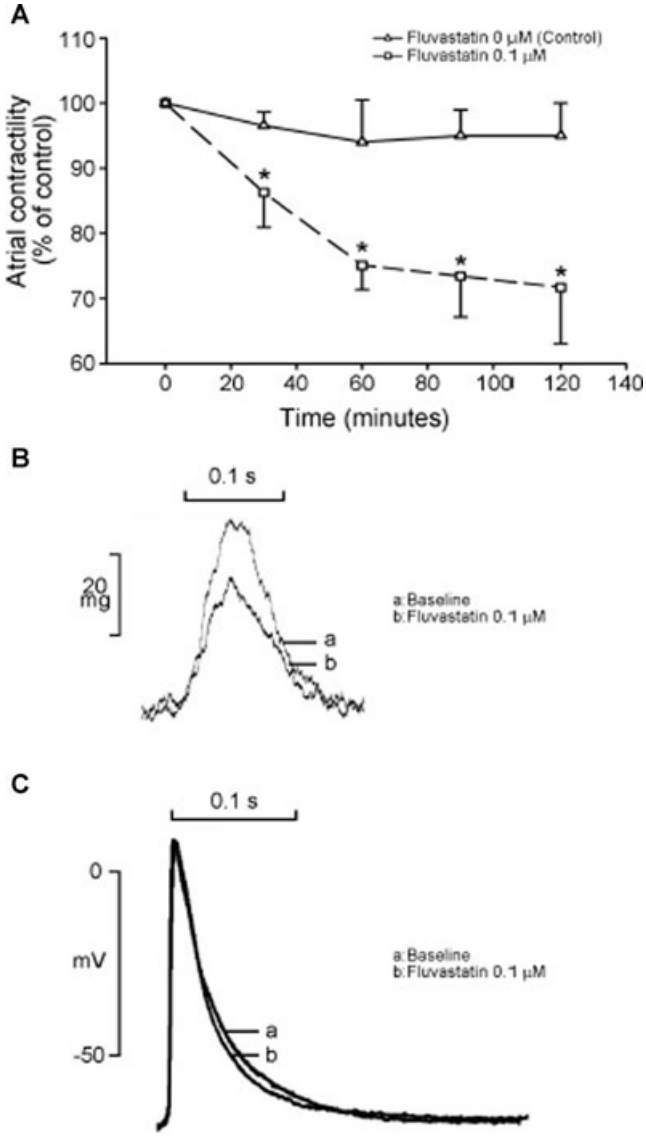

Figure 3. *Effects of fluvastatin on the atrial electrical activity. Panel (A) shows the atrial contractility from the fluvastatin (0.1 μM and 1.0 μM, n = 5) treatment and control group (fluvastatin 0 μM, n = 3). Panel (B) shows the superimposed tracings of the atrial contractility before and after the administration of fluvastatin. Panel (C) shows the superimposed tracings of the AP configuration before and after the administration of fluvastatin.* $^{*}P < 0.05$ *versus before the fluvastatin administration.*

which further suggested that the mechanoelectrical feedback through the nitric oxide-cGMP pathway played a key role in the antiarrythmogenesis of statins. Since our study suggests that a link between a statin-induced nitric oxide mediated mechanism for the suppression of PV electrical activity, the addition of an nitric oxide-releasing moiety to the structure of pravastatin and fluvastatin may have more anti-AF potential.[22] These new drugs released nitric oxide slowly and had increased anti-inflammatory effects relative to their parent molecule.

Nitric oxide was reported to shorten the AP duration through inhibiting the calcium currents and enhancing the potassium currents.[23] An increasing stretch of the PVs was reported to shorten the AP duration, and AP will lengthen after the relief of PV tension.[13] The balance of influence of AP duration between the direct ion effect of nitric oxide and the relief of stretch explains why the AP duration did not change. In this study, the dosage of fluvastatin is close to the known serum concentration of fluvastatin in humans.[24] Therefore, our findings might be clinically relevant as they support the concept that statins might have anti-AF effects *in vivo*.

Statin Activates eNOS Through PI3 K/Akt Pathway

There are three cardiac isoforms of NOS: NOS1 (neuronal NOS), NOS2 (inducible NOS), and NOS3 (endothelial NOS), where NOS2 is the inducible one. A previous study

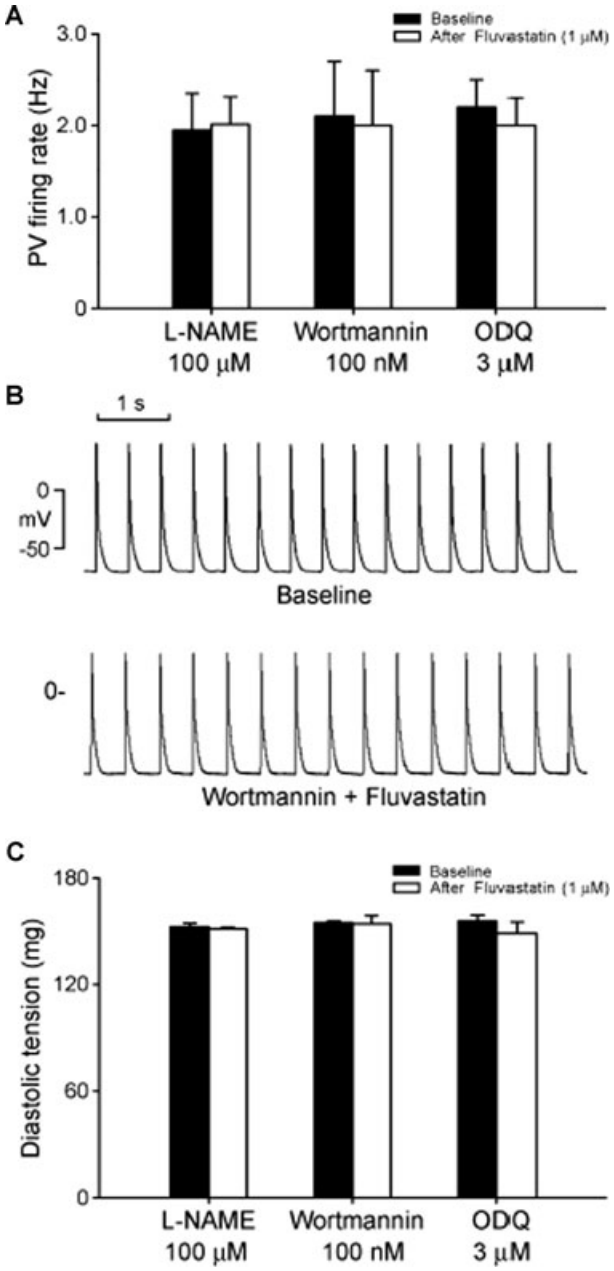

Figure 4. *Interactions of L-NAME, wortmannin, and ODQ on fluvastatin-induced electrical activity in the PVs with spontaneous activity. Panel (A) shows that fluvastatin (1 μM) did not change the PV firing rates in the presence of L-NAME (100 μM, n = 3), wortmannin (100 nM, n = 4), or ODQ (10 μM, n = 4). Panel (B) shows the tracings in which the PV firing rate was not changed by fluvastatin in the presence of wortmannin. Panel (C) shows that fluvastatin (1 μM) did not change the diastolic tension in the presence of L-NAME (100 μM, n = 3), wortmannin (100 nM, n = 4), or ODQ (10 μM, n = 4).*

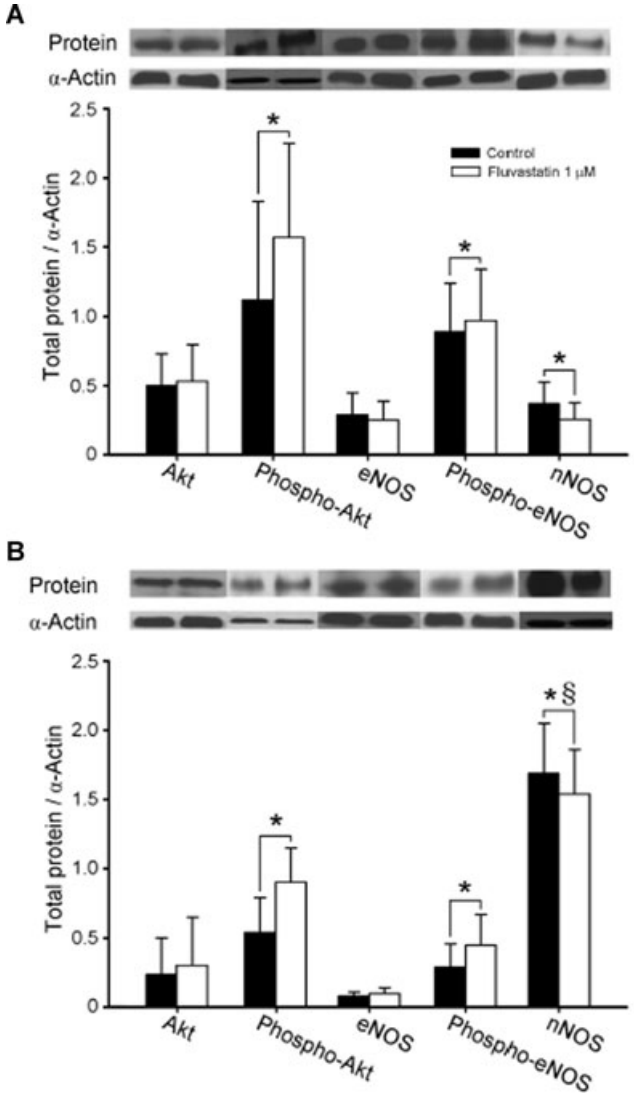

Figure 5. *Western blot analysis of the PVs and atrium after the incubation of fluvastatin (1 μM) for 2 hours. Panel (A) shows that the phosphorylation of Akt and eNOS (n = 5) was increased without changes in the total protein level of Akt (n = 3) and eNOS (n = 4) in the PV tissue preparations. The total protein level of the nNOS (n = 6) decreased. Panel (B) shows similar results in the atrium. The expression of nNOS was higher in the atrium than in the PVs. $^*P < 0.05$ versus without the fluvastatin administration. §$P < 0.05$ versus the baseline expression in the PVs.*

had shown that statins activate the eNOS with the production of nitric oxide through various pathways.[19] One of the pathways is to induce the phosphorylation and activation of the eNOS via the PI3 K/Akt pathway to produce nitric oxide in the endothelial cells without changing the expression of the Akt and eNOS.[25,26] Similarly, in the present study, fluvastatin increased the phosphorylation of Akt and eNOS in the PVs and atrium from the Western blot analysis. The total amount of the Akt and eNOS was not changed by fluvastatin. The fluvastatin-induced phosphorylation of Akt and eNOS would increase nitric oxide release. Furthermore, the administration of ODQ could attenuate the nitric oxide-induced vasodilatation through preventing cGMP production.

Regulation of nNOS

The nNOS is expressed in cholinergic, nonadrenergic and noncholinergic nerve terminals, specialized conduction tissues of the heart, and sympathetic nerve terminals, where it has been postulated to play a role in a catecholamine release and reuptake.[27,28] Basic research has shown that both sympathetic and parasympathetic inputs with a neurotransmitter release may serve as triggers for AF.[29,30] In the present study, the statins decreased the nNOS in either the atrium or

the PVs. Through the regulation of the nNOS, the statins can prevent AF by modulating the autonomic function.

This was the first study to demonstrate the different regulation effects of eNOS and nNOS caused by statins. Both eNOS and nNOS were reported to decrease the cardiac inotropic function[31,32] and induce vasorelaxation.[16,17] The increased phosphorylation of eNOS and decreased expression of nNOS by statins lead to the hypothesis that the decreased PV vascular tension and contractility of the atrium and PVs were mainly caused by the eNOS activation rather than that of nNOS. The antiarrhythmic effects, exerted by statins through the nitric oxide and mechanoelectrical feedback, were due to eNOS rather than nNOS. Moreover, we compared the expression of the Akt and NOS in the atrium and PVs and found a higher expression of nNOS in the atrium. This finding may be caused by the sparse expression of nNOS in the smooth muscle.[33,34] However, statins have a similar electrophysiological effect and protein expression in the PV and atrium. These results suggest that statins could not only prevent PV arrhythmogenesis, but also might modify the atrial substrate through a similar pathway.

Clinical Implications

The spontaneous activity of the PVs was reported to have a arrhythmogenic potential for initiating and maintaining atrial arrhythmias including atrial tachycardia from pulmonary vein and AF.[8-11,35] Suppression of spontaneous ectopic PV activity by beta-blocker and calcium channel blocker was associated with lower frequency of AF attack and focal atrial tachycardia from PVs.[8] Fluvastatin produced nitric oxide through a PI3kinase/Akt pathway that reduced the PV vascular diastolic tension and decreased the PV spontaneous firing, which might contribute to the prevention of atrial arrhythmias from PVs.

Limitations

The present study suggests that statins may regulate the PV electrical activity through a mechanoelectrial feedback. However, more mechanisms should be involved in the statin's effects since there is an entire group of preparations absent PV firing in which the statin affected the vascular diastolic tension. It is possible that antioxidation or anti-inflammation might also play a role. In addition, it is unclear to what extent this observation represents a new mechanism or that it is part of the antioxidative pathway of fluvastatin. Moreover, we studied the effects of fluvastatin in superfused preparations; thus, it is not elucidated about the effects of fluvastatin on intravascular pressure or flow *in vivo*.

Conclusions

Fluvastatin produced nitric oxide through a PI3kinase/Akt pathway that reduced the PV vascular diastolic tension and decreased the PV arrhythmogenesis. These results may contribute to the beneficial effects of statins on atrial arrhythmias from PVs.

References

1. Fauchier L, Pierre B, de Labriolle A, Grimard C, Zannad N, Babuty D: Antiarrhythmic effect of statin therapy and atrial fibrillation a meta-analysis of randomized controlled trials. J Am Coll Cardiol 2008;51:828-835.
2. Maack C, Kartes T, Kilter H, Schäfers HJ, Nickenig G, Böhm 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;108:1567-1574.
3. Kumagai K, Nakashima H, Saku K: The HMG-CoA reductase inhibitor atorvastatin prevents atrial fibrillation by inhibiting inflammation in a canine sterile pericarditis model. Cardiovasc Res 2004;62:105-111.
4. Korantzopoulos P, Kolettis TM, Kountouris E, Dimitroula V, Karanikis P, Pappa E, Siogas K, Goudevenos JA: Oral vitamin C administration reduces early recurrence rates after electrical cardioversion of persistent atrial fibrillation and attenuates associated inflammation. Int J Cardiol 2005;102:321-326.
5. Vaquero M, Caballero R, Gómez R, Núñez L, Tamargo J, Delpón E: Effects of atorvastatin and simvastatin on atrial plateau currents. J Mol Cell Cardiol 2007;42:931-945.
6. Maeda S, Matsuoka I, Iwamoto T, Kurose H, Kimura J: Down-regulation of Na^+/Ca^{2+} exchanger by fluvastatin in rat cardiomyoblast H9 c2 cells: Involvement of RhoB in Na^+/Ca^{2+} exchanger mRNA stability. Mol Pharmacol 2005;68:414-420.
7. Haïssaguerre M, Jaïs P, Shah DC, Takahashi A, Hocini M, Quiniou G, Garrigue S, Le Mouroux A, Le Métayer P, Clémenty J: Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998;339:659-666.
8. Chen SA, Hsieh MH, Tai CT, Tsai CF, Prakash VS, Yu WC, Hsu TL, Ding YA, Chang MS: Initiation of atrial fibrillation by ectopic beats originating from the pulmonary veins: Electrophysiological characteristics, pharmacological responses, and effects of radiofrequency ablation. Circulation 1999;100:1879-1886.
9. Chen YJ, Chen SA, Chang MS, Lin CI: Arrhythmogenic activity of cardiac muscle in pulmonary veins of the dog: Implication for the genesis of atrial fibrillation. Cardiovasc Res 2000;48:265-273.
10. Weerasooriya R, Jaïs P, Scavée C, Macle L, Shah DC, Arentz T, Salerno JA, Raybaud F, Choi KJ, Hocini M, Clémenty J, Haïssaguerre M: Dissociated pulmonary vein arrhythmia: Incidence and characteristics. J Cardiovasc Electrophysiol 2003;14:1173-1179.
11. Marrouche N, Wazni OM, Martin DO, Rossillo A, Saliba W, Erciyes D, Schweikert R, Khaykin Y, Burkhardt D, Bhargava M, Verma A, Abdul-Karim A, Natale A: Response to pharmacological challenge of dissociated pulmonary vein rhythm. J Cardiovasc Electrophysiol 2005;16:122-126.
12. Kalifa J, Jalife J, Zaitsev AV, Bagwe S, Warren M, Moreno J, Berenfeld O, Nattel S: Intra-atrial pressure increases rate and organization of waves emanating from the superior pulmonary veins during atrial fibrillation. Circulation 2003;108:668-671.
13. Chang SL, Chen YC, Chen YJ, Wangcharoen W, Lee SH, Lin CI, Chen SA: Mechanoelectrical feedback regulates the arrhythmogenic activity of pulmonary veins. Heart 2007;93:82-88.
14. Bode F, Katchman A, Woosley RL, Franz MR: Gadolinium decreases stretch-induced vulnerability to atrial fibrillation. Circulation 2000;101:2200-2205.
15. Ravelli F, Allessie M: Effects of atrial dilatation on refractory period and vulnerability to atrial fibrillation in the isolated Langendorff-perfused rabbit heart. Circulation 1997;96:1686-1695.
16. Gao Y, Raj JU: Role of veins in regulation of pulmonary circulation. Am J Physiol Lung Cell Mol Physiol 2005;288:213-226.
17. Shiga N, Hirano K, Hirano M, Nishimura J, Nawata H, Kanaide H: Long-term inhibition of RhoA attenuates vascular contractility by enhancing endothelial NO production in an intact rabbit mesenteric artery. Circ Res 2005;96:1014-1021.
18. Janssen LJ, Mardi K, Netherton S, Betti PA: Nitric oxide inhibits human and canine pulmonary vascular tone via a postjunctional, nonelectromechanical, cGMP-dependent pathway. Can J Physiol Pharmacol 1999;77:320-329.
19. Rikitake Y, Liao JK: Rho GTPases, statins, and nitric oxide. Circ Res 2005;97:1232-1235.
20. Huang A, Sun D, Shesely EG, Levee EM, Koller A, Kaley G: Neuronal NOS-dependent dilation to flow in coronary arteries of male eNOS-KO mice. Am J Physiol Heart Circ Physiol 2002;282:429-436.
21. Hare JM, Loh E, Creager MA, Colucci WS: Nitric oxide inhibits the positive inotropic response to β-adrenergic stimulation in humans with left ventricular dysfunction. Circulation 1995;92:2198-2203.
22. Ongini E, Impagnatiello F, Bonazzi A, Guzzetta M, Govoni M, Monopoli A, Del Soldato P, Ignarro LJ: Nitric oxide (NO)-releasing statin derivatives, a class of drugs showing enhanced antiproliferative and antiinflammatory properties. Proc Natl Acad Sci U S A 2004;101:8497-8502.

23. Bai CX, Namekata I, Kurokawa J, Tanaka H, Shigenobu K, Furukawa T: Role of nitric oxide in Ca^{2+} sensitivity of the slowly activating delayed rectifier K^+ current in cardiac myocytes. Circ Res 2005;96:64-72.
24. Sabia H, Prasad P, Smith HT, Stoltz RR, Rothenberg P: Safety, tolerability, and pharmacokinetics of an extended-release formulation of fluvastatin administered once daily to patients with primary hypercholesterolemia. J Cardiovasc Pharmacol 2001;37:502-511.
25. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, Sessa WC, Walsh K: The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med 2000;6:1004-1010.
26. Harris MB, Blackstone MA, Sood SG, Li C, Goolsby JM, Venema VJ, Kemp BE, Venema RC: Acute activation and phosphorylation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. Am J Physiol Heart Circ Physiol 2004;287:560-566.
27. Tanaka K, Hassall CJ, Burnstock G: Distribution of intracardiac neurons and nerve terminals that contain a marker for nitric oxide, NADPH-diaphorase, in the guinea-pig heart. Cell Tissue Res 1993;273:293-300.
28. Paterson DJ: Nitric oxide and the autonomic regulation of cardiac excitability. Exp Physiol 2001;86:1.
29. Patterson E, Po SS, Scherlag BJ, Lazzara R: Triggered firing in pulmonary veins initiated by *in vitro* autonomic nerve stimulation. Heart Rhythm 2005;2:624-631.
30. Zhou J, Scherlag B, Edwards J, Jackman WM, Lazzara R, Po SS: Gradients of atrial refractoriness and inducibility of atrial fibrillation due to stimulation of ganglionated plexi. J Cardiovasc Electrophysiol 2007;18:83-90.
31. Burkard N, Rokita AG, Kaufmann SG, Hallhuber M, Wu R, Hu K, Hofmann U, Bonz A, Frantz S, Cartwright EJ, Neyses L, Maier LS, Maier SK, Renne T, Schuh K, Ritter O: Conditional neuronal nitric oxide synthase overexpression impairs myocardial contractility. Circ Res 2007;100:32-44.
32. Massion PB, Dessy C, Desjardins F, Pelat M, Havaux X, Belge C, Moulin P, Guiot Y, Feron O, Janssens S, Balligand JL: Cardiomyocyte-restricted overexpression of endothelial nitric oxide synthase (NOS3) attenuates beta-adrenergic stimulation and reinforces vagal inhibition of cardiac contraction. Circulation 2004;26: 2666-2672.
33. Magness RR, Shaw CE, Phernetton TM, Zheng J, Bird IM: Endothelial vasodilator production by uterine and systemic arteries: II. Pregnancy effects on NO synthase expression. Am J Physiol 1997;272:1730-1740.
34. Teng B, Murthy KS, Kuemmerle JF, Grider JR, Sase K, Michel T, Makhlouf GM: Expression of endothelial nitric oxide synthase in human and rabbit gastrointestinal smooth muscle cells. Am J Physiol 1998;275:342-351.
35. Kistler PM, Sanders P, Fynn SP, Stevenson IH, Hussin A, Vohra JK, Sparks PB, Kalman JM: Electrophysiological and electrocardiographic characteristics of focal atrial tachycardia originating from the pulmonary veins: Acute and long-term outcomes of radiofrequency ablation. Circulation 2003;108:1968-1975.