Effect of Simvastatin and Antioxidant Vitamins on Atrial Fibrillation Promotion by Atrial-Tachycardia Remodeling in Dogs

Akiko Shiroshita-Takeshita, MD; Gernot Schram, MD; Joel Lavoie, PhD; Stanley Nattel, MD

Background—There is evidence for a role of oxidant stress and inflammation in atrial fibrillation (AF). Statins have both antioxidant and anti-inflammatory properties. We compared the effects of simvastatin with those of antioxidant vitamins on AF promotion by atrial tachycardia in dogs.

Methods and Results—We studied dogs subjected to atrial tachypacing (ATP) at 400 bpm in the absence and presence of treatment with simvastatin, vitamin C, and combined vitamins C and E. Serial closed-chest electrophysiological studies were performed in each dog at baseline and 2, 4, and 7 days after tachypacing onset. Atrioventricular block was performed to control ventricular rate. Mean duration of induced AF was increased from 42 ± 18 to 1079 ± 341 seconds at terminal open-chest study after tachypacing alone (P < 0.01), and atrial effective refractory period (ERP) at a cycle length of 300 ms was decreased from 117 ± 5 to 76 ± 6 ms (P < 0.01). Tachypacing-induced ERP shortening and AF promotion were unaffected by vitamin C or vitamins C and E; however, simvastatin suppressed tachypacing-induced remodeling effects significantly, with AF duration and ERP averaging 41 ± 15 seconds and 103 ± 4 ms, respectively, after tachypacing with simvastatin therapy. Tachypacing downregulated L-type Ca2+-channel α-subunit expression (Western blot), an effect that was unaltered by antioxidant vitamins but greatly attenuated by simvastatin.

Conclusions—Simvastatin attenuates AF promotion by atrial tachycardia in dogs, an effect not shared by antioxidant vitamins, and constitutes a potentially interesting new pharmacological approach to preventing the consequences of atrial tachycardia remodeling. (Circulation. 2004;110:2313-2319.)

Key Words: antioxidants ■ antiarrhythmia agents ■ electrophysiology

Atrial fibrillation (AF) is a common and troublesome arrhythmia that is difficult to treat. Atrial tachyarrhythmias alter atrial electrophysiology in a way that promotes AF, and these alterations are believed to contribute to both the occurrence and the persistence of the arrhythmia.1-4 Prevention of atrial tachycardia–induced remodeling is an attractive therapeutic approach,5 but to date, the only drugs shown to prevent experimental remodeling caused by several days or more of atrial tachycardia are mibefradil,6,7 which is no longer on the market, and amiodarone.8

There is evidence for enhanced oxidative stress in atrial tissue samples from AF patients9 and for a benefit from antioxidant vitamins in preventing atrial tachycardia remodeling.10 In addition, there is evidence for a role of inflammation in AF.11,12 Statins have both anti-inflammatory and antioxidant properties.13,14 The present study was designed to assess the effects of simvastatin on atrial remodeling caused by 1 week of atrial tachycardia. As comparator agents, we used vitamin C and combined vitamin C and E therapy, because these also have some antioxidant properties and vitamin C has shown some value in preventing atrial tachycardia remodeling.10

Animal Model

Thirty-nine mongrel dogs (20 to 37 kg) were anesthetized with ketamine (5.3 mg/kg IV), diazepam (0.25 mg/kg IV), and halothane (1.5%). Unipolar leads were inserted through jugular veins into the right ventricular (RV) apex and right atrial (RA) appendage and connected to pacemakers (Medtronic) in subcutaneous pockets in the neck. A bipolar electrode was inserted into the RA for stimulation and recording during serial electrophysiological study (EPS). AV block was created by radiofrequency ablation to control ventricular response during atrial tachypacing (ATP). The RV pacemaker was programmed to 80 bpm.

After 24 hours for recovery, a baseline closed-chest EPS was performed under ketamine/diazepam/isoflurane anesthesia, and then ATP (400 bpm) was initiated. Closed-chest EPS was repeated at 2, 4, and 7 days of ATP, and a final open-chest EPS was performed on day 8 under morphine/α-chloralose anesthesia.

Groups

Results in 7 ATP dogs without any treatment (ATP-only group) and 9 nonpaced control dogs were compared with dogs subjected to ATP during oral treatment with (1) simvastatin, 80 mg/d (n = 6), beginning 3 days before ATP onset; (2) vitamin C, 500 mg twice daily (n = 6); and (3) combined vitamin C, 500 mg, and vitamin E, 200 IU, twice daily (n = 6), beginning 1 day before ATP onset and continued throughout the study period. In addition, because no clear effect of

Methods

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vitamin C was observed at this dose, we studied 5 additional dogs receiving oral sustained-release vitamin C, 500 mg at 6 AM and 1 g at 4 PM, beginning 1 day before ATP.

Study Protocol

On closed-chest EPS days, dogs were anesthetized with ketamine/diazepam/isoflurane and ventilated mechanically. The atrial pacemaker was deactivated and the RA appendage effective refractory period (ERP) was measured at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms with 10 basic stimuli (S1) followed by a premature extrastimulus (S2) followed by a premature extrastimulus (S3) with 5-ms decrements. The longest S3–S3 failing to capture defined the ERP. AF was induced with atrial burst pacing at 10 Hz and 4 times threshold current and then averaged. If AF lasted longer than 30 minutes, it was considered sustained and was terminated by DC cardioversion. A 20-minute rest period was then allowed before continuing measurements. If sustained AF was induced twice, no further AF induction was performed.

For open-chest EPS, dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV, followed by 29.25 mg · kg⁻¹ · h⁻¹), and ventilated mechanically. Body temperature was maintained at 37°C, and a femoral artery and both femoral veins were cannulated for pressure monitoring and drug administration. A median sternotomy was performed, and bipolar electrodes were hooked to the RA and left atrial (LA) appendages for recording and stimulation. A programmable stimulator (Digital Cardiovascular Instruments) was used to deliver twice-threshold currents. Five silicon sheets containing 240 bipolar electrodes were sutured onto the atrial surfaces as previously described. Atrial ERPs were measured at multiple BCLs in the RA and LA appendages and at BCL 300 ms in 6 additional sites: RA and LA posterior wall, RA and LA inferior wall, and RA and LA Bachmann’s bundle. AF vulnerability was determined as the percentage of atrial sites at which AF could be induced by single extrastimuli.

Blood samples were collected on the final open-chest study day. Serum was removed after centrifugation (3000 rpm, 20 minutes) and stored at −80°C for subsequent C-reactive protein (CRP) analysis. CRP was measured with the Phase Range canine CRP ELISA kit (Tri-delta Diagnostics).

After open-chest studies, RA and LA tissue samples were fast-frozen in liquid nitrogen and stored at −80°C. To isolate proteins, tissues were homogenized in RIPA buffer with a protease-inhibitor cocktail (5 µg/µL leupeptin, 5 µg/µL soybean trypsin inhibitor and 10 µg/mL benzamidine; Sigma). The suspension was incubated on ice and then centrifuged (14 000 g, 10 minutes, 4°C). The soluble fraction was stored at −80°C. Protein concentrations were measured by Bradford assay with BSA as a standard. Proteins (200-µg samples) were denatured in Laemmli buffer, electrophoresed on 7.5% SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride membranes overnight, blocked for 2 hours with 0.1% Tween-80–Tris-buffered saline (TTBS) at room temperature, and then incubated with primary antibody (Alomone, anti-cardiac Cav1.2, 1:100) at 4°C overnight. After 3 washes, membranes were reblocked in 1% nonfat dry milk in TTBS for 10 minutes and incubated with secondary antibody (Jackson Laboratories, goat anti-rabbit) for 90 minutes at room temperature. After 3 additional washes in TTBS, antibody detection was performed with chemiluminescence detection. Band densities were quantified by densitometry, standardized to GAPDH, and normalized to the control sample on each gel.

Data Analysis

Data are presented as mean±SEM. Multiple-group comparisons were obtained by ANOVA. AF duration data were analyzed after...
**Results**

There were no significant differences among groups in body weight or hemodynamic variables at final open-chest study (Table). Although CRP tended to be slightly higher at the end of the study in ATP-only dogs, CRP varied widely, and there were no statistically significant CRP differences among groups.

**Effects of Interventions on Atrial Tachycardia–Induced Changes During Serial Closed-Chest Studies**

Changes in ERP caused by 7 days of ATP in ATP-only dogs are shown in Figure 1. ERP decreased substantially within 2 days and reached steady state at 4 days (Figure 1A). AF duration increased substantially from 10 ±7 seconds before ATP to values averaging hundreds of seconds on days 4 and 7 of atrial tachycardia (Figure 1B).

Figure 2 compares ERP changes as measured at cycle lengths of 300 (A) and 150 (B) ms in dogs subjected to ATP only with dogs subjected to ATP in the presence of drug interventions. Under baseline conditions (day 0), there were no significant differences in ERP among groups. With the onset of ATP, ERP decreased rapidly and similarly in ATP-only dogs and dogs subjected to ATP in the presence of each of the antioxidant vitamin regimens. In simvastatin-treated dogs, the ERP changes were smaller and ERP values were larger than in ATP-only dogs for both BCLs.

**Differences at Final Open-Chest Study**

ERPs as a function of BCL during the final open-chest study are illustrated in Figure 4. Dogs subjected to ATP without drug intervention had atrial ERPs averaging <80 ms at all BCLs, and virtually no ERP rate adaptation was detectable. No significant differences were observed between ATP-only dogs and dogs subjected to ATP in the presence of vitamin C (Figure 4A), vitamins C and E (B), or sustained-release vitamin C (C). Dogs subjected to ATP in the presence of simvastatin showed ERP values that were significantly greater than those subjected to ATP without drug intervention (D).

In nonpaced control dogs, AF always terminated spontaneously within 5 minutes. AF lasting 30 minutes and requiring cardioversion for termination was induced after ATP in 57% of ATP-only dogs, 33% of vitamin C–treated dogs, 50% of combined vitamin C and E–treated dogs, and 40% of sustained-release vitamin C–treated dogs. No sustained AF requiring cardioversion occurred in ATP dogs treated with simvastatin. Figure 5 summarizes differences in mean AF duration and atrial vulnerability at open-chest study. Nonpaced control dogs had mean AF durations averaging 42 seconds (Figure 5A), and ATP increased mean AF duration at open-chest study to more than 1000 seconds. Dogs subjected to ATP in the presence of simvastatin showed ERP values that were significantly greater than those subjected to ATP without drug intervention (D).

**Figure 3.** Individual-dog and mean ± SEM AF duration (DAF) during 7-day atrial tachypacing and treatment with vitamin C (A), vitamins C and E (B), sustained-release vitamin C (C), and simvastatin (D). P0, P2, P4, P7 indicate pacing for 0, 2, 4, and 7 days, respectively.

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NP indicates nonpaced control; ATP, ATP-only; ATP + VitC, ATP with vitamin C treatment; ATP + VitC&E, ATP with combined vitamin C and E; ATP + SR-VitC, ATP with sustained-release vitamin C; ATP + SIM, ATP with simvastatin; HR, heart rate; BP, blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LAP, LA pressure; and NA, not available.
of each antioxidant vitamin regimen had mean AF durations >500 seconds and not significantly different from ATP-only. Dogs subjected to ATP in the presence of simvastatin had substantial attenuation of the AF maintenance-promoting effect of ATP, with a mean AF duration (≈40 seconds) equivalent to that of nonpaced controls. AF vulnerability changes are shown in B. AF was induced by single extrastimuli at a mean of more than 50% of atrial sites in ATP-only dogs, significantly greater than the ≤15% of sites at which AF could be induced in nonpaced controls. In dogs subjected to ATP during therapy with antioxidant vitamins, AF was induced at an average of >50% of sites in each group. In simvastatin-treated dogs exposed to ATP, atrial vulnerability was significantly reduced, to an average of ≈20%.

Figure 5. AF promotion as measured during final open-chest study. A, Mean±SEM duration of AF (DAF). B, AF vulnerability (percentage of sites at which AF could be induced by single premature extrastimuli). *P<0.05, **P<0.01 vs ATP-only. NP indicates nonpaced controls; ATP, ATP-only; SIM, VitC, VitC&E, SR-VitC, atrial tachypaced dogs treated with simvastatin, vitamins C and E, and sustained-release vitamin C, respectively.

Changes in L-Type Ca2+-Channel α-Subunit Protein Expression

Reductions in L-type Ca2+ current,16 apparently caused by transcriptional downregulation of the α1c pore-forming Ca2+-channel subunit, Ca1.2,17–19 are important in mediating electrophysiological changes caused by atrial tachycardia remodeling. We therefore quantified the expression of Ca1.2 protein in the RA and LA appendages in nonpaced dogs and dogs subjected to ATP during treatment with simvastatin, vitamin C, and vitamins C and E. A clear signal was obtained at 207 kDa, corresponding to the expected molecular mass of Ca1.2 protein (Figure 7A). GAPDH signals are shown in Figure 7B. ATP alone significantly reduced Ca1.2 protein expression in both RA (Figure 7C, left) and LA (Figure 7C, right) tissue samples. Neither vitamin C alone nor vitamins C plus E significantly altered the tachypacing-induced Ca1.2 changes. In contrast, simvastatin significantly attenuated Ca1.2 downregulation.

Figure 6 shows atrial ERPs in different atrial regions. ERP decreases caused by ATP were regionally variable, as previously described,15 with the largest changes occurring in the RA inferior wall, posterior wall, and appendage, as well as the LA appendage. There were no significant differences between ERPs in ATP-only dogs and dogs in each of the antioxidant vitamin groups (A–C). Simvastatin significantly attenuated ATP effects on ERP in the RA appendage, posterior wall, and inferior wall. LA ERP reductions induced by ATP were not significantly altered by simvastatin therapy.
Discussion

Main Findings
We have found that simvastatin prevents AF promotion by 1 week of ATP in dogs. This action was associated with significant attenuation of RA ERP abbreviation and of atrial tachycardia–induced effects on Cav1.2 protein expression. These actions were not shared by the antioxidant vitamin C or by vitamins C and E in combination.

Comparison With Previous Studies of Drug Effects on Atrial Tachycardia–Induced Remodeling
Although several articles have demonstrated beneficial effects of L-type Ca channel blockers on short-term atrial tachycardia–induced remodeling, they appear to be ineffective against longer-term (>24-hour) remodeling. A variety of other compounds, including Na⁺, H⁺-exchange blockers, and ACE inhibitors, have also been found effective in short-term but not longer-term AF. Carnes et al demonstrated effectiveness of vitamin C at doses equivalent to those in the present study in attenuating ERP changes caused by 48-hour atrial tachycardia in the dog (changes in arrhythmia promotion were not reported). We did not observe effectiveness of vitamin C, alone or in combination with vitamin E, in preventing ERP or AF-promoting effects of 7-day atrial tachycardia.

The T-type Ca²⁺-channel blocker mibefradil and the broad-spectrum antiarrhythmic amiodarone do prevent the effects of 1-week atrial tachycardia in the dog. Mibefradil

![Figure 6](image1.png)

**Figure 6.** ERPs in different atrial regions at BCL 300 ms at final open-chest study. In each panel, results from nonpaced dogs are shown by dotted lines and results from ATP-only dogs by dashed lines. These are compared with results in ATP dogs treated with vitamin C (A), vitamins C and E (B), sustained-release vitamin C (C), and simvastatin (D). *P<0.05, **P<0.01 vs nonpaced. RAA, RAPW, RAIW, RABB, LAA, LAPW, LAIW, and LABB indicate RA and LA appendage, posterior wall, inferior wall, and Bachmann’s bundle.

![Figure 7](image2.png)

**Figure 7.** A, Representative results for expression of L-type Ca²⁺-channel α-subunit protein. B, GAPDH bands corresponding to first 6 lanes of A, C, Mean±SEM Cav1.2 band intensities in RA (left) and LA (right) appendages. Abbreviations as in Figure 5; MWM indicates molecular-weight marker (210 kDa); lanes C, vitamin C; C&E, combined vitamin C and E; and NP+CA, nonpaced plus control antigen.
also has antioxidant properties,28 which may contribute to its efficacy in atrial remodeling. However, mibefradil has been removed from the market because of adverse drug interactions, and the value of amiodarone is limited by a range of potentially serious adverse effects. To the best of our knowledge, the present study is the first demonstration of the effectiveness of simvastatin in atrial tachycardia remodeling and AF prevention.

Potential Underlying Mechanisms
Simvastatin acts as antioxidants by inhibiting superoxide production,29 as well as by increasing nitric oxide bioavailability.30,31 Simvastatin increases catalase and glutathione peroxidase activity.32 Thus, it is possible that the efficacy of simvastatin is due to an antagonism of oxidant pathways involved in atrial tachycardia remodeling.9 The antioxidant properties of both vitamin C and E are well recognized33,34; however, the ability of exogenous vitamin C and E to increase the body’s already substantial stores of these important endogenous antioxidants may be insufficient to significantly alter atrial antioxidant capacity. An alternative explanation lies in the antiinflammatory properties of statins,13,14 in the context of the potential role of inflammation in AF.11,12 We measured CRP concentrations in our dogs but did not observe significant changes. Although these results do not support the involvement of inflammatory mechanisms, they are insufficient to exclude them.

Limitations of the Study
Simvastatin was much more effective in preventing tachycardia-induced RA ERP changes than those in the LA (Figure 6D). The basis of this regionally determined efficacy is unclear, particularly in view of the ability of simvastatin to prevent LA Ca,1.2 downregulation (Figure 7C). These observations point to a role for factors other than Ca,1.2 downregulation in contributing to atrial tachycardia–induced ERP changes and may be related to the observation that nitric oxide synthase downregulation with atrial tachycardia remodeling is more significant in the LA than in the RA.35

Statins have pleiotropic effects, in addition to their antioxidant or antiinflammatory action, and the precise mechanisms of simvastatin action in tachycardia remodeling remain to be established.

Potential Clinical Implications
Atrial tachycardia remodeling has significant clinical consequences, particularly for AF occurrence and maintenance, and inhibition of such remodeling may be an interesting novel approach to AF therapy, but this remains to be established clinically.5 To date, the drugs shown to prevent atrial tachycardia remodeling in dog models either have been unavailable clinically (mibefradil) or have a variety of other potent electrophysiological and extracardiac actions (amiodarone). Simvastatin is widely used in clinical management, and if it is found to prevent atrial tachycardia remodeling in humans, it might prove a useful tool to test the value of atrial remodeling prevention in AF therapy. The doses of simvastatin that we studied (2 · kg−1 · d−1) are equal to those used in some experimental dog studies36 and smaller than in others37 but are somewhat higher than those in common clinical use (0.3 to 1 mg/kg). It remains to be determined whether clinically used doses of simvastatin are able to prevent atrial tachycardia remodeling in humans.

Conclusions
Simvastatin prevents the AF-promoting actions of atrial tachycardia in dogs and may open up interesting new approaches to preventing the arrhythmic consequences of atrial tachycardia remodeling in humans.

Acknowledgments
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