Effects of simvastatin on the development of the atrial fibrillation substrate in dogs with congestive heart failure ☆

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Abstract

Background: Congestive heart failure (CHF) is a common cause of atrial fibrillation (AF). Oxidative stress and inflammation (profibrotic) and peroxisome proliferator-activated receptor-α (PPAR-α, antifibrotic) factors may be involved in CHF-related remodeling. We evaluated the effects of simvastatin (antioxidant, anti-inflammatory) and fenofibrate (PPAR-α activator) on CHF-related atrial remodeling.
Methods and results: Dogs were subjected to 2-week ventricular tachypacing (VTP) in the absence and presence of simvastatin (20 or 80 mg/day) or fenofibrate. Induced AF duration (DAF) was increased by VTP from 36 ± 14 (non-paced controls) to 1005 ± 257 s ($p<0.01$). Simvastatin prevented VTP-induced DAF increases (147 ± 37 and 84 ± 37 s at 20 and 80 mg/day, respectively), but fenofibrate did not (1018 ± 352 s). Simvastatin also attenuated CHF-induced conduction abnormalities (heterogeneity-index reduced from 1.5 ± 0.1 to 1.1 ± 0.1 and 1.0 ± 0.1 at 20 and 80 mg/day, $p<0.01$) and atrial fibrosis (from $19.4\pm1.3\%$ to $10.8\pm0.8\%$ and $9.9\pm0.8\%$ at 20 and 80 mg/day, $p<0.01$), while fenofibrate did not. Simvastatin (but not fenofibrate) also attenuated VTP-induced left-ventricular nitric-oxide synthase and nitrotyrosine increases, along with hemodynamic dysfunction. Atrial fibroblast proliferation increased with 24-h fetal bovine serum (FBS) stimulation from 654 ± 153 to 7264 ± 1636 DPM ($p<0.001$). Simvastatin, but not fenofibrate, suppressed fibroblast proliferation (664 ± 192 DPM, $p<0.001$). Simvastatin also significantly attenuated transforming growth factor-β1-stimulated α-smooth muscle actin (α-SMA) expression (indicating myofibroblast differentiation) from 1.3 ± 0.1 to 1.0 ± 0.1 times baseline ($p<0.05$).
Conclusions: CHF-induced atrial structural remodeling and AF promotion are attenuated by simvastatin, but not fenofibrate. Statin-induced inhibition of profibrotic atrial fibroblast responses and attenuation of left-ventricular dysfunction may contribute to preventing the CHF-induced fibrotic AF substrate.

This article is referred to in the Editorial by I.C. Van Gelder (pages 8–10) in this issue.

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. Congestive heart failure (CHF) is a particularly common clinical cause of AF [1]. Experimental CHF produces a

substrate for AF maintenance characterized by atrial interstitial fibrosis and local conduction abnormalities [2]. The AF substrate is an interesting potential target for AF therapy [3].

Recent evidence suggests the possible involvement of oxidative stress [4,5] and inflammation [6,7] in the pathogenesis of CHF and AF. Statins have antioxidant and anti-inflammatory properties [8,9]. Recent studies suggest that statins can prevent ventricular dysfunction and remodeling in human and experimental models [10,11]. Simvastatin prevents AF promotion induced by atrial tachycardia remodeling [12]. Peroxisome proliferator-activated receptor-alpha (PPAR-α) is a modulator of inflammation and fibrosis [13,14]. The PPAR-α activator, fenofibrate, inhibits myocardial inflammation and fibrosis in angiotensin II-infused rats [15] and diastolic dysfunction in hypertensive rats [16]. A recent data-base analysis showed that patients with left ventricular dysfunction taking lipid-lowering drugs (98% statins and fibrates) had a significantly lower prevalence of AF [17]. We could not identify data in the experimental literature regarding the effects of statins or fibrates on AF related to CHF. Accordingly, this study was designed to determine whether statin or PPAR-α agonist hypolipidemic agents protect against CHF-induced atrial arrhythmogenic remodeling. A preliminary presentation of this work has appeared in abstract form [18].

2. Methods

2.1. Animal preparation

Animal-handling procedures were in accordance with the guidelines of the National Institutes of Health. Twenty-nine mongrel dogs (20–35 kg) were studied. Dogs were anesthetized with ketamine (5.3 mg/kg IV), diazepam (0.25 mg/kg IV), and halothane (1.5%). A unipolar pacing lead was inserted into the right ventricular apex and connected to a pacemaker in a subcutaneous pocket in the neck. After 24-hour recovery, the ventricular pacemaker was programmed for 2 weeks of ventricular tachypacing (VTP) at 240 bpm, following which the dog was subjected to electrophysiological study (EPS).

2.2. Groups and protocols

Results in VTP dogs treated with simvastatin (lower dose: 20 mg/day, $n=5$; or higher dose: 80 mg/day, $n=6$) or fenofibrate (800 mg/day, $n=6$) were compared with 9 untreated (VTP-only) dogs and 8 non-paced controls. Drugs were given orally (2 equal daily doses), starting 3 days before VTP-onset and continued throughout VTP. The higher simvastatin dosage was based on previous studies in dogs [12,19,20] and the lower one was based on human doses. The dose of fenofibrate was of the same order as in previous rabbit [21] and pig [22] studies, which produced clinically-relevant plasma concentrations [22].

For open-chest EPS, the ventricular pacemaker was de-activated. Dogs were anesthetized with morphine (2 mg/kg SC)

and α-chloralose (120 mg/kg IV, followed by 29.25 mg/kg/h), and ventilated mechanically. Body temperature was maintained at 37°C. A femoral artery and both femoral veins were cannulated for pressure monitoring and drug administration. A median sternotomy was performed. Bipolar electrodes were hooked to the right (RA) and left atrial (LA) appendages for recording and stimulation. A programmable stimulator was used to deliver 2-ms, twice-threshold current pulses.

Five silicon sheets containing 240 bipolar electrodes were sutured onto the atrial surfaces as previously described [2,12,23,24]. Electrophysiological mapping was conducted with the Cardiomap© system (Research Center, Sacré-Coeur Hospital and Biomedical Engineering Institute, École Polytechnique and Université de Montréal). Atrial ERPs were 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) with 5-ms decrements (RA and LA appendages), and at a BCL of 300 ms at 6 additional sites: RA and LA posterior wall, RA and LA inferior wall, RA and LA sides of Bachmann's bundle. The longest S1–S2 interval failing to capture defined the ERP. The percentage of sites at which AF (>1 s) was induced by single extrastimuli defined AF vulnerability. AF was induced with atrial burst pacing (1–10 s, 10 Hz, 4-times threshold current). Mean AF duration in each dog was based on 10 AF inductions for AF duration <20 min and 5 for AF 20–30 min. AF>30 min was considered sustained and terminated by direct-current cardioversion. A 20-minute rest period was then allowed. If sustained AF was induced twice, no further AF induction was performed. At the end of EPS, hemodynamic data were obtained with a fluid-filled catheter and disposable transducer.

Phase-delay analysis was used to evaluate local conduction abnormalities as previously described [2,23]. The median phase-delay value (P50) reflects overall conduction and P95 (95th percentile of the phase-delay histogram) the longest phase-delays. P5–95 (range between 5th and 95th percentile) represents the range of conduction delays. The P5–95/P50 (the conduction-delay range normalized to P50) is a conduction-heterogeneity index independent of overall conduction–velocity changes.

2.3. Biochemical measurements

At the end of open chest studies, LA and left-ventricular (LV) tissue samples were fast-frozen in liquid nitrogen and stored at −80 °C. Tissue samples were homogenized in RadioImmunoPrecipitationAssay (RIPA) buffer [24,25] and centrifuged (15,000 rpm, 20 min, 4 °C). The supernatant was used for protein concentration measurement by Bradford assay (Bio-Rad) with bovine serum albumin (BSA) as a standard. For endothelial NOS (eNOS, NOS-3) and inducible NOS (iNOS, NOS-2) expression assessment, 40-μg protein samples were separated by 8%-Na-dodecyl-sulfate polyacrylamide-gel (SDS-PAGE) electrophoresis. After transfer to nitrocellulose membranes (Bio-Rad),
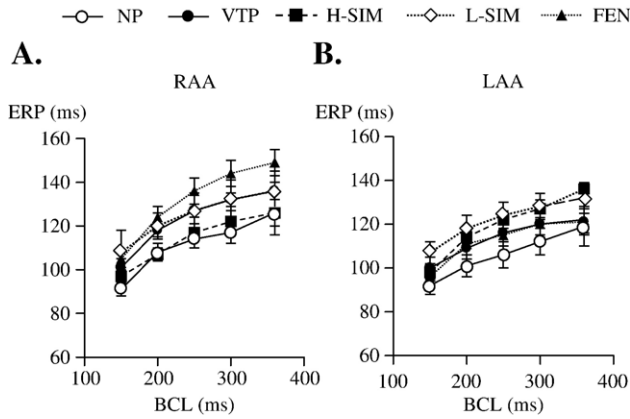
Fig. 1. ERP versus basic cycle length (BCL) in RA (A) and LA appendage (B) during open-chest EP study. NP=non-paced control, VTP=VTP-only group, H-SIM, L-SIM=high-dose and low-dose simvastatin-treated VTP dogs, FEN=fenofibrate-treated VTP dogs.

membranes were incubated with primary antibodies against NOS-3 and NOS-2 (BD Transduction Laboratories) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Research Diagnostics). Horseradish peroxidase-conjugated anti-mouse IgG (Santa-Cruz Biotechnology) was the secondary antibody. Signals were detected with Western Lightning Chemiluminescence Reagent-Plus (Perkin-Elmer Life Sciences) and quantified by densitometry.

For nitrotyrosine slot blot analysis, 30-μg heat-denatured protein samples (producing signals in the linear immunoreactive area) were spotted on nitrocellulose membranes (Stratagene). Membrane blocking (10% nonfat milk, Tris-buffered saline, 0.1% Tween-20) was followed by incubation with anti-nitrotyrosine primary antibody (Oxis Research) or GAPDH. Primary-antibody immunodetection was performed with peroxidase-conjugated anti-mouse IgG secondary antibody (Santa Cruz) or anti-sheep IgG (Sigma). Blots were incubated

with chemiluminescence-detection reagent (Perkin Elmer) for 1 min and exposed (Kodak X-ray films) for 15–90 s, followed by densitometric scanning.

Serum CK was measured by clinical enzyme-activity assay.

2.4. Histochemistry

Atrial samples from 4 regions (RA and LA appendage, LA and RA free wall) were immersed in 10% neutral buffered formalin for >24 h and stained with Masson-trichrome. Fibrous tissue was quantified with SigmaScan 4.0 (Jandel Scientific) as previously described [2,23].

2.5. Atrial fibroblast culture and characterization

Left atrial tissues from another series of non-paced dogs ($n=12$) were immersed in oxygen-saturated, Ca^{2+}-containing Tyrode solution at room temperature. The left circumflex coronary artery was cannulated and perfused with Ca^{2+}-containing Tyrode-solution (37 °C, 10 min), then perfused with Ca^{2+}-free Tyrode-solution (15 min), followed by 40-minute perfusion with Tyrode-solution containing collagenase (Worthington, type II) and 1% BSA (Sigma). Cells were dispersed by trituration in Medium 199 (Invitrogen) supplemented with 7% fetal bovine serum (FBS, Gibco), penicillin and streptomycin. Filtration (80-μm nanomesh) and centrifugation (500 rpm) were used to remove debris and cardiomyocytes. Following an additional 1000-rpm centrifugation and wash, cells were preincubated on uncoated culture flasks for 1 h to remove remaining myocytes. The culture medium was replaced and cells grown to confluence in 5% CO_2/95%-humidified air (37 °C). First-passage cells were used for all studies. Cells were plated and allowed to adhere for 24 h, then rendered quiescent in serum-free medium for 24 h. Following serum-starvation, cells received an 18-hour pretreatment with fenofibrate (10 μM, Sigma), simvastatin (10 μM, Merck), or vehicle, with all
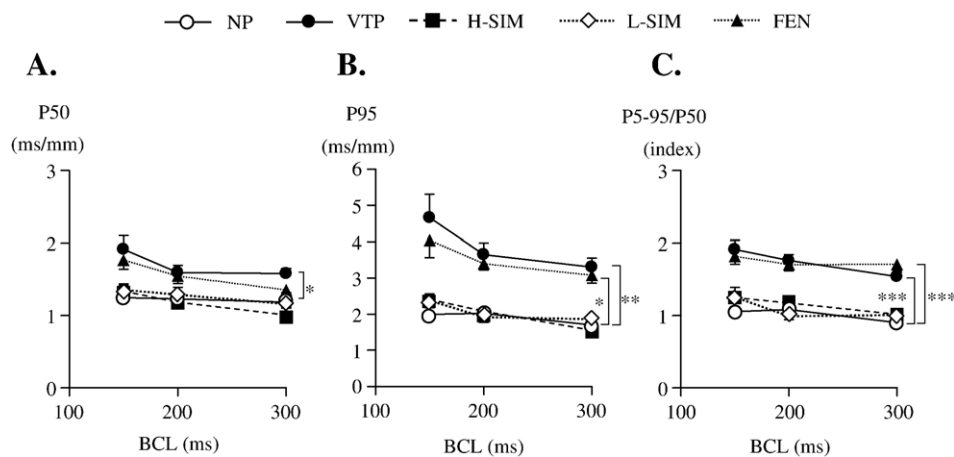

Fig. 2. Results of phase-delay histogram atrial-conduction analyses. A: P50, an index of mean conduction velocity. B: P95, an index of conduction times in slowest-conduction regions. C: P5–95/P50, a conduction-heterogeneity index independent of overall conduction changes. *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus non-paced controls. Abbreviations as in Fig. 1.
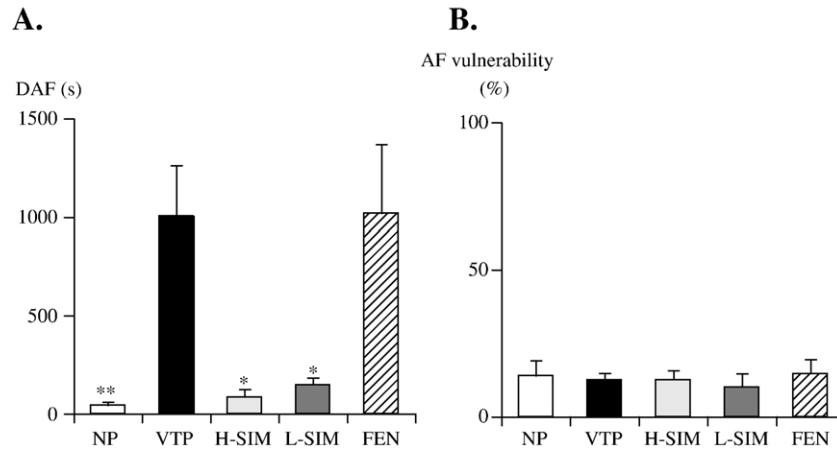
A.

B.

Fig. 3. AF promotion indices. A: Mean±SEM AF duration. B: AF vulnerability (percentage of sites at which AF was induced by single extrastimuli). *$p<0.05$, **$p<0.01$ versus VTP-only. Abbreviations as in Fig. 1.

interventions applied to cells from each of 6 dogs. We chose fenofibrate concentration based on concentrations in previous in vitro studies [26,27], which were comparable to concentrations achieved clinically [28]. The simvastatin concentration was based on concentrations used previously in vitro, which were in the range achieved at clinical doses [29].

Cultured atrial fibroblasts were plated on 24-well plates at 50 cells/mm^2. After 18-hour pretreatment, cells were stimulated by 7% FBS for 24 h. Four hours before the end of the treatment period, 1 μCi/ml [^{3}H]thymidine was added [29]. Cells were washed twice with 4 °C phosphate-buffered saline

(PBS) and treated with cold 5%-tricholoracetic acid for 30 min to precipitate protein and DNA. The precipitates were washed twice with cold water and resuspended in 0.4-M NaOH for scintillation-counting.

In another 6 dogs, cultured atrial fibroblasts pretreated with simvastatin, fenofibrate, or vehicle were stimulated with 5-ng/ml transforming growth factor-β_1 (TGFβ_1, Sigma) for 24 h. Cells were then washed twice with ice-cold PBS, then lysed in 500-μl ice-cold lysis buffer, pH 7.4 (10-mM Tris/HCl, 1-mM EDTA, 1-mM EGTA, 150-mM NaCl), 1% Triton X-100, 0.5% Igepal, protease inhibitors (1-μg/ml aprotinin, 0.5-mM
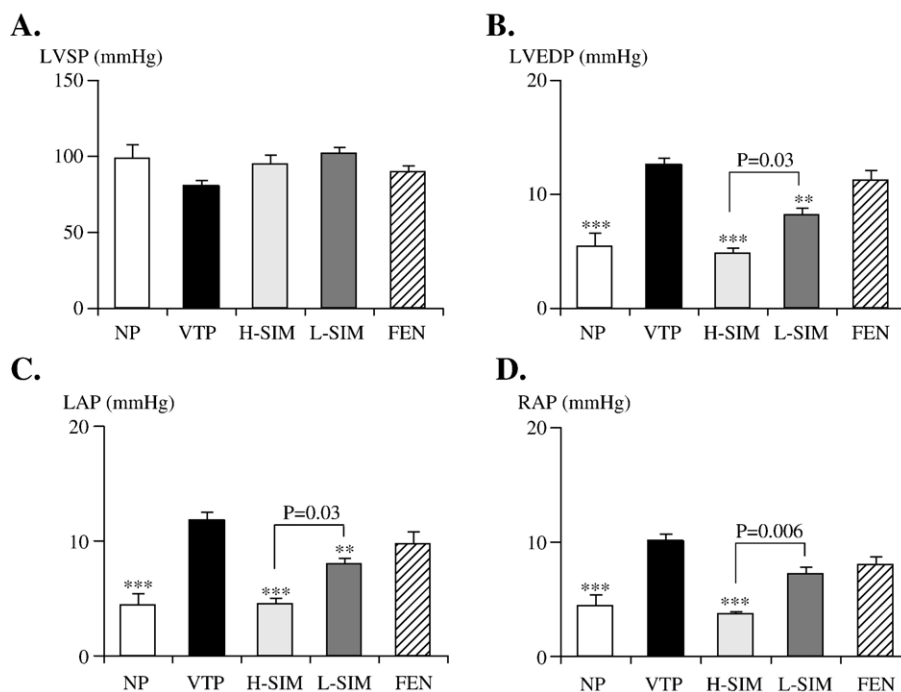
A.

B.

C.

D.

Fig. 4. Hemodynamic indices after open-chest EP study. A: Left ventricular systolic pressure (LVSP), B: LV end diastolic pressure (LVEDP), C: Left atrial pressure (LAP), D: Right atrial pressure (RAP). **$p<0.01$, ***$p<0.001$ versus VTP-only. Abbreviations as in Fig. 1.
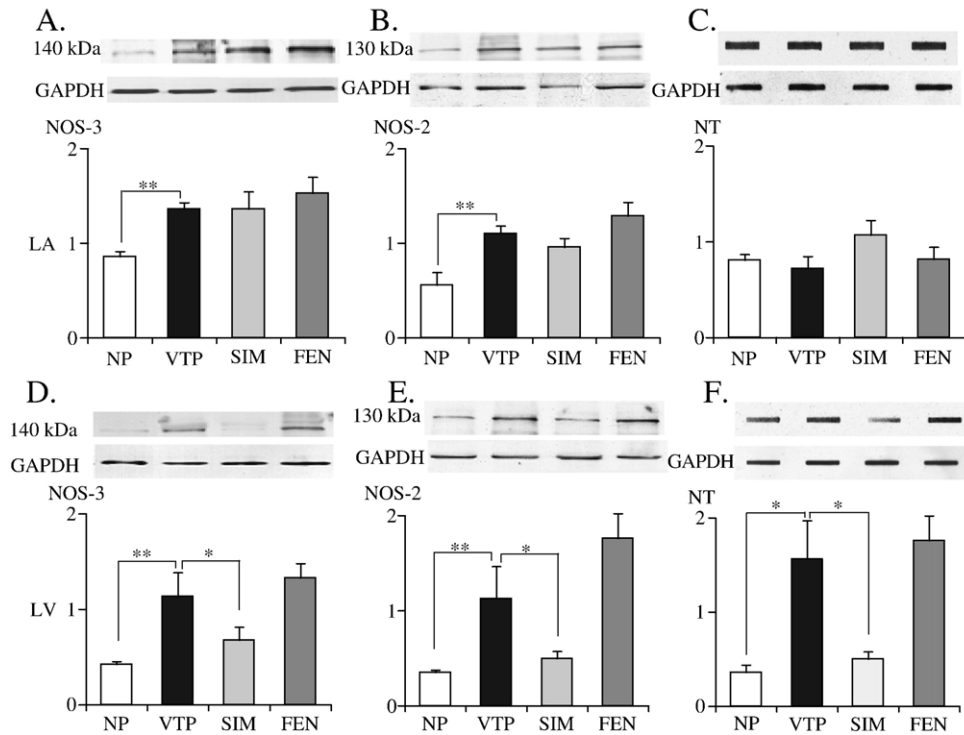
Fig. 5. Changes in endothelial NOS (NOS-3, left column), inducible NOS (NOS-2, middle column), and nitrotyrosine (NT, right column) expression in left atria (top, A–C) and left ventricles (bottom, D–F). Representative bands are shown along with corresponding GAPDH bands, and mean±SEM band intensities relative to GAPDH. *$p<0.05$, **$p<0.01$ versus VTP-only. Abbreviations as in Fig. 1.

phenylmethylsulfonyl fluoride, 1-µg/ml leupeptin), and phosphatase inhibitors (50-mM NaF, 1-mM Na-orthovanadate). Solubilized proteins were centrifuged (14,000 rpm, 4 °C, 10 min), and supernatants stored at −80 °C. Protein concentration was measured by Bradford assay. Protein samples (30 µg) were separated on 10%-polyacrylamide gels using SDS-PAGE and transferred to PVDF membranes (Bio-Rad). Membranes were incubated with primary antibodies against αSMA (Sigma) and GAPDH, with horseradish peroxidase-conjugated anti-mouse IgG (Santa-Cruz) secondary antibody. Cells from each dog were exposed to each of the three conditions, with and without TGFβ$_1$ stimulation.

2.6. Data analysis

Data are presented as mean±SEM. For all Western and slot blots, proteins were loaded in the linear immunoreactive-signal range and target-band intensities expressed relative to GAPDH-intensity from the same sample. Multiple group comparisons were obtained by a two-factor mixed design ANOVA with repeated measures on one factor for ERP and conduction-heterogeneity data, a one way factorial ANOVA for AF duration, AF vulnerability, hemodynamic data, NOS and nitrotyrosine measurements, and atrial fibrosis assessment, and a one-way ANOVA with repeated measures for fibroblast proliferation and αSMA data. AF-duration data were normalized by log-transformation. Bonferroni-cor-rected t-tests were used to evaluate individual mean differences when ANOVA revealed significant group effects. A two-tailed $p<0.05$ was considered statistically significant.

3. Results

3.1. Electrophysiology

ERPs at RA and LA appendage during the open-chest EP study are illustrated in Fig. 1. There were no significant ERP changes in any groups. Mean conduction times, as indicated by P50, were significantly increased by VTP, an effect prevented by both doses of simvastatin but not fenofibrate (Fig. 2A). Maximum phase-delay (P95) and conduction-heterogeneity index (P5–95/P50) were increased substantially in VTP-only dogs (Fig. 2B and C), reflecting local conduction delays characteristic of CHF-related AF [2,23]. These increases were

Table 1
Serum creatine kinase values (U/L) at end-study

	Mean±SE	Range
Non-paced controls	65±14	41–91
VTP-only	55±5	45–75
High dose simvastatin treated	68±13	37–123
Fenofibrate-treated	87±34	21–248
P	NS	

prevented by both doses of simvastatin, but unaffected by fenofibrate. P-wave duration, a measure of atrial conduction speed, was not changed by the interventions applied, averaging 59 ± 3, 59 ± 5, 60 ± 5, and 60 ± 4 ms in VTP-only, lower-, higher-dose simvastatin, and fenofibrate dogs ($p = $NS) respectively before VTP-onset.

In non-paced control dogs, AF was short-lasting and always terminated spontaneously within 5 min. AF >30 min requiring cardioversion was induced in 78% of VTP-only dogs and 83% of fenofibrate-treated dogs, but did not occur in higher-dose simvastatin-treated dogs. Only one episode of sustained AF (>30 min) was seen in a lower-dose simvastatin-treated dog. Mean duration of induced AF (DAF) averaged 36 s in non-paced control dogs, and VTP-only increased DAF to over 1000 s ($p < 0.001$, Fig. 3A). Fenofibrate-treated VTP dogs showed a similar increase. However, VTP-induced AF-duration increases were prevented by both doses of simvastatin. As previously observed [2], VTP did not increase atrial vulnerability to premature extrastimuli (Fig. 3B).

3.2. Hemodynamic indices

There were no significant differences in LV systolic pressure among groups (Fig. 4A). Simvastatin attenuated VTP-induced increases in LVEDP (Fig. 4B), LA (Fig. 4C) and RA (Fig. 4D) pressures, with the lower dose having significantly smaller effects than the higher. Fenofibrate had no effects on hemodynamics.

3.3. Biochemical measures

Fig. 5 shows the expression of NOS-3 (left panels), NOS-2 (middle), and nitrotyrosine (right) in LA (top panels) and LV (bottom). Representative bands are shown above the mean data bars for each group. Clear bands were obtained at 140 (left panels) and 130 kD (middle panels), corresponding respectively to expected molecular masses of NOS-3 and NOS-2 protein. Atrial NOS-3 and NOS-2 protein expression levels were increased by 2-week VTP, a response not affected by treatment with simvastatin or fenofibrate (panel A–B). Atrial nitrotyrosine expression was unaffected by 2-week VTP or drug treatment (panel C). LV NOS-3, NOS-2, and nitrotyrosine were significantly increased by VTP: these increases were prevented by simvastatin but not fenofibrate (panels D and E). Serum CK was not affected by any of the interventions applied (Table 1).

3.4. Atrial fibrosis

Representative Masson-trichrome stained tissue sections from each group are shown in Fig. 6A–E. In non-paced controls (panel A), atria appeared grossly normal. In VTP-only dogs (panel B), bundles of myofibers were separated by fibrous tissue. VTP-induced atrial fibrosis was attenuated in simvastatin-treated dogs (panels C and D), but not in fenofibrate-treated dogs (panel E). The almost 10-fold mean fibrous-tissue increase in VTP-only dogs was suppressed by simvastatin, but not by fenofibrate (Fig. 6F).

3.5. Atrial fibroblast responses

There were no inter-group differences in atrial-fibroblast proliferative response in the absence of a proliferative stimulus (Fig. 7A). However, 7% FBS stimulation significantly increased [^{3}H]thymidine uptake, a phenomenon prevented by simvastatin but not by fenofibrate.

Fig. 7B (top) shows representative αSMA immunoblots in TGFβ$_1$-stimulated atrial-fibroblast preparations, above bar graphs (bottom) showing corresponding mean data. There were no inter-group differences in mean αSMA
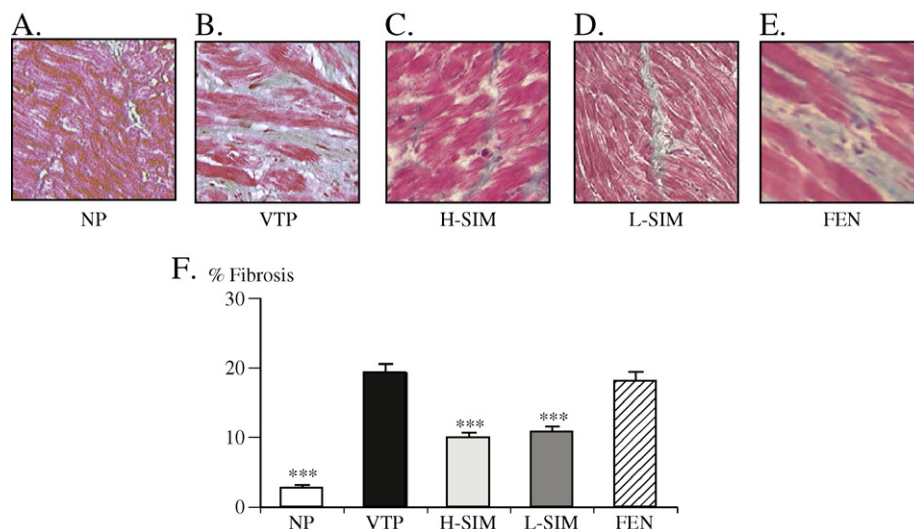

Fig. 6. A–E: Masson's trichrome-stained light micrographs from 1 representative dog per group (original magnification ×400). F: Mean ± SEM connective-tissue content. ***$p < 0.001$ versus VTP-only. Abbreviations as in Fig. 1.
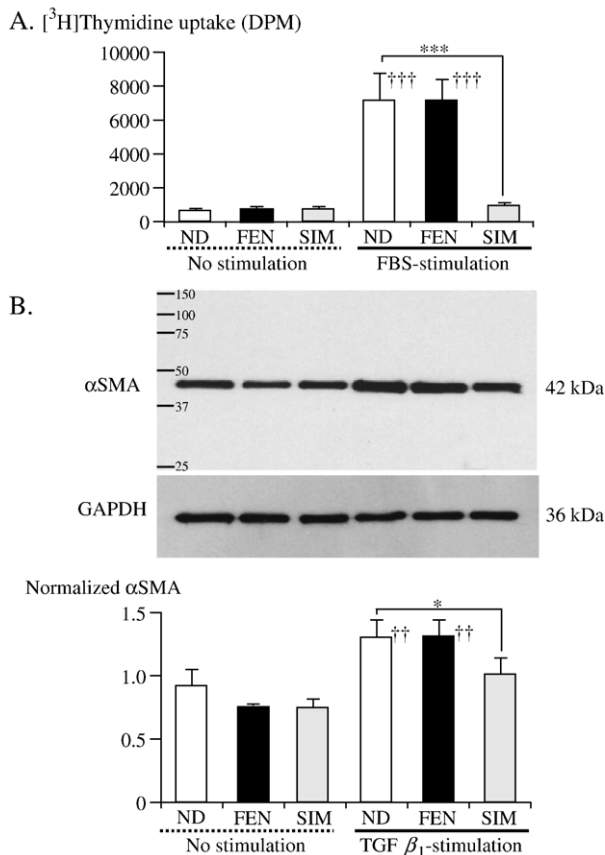
Fig. 7. A: Mean±SEM atrial fibroblast proliferation in each group, with and without 7%-FBS stimulation. B: Representative αSMA (Top, MW markers indicated) and GAPDH (Middle) bands corresponding to bars in bottom panel. Bottom: Mean±SEM αSMA band intensities relative to corresponding GAPDH signals for each sample. DPM=disintegrations per minute. ND=no-drug control; FEN=fenofibrate-treated cells; SIM=simvastatin-treated cells. $*p < 0.05$, $***p < 0.001$ versus ND with stimulation. $\dagger\dagger p < 0.01$, $\dagger\dagger\dagger p < 0.001$ versus ND without simulation.

expression without TGFβ1-stimulation. TGFβ$_1$-stimulation increased αSMA expression, a response attenuated by simvastatin, but not by fenofibrate.

4. Discussion

In this study, we found that simvastatin, but not fenofibrate, prevents AF promotion related to CHF by attenuating atrial arrhythmogenic remodeling as reflected by atrial fibrosis and conduction abnormalities. The beneficial effects of simvastatin were associated with attenuation of LV dysfunction and direct suppressant effects on atrial fibroblast proliferation and differentiation.

4.1. Previous observations regarding atrial structural remodeling prevention

Atrial fibrosis is common in patients with AF [30] and is seen in animal models of AF associated with heart disease [2,23,31]. Goette et al. reported that AT1 receptors are

downregulated, and AT2 receptors upregulated, in AF patients [32]. They also observed increased expression of angiotensin converting enzyme (ACE) and extracellular signal-regulated kinase (ERK) in atria of AF patients [33]. Angiotensin II is important for CHF-related atrial ERK phosphorylation and arrhythmogenic structural remodeling in experimental CHF [23].

ACE inhibitors [23,34] and angiotensin II-receptor blockers (ARBs) [35] prevent CHF-related arrhythmogenic atrial structural remodeling in dogs. Clinical studies suggest that ACE inhibition prevents AF in patients with left ventricular dysfunction [36,37] and hypertension [38,39]. A recent study showed that patients with left ventricular dysfunction taking lipid-lowering drugs (92% statins, 6% fibrates) have a reduced prevalence of AF [17]. Our findings suggest the prevention of structural remodeling as a potential mechanism, and further suggest that statins may be more effective than fibrates. Further work on the relationship between mechanisms of statin efficacy in CHF-remodeling and those of other agents, like those that inhibit the renin–angiotensin system, is needed. In addition, it would be interesting to study the effects of a combination of statins and ACE inhibitors or ARBs compared to that of statins alone and ACE inhibitors or ARBs alone.

4.2. Potential mechanisms of simvastatin effects

Statins act as antioxidants by inhibiting superoxide production via Rac1 [40]. In failing human myocardium NADPH oxidase-related reactive oxygen species production increases, with enhanced expression and activity of Rac1, and oral statin treatment inhibits myocardial Rac1-GTPase activity [41]. Simvastatin attenuates cytokine-inducible NOS expression [42] and prevents myocardial nitrotyrosine increases and left ventricular hypertrophy in pressure-overloaded rats [43]. Cerivastatin improves LV remodeling and decreases tyrosine-nitration in myocardial infarction-induced heart failure [44]. NOS-2 is expressed at high levels in the failing myocardium [45]; however, the role of NOS-2 in the pathophysiology of CHF remains unclear. Conflicting results have been reported in murine models of NOS-2 overexpression [46,47]. Nitric oxide reacts with superoxide to form peroxynitrite, a toxic reactive nitrogen intermediate that leads to protein tyrosine nitration [48] and increases nitrotyrosine expression. The role of NOS-3 in CHF is also controversial [49]. NOS-3 expression increases in CHF [50] and NOS-3 uncoupling occurs [51]. NOS-3 uncoupling leads to reactive oxygen-species generation and adverse ventricular remodeling [52]. In the present study, ventricular NOS and nitrotyrosine overexpression was attenuated by simvastatin and this may have contributed to preventing tachypacing-induced ventricular dysfunction. This attenuation of ventricular dysfunction, as evidenced by reduced filling pressures in simvastatin-treated VTP dogs, could have contributed to reducing atrial remodeling. That simvastatin attenuation of LV-dysfunction may not be the only

mechanism is suggested by the observation that lower-dose simvastatin prevented AF promotion, conduction abnormalities and atrial fibrosis as well as the higher dose, but had significantly-weaker effects on hemodynamics.

Simvastatin reduces human atrial myofibroblast proliferation via a RhoA pathway [53]. Cytokine-induced atrial myofibroblast proliferation is also prevented by therapeutic concentration of simvastatin [54]. In the present study, we observed that simvastatin, but not fenofibrate, inhibits canine atrial fibroblast proliferation and αSMA expression, which parallels collagen-synthetic fibroblast function [55]. We elected to compare simvastatin to fenofibrate because both are lipid-lowering drugs and PPAR-α agonists like fenofibrate also have pleiotropic anti-inflammation and antiproliferative effects [13,14]. Our results show that despite some similarities, statins and PPAR-α agonists have very different efficacy in preventing CHF-related atrial structural remodeling.

The ability of statins to prevent both CHF-induced and atrial tachycardia remodeling, which have very different electrophysiological manifestations [2], is interesting. There are suggestions of involvement of inflammation and oxidant stress in both [4–7], and statins have antioxidant and anti-inflammatory properties [8,9]. Further work on the detailed molecular basis of statin-protection in both models is needed to clarify the inter-relationships.

4.3. Limitations of the study

Although NOS-3 and NOS-2 were upregulated in left atria and ventricles of VTP dogs, nitrotyrosine was upregulated only in left ventricles. This may suggest that oxidative and/or nitration stress occurs primarily at the ventricular level. However, the lack of NOS overexpression reversal by simvastatin at the atrial level is puzzling and remains unexplained. Despite its lack of effect on atrial NOS, simvastatin prevented atrial fibrosis, suggesting that atrial NOS upregulation may not be directly involved in CHF-related atrial fibrosis. Further work is needed to probe the role of antioxidant and NOS-related effects of statins in preventing CHF-induced structural remodeling.

Our doses of simvastatin covered the range from standard clinical doses to the equivalent of higher doses (up to 160 mg/day) shown to provide additional efficacy without additional adverse effects [56]. Our fenofibrate doses were based on previous animal studies [21,22], reported to produce clinically-relevant concentrations [22]. Neither drug produced myotoxicity based on CK measurements. We cannot exclude potential direct ion-channel blocking effects of simvastatin or fenofibrate. However, neither drug altered atrial repolarization as reflected by ERP or atrial conduction as reflected by P-wave duration, making a significant role of direct ion-channel effects unlikely. Simvastatin almost completely reversed VTP effects on AF duration and conduction abnormalities, but decreased fibrosis by only ~50%. Further assessment of the quantitative relation between drug-induced antifibrotic effects and the AF-substrate will be important to understand this apparent discrepancy.

4.4. Potential clinical implications

The elucidation of the mechanisms and responses of atrial remodeling may lead to novel approaches to AF therapy [3]. We previously demonstrated that simvastatin prevents atrial tachycardia-induced AF promotion [12]. Another report has described statin efficacy in sterile pericarditis-related AF [57]. In the present study, we analyzed the effects of simvastatin and another lipid-lowering agent with potential anti-inflammatory and anti-fibrotic actions, fenofibrate, in the AF paradigm associated with CHF-related arrhythmogenic structural remodeling. The results support the potential utility of simvastatin, in contrast to the inefficacy of PPAR-α activation, in AF occurring in the setting of CHF, one of the most common clinical contexts for AF occurrence.

To our knowledge, our study is the first to demonstrate the effectiveness of simvastatin on arrhythmogenic atrial structural remodeling. Since statins are commonly used in clinical practice and have limited, well-recognized adverse effects, they may be an interesting adjunct in the treatment of CHF-related AF.

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