The HMG-CoA reductase inhibitor atorvastatin prevents atrial fibrillation by inhibiting inflammation in a canine sterile pericarditis model

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Abstract

Objective: It has been recently reported that AF is associated with tissue inflammation. Statins reduce C-reactive protein (CRP) levels. However, the effect of statin on atrial fibrillation (AF) is unclear. The purpose of the present study was to evaluate the effect of statin on AF in a canine sterile pericarditis model.

Methods: Sterile pericarditis was created in 20 dogs randomly assigned to two groups: a control group (10 dogs) and an atorvastatin treatment group (10 dogs). Atorvastatin was administered orally (2 mg/kg/day) beginning 1 week before the operation until the end of the study. Before and 2 days after the operation, CRP levels, the duration of induced AF, the atrial effective refractory period (AERP), and intra-atrial conduction time were determined.

Results: Before the operation, there were no significant differences in any of the parameters between the two groups. On the second postoperative day, the atorvastatin group had a lower CRP level (7.6 ± 0.5 versus 11.7 ± 1.3 mg/dl, P < 0.0001), a shorter AF duration (177 ± 57 versus 534 ± 189 s, P < 0.0001), a longer AERP (138 ± 6 versus 130 ± 6 ms, P < 0.01), and a shorter intra-atrial conduction time (46 ± 3 versus 51 ± 5 ms, P < 0.01) than the control group.

Conclusions: Atorvastatin can prevent maintenance of AF by inhibiting inflammation in the canine sterile pericarditis model. Atorvastatin may thus be a novel therapeutic agent for AF.

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Keywords: Arrhythmias; Inflammation; Statins

1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in humans. Although its maintenance is thought to be due to multiple wavelets reentry occurring in both atria [1], the pathophysiological mechanism underlying the genesis of reentrant substrate is not well understood. AF may persist due to structural changes in the atria that are promoted by inflammation. Recently, Chung et al. [2] reported that C-reactive protein (CRP) was elevated in patients with AF and higher in patients with persistent AF compared to those with paroxysmal AF. However, the effect of inflammation on atrial electrophysiological properties is unclear.

Moreover, whether CRP elevation is a cause rather than a result of AF could not be determined based on their results.

Evidence for an inflammatory contribution to at least some forms of AF was initially suggested by the high incidences (25–40%) of AF after cardiac surgery. According to this fact, Page et al. [3] developed the canine sterile pericarditis model. In this model, AF can be induced and peaks on the second postoperative day [4].

It is conceivable that the prevention of AF with elevated CRP might be improved by using anti-inflammatory agents or other CRP-lowering drugs. Recent studies have shown that it is possible to modulate CRP levels with pharmacological interventions, including HMG-CoA reductase inhibitor (statin) drugs [5–7]. Statin reduces cardiovascular risk, the mechanism of which may include diminished arterial inflammation, as suggested by a reduction in levels of CRP in serum that appear to be independent of a reduction in LDL cholesterol levels [8,9]. However, it remains unclear whether a reduction...
in CRP levels would have a beneficial effect on the inducibility or maintenance of AF.

Therefore, we hypothesized that a statin could prevent maintenance of AF by inhibiting inflammation in a canine sterile pericarditis model.

2. Methods

2.1. Animal preparation

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Twenty mongrel dogs of either sex, weighing 18–23 kg, were randomly divided into two groups. In the atorvastatin group (n=10), oral administration of atorvastatin (2 mg/kg/day) was started 1 week before the baseline study, and was continued until the end of the study. The dogs in the control group (n=10) did not receive atorvastatin. Investigators were blinded to treatment.

All dogs were anesthetized with an intravenous injection of pentobarbital (25 mg/kg). Anesthesia was maintained with halothane after intubation and mechanical ventilation. The chest was opened through the right fourth intercostal space, and four electrodes pair were sutured to four sites in the right atrium (appendage, high lateral, low lateral, and anterior walls).

2.2. Baseline electrophysiological study

The arterial blood gases were adjusted to between pH 7.35 and 7.45 during ventilation. The surface ECG lead II, intracardiac electrograms and blood pressure were continuously monitored and recorded during the experiment. Bipolar intracardiac electrograms were recorded at a filter setting of 30–500 Hz and stored digitally on an EPLab system (Quinton Electrophysiology, Canada) simultaneously with the surface ECG. A programmed stimulator (Fukuda Denshi BC02A, Japan) was used to deliver square-wave impulses of 1-ms duration.

The atrial effective refractory period (AERP) at four sites was measured at three basic cycle lengths (200, 300 and 400 ms). Five basic drive stimuli were followed by a single premature stimulus, and all stimuli were twice the diastolic threshold. The S1–S2 interval was increased in steps of 2 ms, and AERP was determined to be the shortest S1–S2 interval that resulted in a propagated atrial response. Intra-atrial conduction times (CT) from the appendage to the other three sites (high lateral, low lateral, and anterior walls) were measured during appendage pacing at each basic cycle length.

After measuring the baseline AERP and CT, AF was repeatedly induced. Induction of AF was attempted for 1 h using burst atrial pacing of 90–110 ms for 20 beats from each electrode site. AF was defined as a rapid (mean cycle length <150 ms) atrial rhythm with variability of the beat-to-beat cycle length, polarity, morphology, and/or amplitude of the recorded bipolar atrial electrograms.

The canine sterile pericarditis model was prepared as previously described [3,4]. Upon completion of the surgery, the dogs were given antibiotics and then allowed to recover. Postoperative care included the administration of antibiotics and analgesics.

2.3. Postoperative electrophysiological study

On the second postoperative day, the dogs were reanesthetized with pentobarbital (25 mg/kg) and ventilated with halothane. After measuring the AERP and CT, AF was repeatedly induced. AF induction was attempted for 1 h using burst atrial pacing of 90–110 ms for 20 beats from each electrode site. If AF was sustained >15 min, it was defibrillated and re-induced after 5 min.

2.4. Histology

At the end of the experiments, the heart was quickly removed. To investigate the influence of pericarditis on pathological properties, the atrial tissues of five sham dogs without pericardial incision were observed on the second postoperative day in the same operation. The tissues of the left and right atrial free wall and appendages were cut into small blocks about 10 × 5 mm and immersed in 10% phosphate-buffered formalin for 24 h. After dehydration, the each section was cut into 4-μm-thick slices. Deparaffinized sections were stained with haematoxylin–eosin and Masson’s trichrome. Microscopic images were scanned into a personal computer with Photoshop. Image files were analyzed with NIH software. Connective tissue was differentiated on the basis of its color and expressed as a percentage of the reference tissue area. These analyses were performed by a pathologist who is blinded to treatment.

2.5. CRP assay

Plasma samples were obtained at the beginning of the study and on the second postoperative day. Canine CRP concentrations were measured by a laser nephelometric immunoassay using Laser CRP-2 (Arrows, Osaka, Japan).

2.6. Statistical analysis

All values are expressed as the mean ± S.D. Continuous variables before and after the operation were compared by the paired Student’s t-test. An unpaired t-test was used to evaluate the differences in discrete variables between the sustained AF and nonsustained AF, or...
between the atorvastatin group and the control group. A $P<0.05$ was considered to be statistically significant.

3. Results

3.1. Characteristics of induced AF

Before the induction of pericarditis, single extrastimulation and the atrial burst pacing could not induce AF lasting more than 10 s in all dogs. On the second postoperative day, in the control group, a total of 42 episodes of AF lasting more than 300 s (range, 340–900 s; mean duration, 534 ± 189 s) were induced in all 10 dogs.

3.2. Atorvastatin versus control

Before the operation, there were no significant differences in any of the parameters between the atorvastatin group and control groups (Table 1). On the second postoperative day, CRP and AF duration were significantly increased in both groups; however, CRP was significantly lower in the atorvastatin group than in the controls ($7.6 ± 0.5$ versus $11.7 ± 1.3$ mg/dl, $P<0.0001$, Fig. 1) and the atorvastatin group had a shorter AF duration than the

![Graph showing CRP levels comparison between control and atorvastatin groups.](Image 309x139 to 542x358)

![Graph showing duration of induced atrial fibrillation comparison.](Image 309x503 to 542x728)

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group (n = 10)</th>
<th>Atorvastatin group (n = 10)</th>
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<tr>
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<td>after</td>
</tr>
<tr>
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<td>135 ± 3</td>
<td>124 ± 8</td>
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<tr>
<td>300</td>
<td>141 ± 3</td>
<td>130 ± 6</td>
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<td>LRA 200</td>
<td>125 ± 5</td>
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</tr>
<tr>
<td>300</td>
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<td>137 ± 8</td>
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<td>143 ± 8</td>
<td>130 ± 9</td>
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**Intra-atrial CT (ms)**

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<td>RAA-LRA 200</td>
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<td>44 ± 4</td>
<td>47 ± 3*</td>
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<td>36 ± 3</td>
<td>38 ± 4*</td>
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<td>28 ± 4*</td>
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<td>25 ± 4</td>
<td>33 ± 6</td>
<td>26 ± 3</td>
<td>28 ± 4*</td>
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AERP = atrial effective refractory period; CT = conduction time; RAA = right atrial appendage; LRA = low lateral right atrium; HRA = high lateral right atrium; ARA = anterior right atrium.

* $P<0.05$ compared with the control group.

** $P<0.01$ compared with the control group.

1 $P<0.05$ compared with before the operation.

1 $P<0.01$ compared with before the operation.

1 $P<0.0001$ compared with before the operation.
control group (177 ± 57 versus 534 ± 189 s, \( P < 0.0001 \), Fig. 2). In both groups, AERP at each site was significantly shortened. In the control group, intra-atrial CT was significantly prolonged after operation, whereas in atorvastatin group, there was no significant difference in intra-atrial CT before and after the operation (Table 1). However, after the operation the atorvastatin group had a significantly longer AERP, and a significantly shorter intra-atrial CT compared to the control group (Table 1).

### 3.3. Pathological examination

Histological studies were performed to identify the potential pathological substrate underlying the electrophysiological abnormalities in the sterile pericarditis dogs. Representative histological sections from each group are shown in Fig. 3. Atrial myocyte from sham dogs showed a normal composition of sarcomeres distributed throughout the cell, and the intracellular space also appeared normal. In contrast, atrial myocytes of control dogs showed active perimyocarditis, which consisted of inflammatory infiltrate with lipid degeneration. Inflammatory cell foci were multiple and involved the myocardium. In addition, extensive interstitial fibrosis, evidenced by Masson trichrome stain was found in these tissues. Thick layers of fibrous tissue were observed in the epicardium. Furthermore, the amount of connective tissue was increased and this extended around the parenchymal cells. In contrast, these pathological abnormalities in the atrial tissues were attenuated in the atorvastatin group.

Fig. 4. Percentage of fibrosis of the free walls and appendages in both atria after the operation. The percentage of fibrosis in all atrial regions in the atorvastatin group was significantly lower than that in the control, although greater than that in the sham group. Gray bars = sham group; white bars = control group; black bars = atorvastatin group. ***\( P < 0.0001 \), **\( P < 0.001 \), *\( P < 0.01 \), compared with the control group. #\( P < 0.001 \) compared with the sham group. RAFW = right atrial free wall; RAA = right atrial appendage; LAFW = left atrial free wall; LAA = left atrial appendage.

A quantitative analysis of the fibrosis is shown in Fig. 4. The percentage of fibrosis in all atrial regions in the atorvastatin group was markedly lower than that in the control group (16 ± 4% versus 26 ± 7% at the right atrial appendage, \( P < 0.001 \)), although greater than that in the sham group (16 ± 4% versus 7 ± 2% at the right atrial appendage, \( P < 0.001 \)).
4. Discussion

4.1. Inflammation and atrial electrophysiological changes

Activation of the complement system and release of proinflammatory cytokines occur after cardiac surgery, suggesting the presence of an intense inflammatory process. CRP, a prototypic marker of inflammation, is driven by the proinflammatory cytokines interleukin (IL)-1, tumor necrosis factor-α, and IL-6 [10]. Bruins et al. [11] reported that IL-6 levels rise markedly, peaking 6 h after surgery. A second phase then occurs with an increase in CRP, which peaks on the second postoperative day, and increases in complement-CRP complexes peaking on the second or third postoperative day. The incidence of atrial arrhythmias similarly peaks 2–3 days after surgery. In the canine sterile pericarditis model, AF can be induced and it also peaks on the second postoperative day [4]. Mapping studies during AF in the sterile pericarditis model have shown that multiple unstable reentrant circuits are critical for maintaining AF [4]. Since the number of wavelets that can be present simultaneously is determined by the atrial wavelength, which is the product of AERP and the intra-atrial conduction velocity, this parameter has been thought to be important for the perpetuation of AF [1]. Prolonged atrial pacing and repetitive induction of AF have been shown to shorten AERP and the atrial wavelength and to increase the inducibility and stability of AF [12]. This study is the first to evaluate the role of inflammation on atrial electrophysiological properties. Thus, in this model, elevated CRP was associated with sustained AF, suggesting that electrophysiological changes resulting from inflammation perpetuate AF.

4.2. Inflammation and atrial structural changes

Atrial structural remodeling may occur from inflammatory stressors. The anatomic substrate of electrical atrial instability has been investigated in vivo by both surgical and atrial biopsy approaches. Basso et al. [13] reported a 50% incidence of isolated atrial myocarditis in fatal Wolff–Parkinson–White cases. This finding supports the hypothesis that atrial inflammatory foci may act as a trigger of paroxysmal AF. Moreover, Rossi [14] looked at a small series of patients with AF and found striking right atrial inflammatory changes in five of eight cases. Notably, the possibility of an isolated arrhythmogenic atrial mycariditis was put forward by Fromer et al. [15], who studied two cases with drug-refractory ectopic atrial tachycardia; surgically resected atrial tissue showed focal myocarditis at endomyocardial biopsy associated with a minor elevation of antibodies against echovirus in one case. In the present study, active perimyocarditis, which consisted of patchy inflammatory infiltrate with lipid degeneration, occurred in dogs with sustained AF and was confined to the atrial myocardium. Inflammatory cell foci were multiple and involved the myocardium. Thus, inflammatory changes may contribute to atrial structural remodeling and increase the propensity for AF to persist.

4.3. Statin and AF

Recently, numerous trials with the statins demonstrated a significant reduction in cardiovascular events [16]. Numerous prospective epidemiological studies have clearly demonstrated an increased risk with increasing CRP levels [17,18]. In addition, studies have demonstrated that CRP confers risk above that of an abnormal lipid profile [17,19]. Thus, modalities targeting inflammation and reducing proinflammatory cytokines and CRP levels could be a potential additional strategy in the prevention of cardiovascular disease. Ridker et al. [20] demonstrated that patients who have increased CRP levels (increased inflammation) gain a greater benefit from pravastatin therapy [20] and that median CRP levels were reduced 17.4% in the group that received pravastatin [5]. Furthermore, Jialal et al. [9] showed that atorvastatin also results in a significant reduction in CRP levels (mean percent reduction in CRP levels: 28.3%). Therefore, it is conceivable that the prevention of AF with elevated CRP might be improved by the use of statins. The present study is the first to document the prevention of AF with elevated CRP by atorvastatin. Atorvastatin prevented the promotion of atrial electrophysiological and structural changes resulting from inflammation. Thus, atorvastatin could attenuate the substrate of AF by inhibiting inflammation in the present canine sterile pericarditis model.

4.4. Study limitations

In the present study, IL-6 levels were not measured, because it has been reported that there is significant circadian variation in IL-6 levels and statin drugs had no effect on IL-6 levels [9].

We used the high-frequency burst pacing to induce AF, because a single extrastimulus could not induce AF in this model. Therefore, this approach allowed to assess AF maintenance, but not AF inducibility.

Although we proved that atorvastatin prevented maintenance of AF in the present canine pericarditis model, these results could not be extrapolated to other animal AF models or non-postoperative AF patients. However, Chung et al. [2] demonstrated that CRP was also elevated in patients with lone AF in the absence of structural heart disease when compared with the control subjects. Moreover, inflammatory changes have also been reported in patients with non-postoperative AF. In a series of 12 patients with drug-refractory paroxysmal AF studied by atrial endomyocardial biopsy, Frustaci et al. [21] found isolated atrial lymphocytic myocarditis in 66% of the cases. The cause-effect relationship between myocarditis and AF was further supported by
the absence of AF recurrence in patients treated with steroids. Thus, recent data suggest that lone paroxysmal AF may be due to isolated atrial myocarditis. Therefore, anti-inflammatory agents or CRP-lowering drugs such as statins might improve the prevention of AF. Randomized trials of statins in patients with AF may be warranted.

In addition, the pleiotropic effects of statins may be largely mediated by nitric oxide [22], which can induce cardioprotection [23]. It has been demonstrated that statins can attenuate oxidant induced mitochondrial dysfunction in cardiac myocytes [24] and downregulate the activity of small G proteins in cardiomyocytes and, thereby, influence surrogate markers of cardiac dysfunction such as atrial natriuretic factor and myosin light chain-2 [25]. These additional effects of statins may be involved in their anti-arrhythmic activity.

4.5. Clinical implications

Epidemiological studies have shown that increased CRP level predict patients at increased risk for future myocardial infarction and thromboembolic stroke [19,26,27]. These studies suggest an important role for inflammation in the development of and/or risk for coronary atherosclerosis, possibly due in part to direct inflammatory effects of CRP on coronary endothelial cells [28]. The association between CRP and thromboembolic risk could be related to an association of CRP with AF. CRP may have prothrombotic effects by increasing tissue factor expression [29]. Therefore, the prevention of AF or thromboembolism in patients with elevated CRP might be improved by the use of statins. In the present study, we showed that atorvastatin could prevent the maintenance of AF in a canine pericarditis model. In addition, the result of a recent clinical study has shown that the use of statins in patients with lone AF was associated with a decrease in the recurrence of AF after successful cardioversion [30]. Thus, these results suggest that statins may constitute a novel therapeutic approach to preventing AF.

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References


