Prednisone prevents atrial fibrillation promotion by atrial tachycardia remodeling in dogs

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Abstract

Background: There is evidence suggesting involvement of oxidative stress, inflammation, and calcineurin/nuclear factor of activated T cell pathways in atrial fibrillation. This study evaluated the efficacy of anti-inflammatory and calcineurin-inhibitory drugs on promotion of atrial fibrillation by atrial tachycardia-induced remodeling in dogs.

Methods and results: Dogs were subjected to atrial tachypacing at 400 bpm in the absence and presence of treatment with prednisone (15 or 50 mg/day) or ibuprofen (anti-inflammatory) or cyclosporine-A (calcineurin inhibitor). Serial closed-chest electrophysiological studies were performed in each dog at baseline and 2, 4, and 7 days after tachypacing onset. A final open-chest study was performed on day 8. Serum C-reactive protein was measured by ELISA and nitric oxide synthase by Western blotting. The mean duration of induced atrial fibrillation was markedly increased by tachypacing alone, from 26 ± 8 to 962 ± 317 s (p < 0.01), and the atrial effective refractory period was decreased from 117 ± 4 to 73 ± 7 ms (p < 0.001; cycle-length 300 ms). Tachypacing-induced effective refractory period shortening and atrial fibrillation promotion were unaffected by ibuprofen or cyclosporine-A; however, both doses of prednisone suppressed tachypacing-remodeling effects (atrial fibrillation duration to 96 ± 60 s and 28 ± 11 s at higher and lower doses, respectively; effective refractory period to 101 ± 6 ms for higher-dose and 105 ± 3 ms for lower-dose group). In addition, prednisone (but not ibuprofen or cyclosporine) significantly decreased C-reactive protein concentrations and attenuated the increase in endothelial nitric oxide synthase expression caused by atrial tachypacing.

Conclusions: Prednisone prevents the electrophysiological and atrial fibrillation-promoting effects of atrial tachycardia-remodeling, possibly by an anti-inflammatory action, whereas the less potent anti-inflammatory ibuprofen and the calcineurin inhibitor cyclosporine-A are without effect.

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1. Introduction

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. Drug therapy for AF is presently suboptimal and there is interest in developing novel approaches that target specific mechanistic determinants [1]. A role for inflammation was first suggested based on observations in postoperative AF [2]. The time of peak AF occurrence (second to third postoperative day) coincides with peak concentrations of the inflammatory marker, C-reactive protein (CRP) [2]. Histological changes consistent with myocarditis were reported in 66% of biopsy specimens from lone-AF patients [3]. CRP is elevated in AF patients and higher CRP levels are observed in persistent than in paroxysmal AF [4]. CRP concentrations are also a predictor...
of future AF [5]. A small clinical trial indicated that methylprednisolone therapy can prevent AF recurrence, supporting a potential role for inflammation in AF [6].

AF remodels atrial electrophysiology in a way that favors AF maintenance and increases vulnerability to recurrence should AF terminate [7–10], an effect attributable principally to atrial tachycardia [1,8,10]. There is evidence that this process is important in AF pathophysiology, and suppressing it may contribute to the clinical efficacy of amiodarone [11]. Simvastatin, which has anti-inflammatory properties, suppresses atrial-tachycardia remodeling [12]. We wondered whether anti-inflammatory agents, such as glucocorticoids, might also suppress atrial-tachycardia remodeling. We therefore designed this study to assess the effects of prednisone and the non-steroidal anti-inflammatory drug ibuprofen on the electrophysiological consequences of atrial tachycardia in dogs. In addition, evidence has been presented for calcineurin activation as a signal transduction pathway in porcine tachycardia-induced AF [13] and in tissue from AF patients [14]. We therefore added an additional group of dogs to determine the effect on atrial-tachycardia remodeling of inhibiting calcineurin-signaling with the calcineurin-antagonist agent cyclosporine-A.

2. Materials and methods

2.1. Animal preparation

Animal-handling procedures followed guidelines of the National Institutes of Health. Forty-two mongrel dogs (20–37 kg) were anesthetized with ketamine (5.3 mg/kg, i.v.), diazepam (0.25 mg/kg, i.v.), and halothane (1.5%). Unipolar pacing leads were inserted through jugular veins into the right ventricular (RV) apex and right atrial (RA) appendage under fluoroscopic guidance, and were connected to pacemakers (Vitatron, USA) in subcutaneous pockets in the neck. A bipolar electrode was inserted into the RA for stimulation and recording during serial closed-chest electrophysiological studies (EPSs). Atrioventricular block was created by radiofrequency-catheter ablation to avoid excessively rapid ventricular responses during atrial tachypacing (ATP). The RV demand-pacemaker was programmed to 80 bpm.

After 24-h post-operative recovery, a baseline closed-chest EPS was performed and then 7-day ATP at 400 bpm was instituted. Closed-chest EPS was repeated at 2, 4 and 7 days of ATP and the final open-chest EPS was performed on day 8 under morphine/α-chloralose anesthesia.

2.2. Groups and drugs

Results in ATP dogs with various interventions were compared with those in 8 ATP dogs without treatment (ATP-only group) and 10 non-paced control dogs. Treatment groups were: 1) prednisone, 15 and 50 mg/day (n=6/group); 2) ibuprofen, 1500 mg/day (n=6); and 3) cyclosporine-A, 300 mg/day (n=6). All drugs were given orally in 2 divided doses, started 3 days prior to ATP onset and continued until the morning of the final open-chest EPS. We used a larger number of non-paced and ATP dogs than in the drug-intervention groups because we performed concomitant controls for each intervention series.

2.3. Electrophysiological study

For closed-chest EPS, dogs were anesthetized with ketamine (5.3 mg/kg, i.v.), diazepam (0.25 mg/kg, i.v.) and isoflurane (1.5%), and ventilated mechanically. The atrial tachypacemaker was deactivated and effective refractory period (ERP) of the RA appendage was measured at basic cycle lengths (BCLs) of 150, 200, 250, 300, and 360 ms with 10 basic stimuli (S1) followed by premature extrastimuli (S2s) with 5-ms decrements. All stimuli were twice-threshold, 2-ms pulses. The longest S1–S2 interval failing to capture defined the ERP. AF was induced with 1–10 s burst pacing (10 Hz, 4× threshold current). To estimate mean AF duration in each dog, AF was induced 10 times for AF duration <20 min and 5 times for 20–30 min AF. Prolonged AF (>30 min) was terminated by direct current (DC) electrical cardioversion. A 20-min rest period was then allowed before continuing measurements. If prolonged AF was induced twice, no further AF induction was performed.

At the terminal open-chest EPS, the atrial tachypacemaker was deactivated and EPS performed during sinus rhythm. Dogs were anesthetized with morphine (2 mg/kg, s.c.) and α-chloralose (120 mg/kg, i.v., followed by 29.25 mg/kg/h), 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 into the RA and left atrial (LA) appendages for recording and stimulation. A programmable stimulator (Digital Cardiovascular Instruments) was used to deliver 2× threshold current, 2-ms duration pulses. Silicon sheets containing 240 bipolar electrodes were attached to the atria as previously described [10–12]. Atrial ERPs were measured at multiple BCLs in the RA appendage, and at a BCL of 300 ms at 6 additional sites: RA and LA posterior wall, RA and LA inferior wall, RA and LA Bachmann’s bundle. AF vulnerability was determined as the percentage of atrial sites at which AF was induced by single extrastimuli.

2.4. CRP, ibuprofen and cyclosporine-A concentrations

Blood samples were collected in each ATP dog just before the commencement of ATP and at day 7. Serum was removed and stored at −80 °C for subsequent CRP and ibuprofen concentration analysis. CRP concentration was measured with the Phase Range® canine CRP ELISA kit (Tri-delta Diagnostics, USA) [12]. Serum ibuprofen con-
concentration was analyzed by high-performance liquid chromatography with an LC-8-DB column and ultraviolet detection (220 nm, Mayo Medical Laboratories). Cyclosporine-A concentrations were measured in whole-blood samples with an enzyme-multiplied immunoassay technique (EMIT, Syva Co.) on a VIVA analyzer (Dade Behring Diagnostics, Marburg, Germany).

2.5. Nitric oxide synthase (NOS) expression

At the end of open-chest studies, LA tissue samples were fast-frozen in liquid-N₂ and stored at −80 °C. Tissue samples were homogenized in Radio-immuno-precipitation assay buffer as previously described [15]. The homogenate was centrifuged at 15 000 rpm for 20 min at 4 °C. The supernatant was used for protein concentration measurement by Bradford assay with bovine albumin as a standard and 40-µg protein separated with 8% Na–dodecyl-sulfate polyacrylamide–gel electrophoresis. Proteins transferred to nitrocellulose membranes were incubated with primary antibodies against eNOS, iNOS (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 laser densitometry. Protein was loaded in the linear immunoreactive-signal range and target-band intensities expressed relative to GAPDH intensity from the same sample.

2.6. Data analysis

Data are presented as means±S.E.M. Multiple group comparisons were obtained by two-factor mixed-design ANOVA with repeated measures on one factor for ERP data, a one-way ANOVA with repeated measures for time-series of AF duration and rate-adaptation data, or a one-way factorial ANOVA for other data sets. AF duration and CRP data were analyzed after normalization by logarithmic transformation. Bonferroni-corrected *t*-tests were applied 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. Serial assessment of atrial tachycardia-induced electrophysiological changes

Serial ERP changes during ATP are shown in Fig. 1. ERP changes at BCLs of 300 and 200 ms in dogs subjected to ATP-only are compared with those in dogs subjected to ATP

![Fig. 1. Time course of atrial tachypacing-induced atrial ERP changes during serial closed-chest electrophysiological studies as measured in the RA at basic cycle lengths (BCLs) of 300 (A, C) and 200 (B, D) ms. *p<0.05, **p<0.01 vs. ATP-only. ATP= ATP without drug intervention; ATP+H-PDN, ATP+L-PDN, ATP+IBU, ATP+CyA= atrial tachypacing in the presence of higher-dose prednisone, lower-dose prednisone, ibuprofen, and cyclosporine-A treatment, respectively.](image)
in the presence of each drug intervention. There were no significant differences in ERPs among groups at baseline (day 0). ERP shortened substantially within 2 days of ATP and continued to decrease in ATP-only dogs and dogs treated with ibuprofen or cyclosporine-A. ERP decreased slightly in prednisone-treated dogs, but the changes were much smaller than in ATP-only dogs. Although lower-dose prednisone produced slightly smaller effects than higher-dose, both resulted in ERPs significantly greater than AT-only.

ERP rate-adaptation was defined as the difference between ERPs at BCLs 360 and 150 ms. In ATP-only dogs, ERP rate-adaptation decreased significantly within 2 days of ATP onset (Fig. 2A), reflecting rate-adaptation loss typical of ATP-remodeling [7,10]. The loss of ERP rate-adaptation in ibuprofen- (Fig. 2C) and cyclosporine-A- (Fig. 2D) treated dogs was indistinguishable from ATP-only animals, whereas in prednisone-treated dogs rate-adaptation changes were smaller and not statistically significant (Fig. 2B).

Fig. 3 shows the progression of mean AF duration in dogs subjected to ATP in the presence and absence of drug interventions. AF duration increased significantly with ATP-only dogs (Fig. 3A). Both prednisone doses fully suppressed the AF-promoting effect of ATP (Fig. 3B). ATP during treatment with ibuprofen (Fig. 3C) or cyclosporine-A (Fig. 3D) progressively increased AF duration similar to ATP-only dogs.

3.2. Electrophysiological changes at the final open-chest study

There were no significant differences among groups in body weight or hemodynamic variables at final open-chest study, although systolic pressures tended to be higher in the high-dose prednisone group (Table 1). The mean day-7 trough ibuprofen serum concentration was 24.6±3.3 μg/ml (range 15–33 μg/ml), within the range reported to inhibit 96% of cyclooxygenase-1 and 83% of cyclooxygenase-2 [16]. The mean trough cyclosporine-A serum concentration on day 7 was 879±381 μg/L (therapeutic concentration >300 μg/L) [17].

Fig. 4 shows ERPs as a function of BCL during the final open-chest study. In dogs subjected to ATP-only, ERPs were less than 80 ms at all BCLs, and ERP rate-adaptation was virtually abolished. ERPs in dogs subjected to ATP during treatment with prednisone, ibuprofen and cyclosporine-A are shown in Fig. 4A, B and C, respectively. No significant ERP differences were observed between ATP-only dogs and dogs subjected to ATP during ibuprofen or cyclosporine therapy. Dogs treated with prednisone during ATP showed significantly greater ERP values versus ATP-only.

**Fig. 2.** Time course of atrial tachypacing-induced changes in ERP rate-adaptation during serial closed-chest electrophysiological studies. Rate-adaptation was determined by subtracting the ERP at a BCL of 150 ms from the value at a BCL of 360 ms in each dog. ERP rate-adaptation decreased significantly within 2 days of ATP in drugs subjected to ATP without drug therapy (A). A similar effect was seen in ATP+IBU (C) and ATP+CyA dogs (D). ERP rate-adaptation was better preserved during the study period despite atrial tachypacing in prednisone-treated dogs (B). P0, P2, P4, P7=pacing for 0, 2, 4, and 7 days, respectively. ***p<0.001 vs. P0. (Group abbreviations are as in Fig. 1).
Fig. 5 shows regional ERPs at open-chest study. ATP-only reduced ERP in all regions, but the degree of ERP-shortening varied, increasing ERP heterogeneity. The effects of prednisone, ibuprofen and cyclosporine-A on regional ERP changes are shown in Fig. 5A, B and C, respectively. There were no significant differences between in ERP response among ATP-only dogs and ATP dogs treated with ibuprofen or cyclosporine-A. Prednisone significantly attenuated regional ERP-shortening by ATP, with the only remaining statistically significant ATP-induced ERP abbreviation being in the LA appendage.

ERP rate-adaptation in RA (A) and LA (B) appendages at open-chest study are shown in Fig. 6 (top). ERP rate-adaptation was significantly decreased in ATP-only dogs. Dogs treated with either ibuprofen or cyclosporine-A during ATP also showed significant decreases in ERP rate-adaptation. However, ERP rate-adaptation was preserved in prednisone-treated dogs.

In non-paced control dogs, AF was generally short-lasting and always terminated spontaneously within 5 min. Prolonged AF requiring DC-cardioversion was induced in 50% of ATP-only dogs and 33% each of dogs treated with ibuprofen or cyclosporine-A. No prolonged AF requiring cardioversion occurred in prednisone-treated dogs. AF duration averaged \( \sim 25 \) s in non-paced controls (Fig. 6C), and 7-day ATP increased AF duration to almost 1000 s. ATP dogs treated with ibuprofen or cyclosporine-A demonstrated increased AF duration relative to non-paced controls, to the range of 500–1000 s, not significantly different from ATP-only dogs. ATP-induced increases in AF duration were significantly attenuated in prednisone-treated dogs.

### Table 1

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<th>ATP+IBU</th>
<th>ATP+CyA</th>
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NP indicates non-paced control; ATP-only, atrial tachypacing-only; ATP+H-PDN, ATP with higher-dose prednisone; ATP+L-PDN, ATP with lower-dose prednisone; ATP+IBU, ATP with ibuprofen; ATP+CyA, ATP with cyclosporine-A; BP, blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure; LAP, LA pressure.
was induced by single extrastimuli at 61% of atrial sites in ATP-only dogs (Fig. 6D), significantly greater than in non-paced controls (15% of sites, \( p < 0.001 \)).

In dogs subjected to ATP during either ibuprofen or cyclosporine-A treatment, AF was induced at \( \approx 60\% \) of sites, similar to ATP-only dogs. Prednisone prevented AF-vulnerability increases resulting from 7-day ATP, to 8–15% of sites (\( p < 0.01 \) versus ATP-only).

### 3.3. CRP changes

There were no significant CRP differences among groups on day 0 (Fig. 7A). CRP tended to increase over the course of ATP in ATP-only dogs, ibuprofen treated dogs, and cyclosporine-A treated dogs. In prednisone-treated dogs, CRP decreased from day 0 to day 7, becoming significantly lower than for ATP-only dogs at the end of ATP (Fig. 7B).

### 3.4. Atrial NOS

Fig. 8 shows Western blot analyses of LA eNOS and iNOS. Representative blots are shown at the top of each
panel, along with corresponding GAPDH signals. Bands were obtained at expected molecular masses of 140 kDa for eNOS (Fig. 8A) and 130 kDa for iNOS (Fig. 8B). ATP-only increased both eNOS and iNOS expression. Neither ibuprofen nor cyclosporine-A significantly altered the ATP effect. Prednisone suppressed the eNOS enhancing effect of

Fig. 6. Top: ERP rate-adaptation measured at RA (A) and LA (B) appendages during the final open-chest study. ERP rate-adaptation was significantly decreased in dogs subjected to atrial tachypacing without drug therapy (ATP). Rate-adaptation of the ERP was similarly depressed in dogs tachypaced during therapy with ibuprofen (IBU) and cyclosporine-A (CyA). ERP rate-adaptation was relatively preserved (not significantly different from non-paced (NP) dogs) in both atria of prednisone-treated dogs. *p<0.05, **p<0.01, ***p<0.001 vs. NP. Bottom: Indices of AF promotion at the final open-chest study. C: Mean±S.E.M. duration of AF (DAF) as determined with 5–10 AF inductions in each dog. D: AF vulnerability (percentage of atrial sites at which AF could be induced by single extrastimuli). **p<0.01, ***p<0.001 vs. ATP-only. (Abbreviations as in Fig. 5).

Fig. 7. CRP values in various experimental groups on day 0 (P0), immediately prior to tachypacing onset (A), and on day 7 (P7) of tachypacing (B). CRP was not significantly different among study groups on day 0. On day 7, CRP was significantly lower in prednisone-treated tachypaced dogs than in ATP-only dogs. **p<0.01 vs. ATP-only. (Abbreviations as in Fig. 5).
ATP significantly (Fig. 8A), but did not affect ATP’s iNOS-enhancing action.

4. Discussion

4.1. Main findings

In this study, we evaluated the effects of prednisone, ibuprofen, and cyclosporine-A on atrial remodeling due to 1 week of atrial tachycardia. We found that prednisone suppresses both the electrophysiological consequences of atrial tachycardia remodeling and the associated AF promotion, whereas ibuprofen and cyclosporine-A are without effect. Prednisone’s anti-remodeling properties were associated with significant CRP reduction and attenuation of tachycardia-induced eNOS activation.

4.2. Relationship to previous observations regarding drug effects on atrial tachycardia-induced remodeling

Atrial tachyarrhythmias alter atrial electrophysiology, shortening ERP, reducing ERP rate-adaptation and promoting AF occurrence and maintenance [7–10]. Because of clinical evidence for the importance of atrial tachycardia remodeling in the pathophysiology of AF, there have been considerable efforts to define its pathophysiology with an eye to developing pharmacological approaches to its prevention [1]. Calcium overload in cardiomyocytes is considered to play an important role in initiating the process of atrial remodeling [18,19], subsequently leading to atrial ionic, molecular, contractile and ultrastructural changes [20–24]. A number of studies have been performed to pursue pharmacologic approaches to prevent atrial remodeling. L-type Ca\textsuperscript{2+} channel blockers, a Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibitor and an angiotensin-converting enzyme inhibitor are ineffective in preventing remodeling caused by >24 h of atrial tachycardia [25–27]. Drugs with T-type Ca\textsuperscript{2+} channel blocking action, such as mibefradil [26,28] and amiodarone [11], have efficacy in preventing tachycardia remodeling, although both also have a wide range of other properties so that the precise mechanism for their benefit is unclear. Simvastatin prevents atrial tachycardia-induced remodeling in dogs, an effect that could be related to an anti-inflammatory action [12]. In addition, atorvastatin prevents AF induced in the presence of sterile pericarditis in dogs, while decreasing CRP concentrations [29]. The present study is the first of which we are aware showing that glucocorticoids prevent tachycardia-induced remodeling in association with reduced CRP concentrations, and providing one possible mechanism for the results of studies indicating AF suppression by oral glucocorticoid therapy [6,30].

4.3. Relationship to AF pathophysiology

There is evidence for a role of inflammation in several forms of AF. Postoperative AF is associated with CRP increases and complement activation [2], and baseline CRP concentrations are a predictor of postoperative AF for both on-pump and off-pump surgery [31]. CRP concentrations are higher in patients with AF than in sinus rhythm patients [4], and there is an epidemiological association between CRP concentrations and AF prevalence at baseline as well as with AF risk on follow-up [5]. The present study supports a role for inflammatory changes in AF pathophysiology, by indicating that the potent anti-inflammatory compound prednisone suppresses
atrial tachycardia remodeling in association with decreased CRP concentrations. The non-steroidal anti-inflammatory drug (NSAID) ibuprofen did not suppress CRP concentrations or atrial remodeling, consistent with the weaker anti-inflammatory action and lack of CRP-reducing effect known to characterize NSAIDs relative to glucocorticoids [32]. The anti-inflammatory action of NSAIDs is due only to cyclooxygenase inhibition, whereas glucocorticoids act via a variety of mechanisms including redirection of leukocytes and suppression of inflammatory cytokines and leukocyte adhesion molecules [33,34]. Our results are consistent with previous clinical observations of steroid efficacy in AF prevention [6,30], as well as with a recent study showing beneficial effects of glucocorticoids in a model of post-cardiac surgical AF [35]. However, although the present findings are consistent with a role for inflammation in atrial tachycardia remodeling, they do not constitute proof of the notion.

Calcineurin enzyme activity is activated and expression of the downstream signal nuclear factor of activated thymocytes (NFAT) is augmented in pigs subjected to 6 weeks of atrial tachypacing [13]. Calcineurin mRNA expression is also increased in atria of AF patients [14]. Based on this information, we speculated that the calcineurin inhibitor cyclosporine-A would inhibit atrial tachycardia remodeling. However, we were unable to demonstrate any protective effect of cyclosporine-A against atrial tachycardia-induced changes.

We found that ATP enhanced the expression of both eNOS and iNOS. Enhanced nitric oxide production can alter transcripational mechanisms and contribute to inflammatory processes [36]. Barouch et al. showed that eNOS co-localizes with cardiomyocyte L-type Ca\(^{2+}\) channels and can inhibit their function via local formation of nitric oxide [37]. It is therefore conceivable that a component of ATP-induced refractoriness abbreviation and AF promotion is related to eNOS-dependent L-type Ca\(^{2+}\) current inhibition. Glucocorticoids are known to down-regulate eNOS production [38] and prednisone-induced prevention of eNOS up-regulation by ATP may have contributed to the attenuation of ATP-remodeling caused by the drug.

4.4. Novelty and potential significance

This study is to our knowledge the first to assess the effects of glucocorticoids, NSAIDs or calcineurin inhibitors in an animal model of AF promotion by atrial tachycardia. Our results show that prednisone, but not ibuprofen or cyclosporine-A, suppresses atrial tachycardia-induced electrical remodeling and AF promotion. The suppression by prednisone of CRP and eNOS levels may provide potential insights into mechanisms underlying prednisone’s actions. This information is relevant to the pharmacological suppression of atrial remodeling and the development of new approaches to AF prevention, and suggests one potential candidate mechanism for previous observations of glucocorticoid efficacy in AF management [6,30].

4.5. Potential limitations

In contrast to our results, Cai et al. observed down-regulation of left atrial nitric oxide in pigs with pacing-induced AF [39]. However, Carnes et al. demonstrated an increase in 3-nitrotyrosine, a stable biomarker of peroxynitrite production from the reaction of nitric oxide and superoxide anion, in atria of atrial tachypaced dogs, suggesting increased nitric oxide production [40]. Mihm et al. also reported enhanced protein tyrosine nitration in AF patients, pointing to increased nitric oxide production [41]. Recent work shows that superoxide is increased in atria by NADPH oxidase in animal [42] and human models of AF [43]. Kim et al. reported that NO synthases also contribute to atrial superoxide production in AF, suggesting that increased oxidative stress in AF may lead to NOS uncoupling [43]. Increased iNOS by ATP was not suppressed by prednisone in our study, despite its anti-inflammatory action. However, glucocorticoids do not suppress iNOS in some inflammatory states [44]. Further work is needed to elucidate relationships among AF, NOS, inflammation and prednisone action.

The dosage of cyclosporine-A was selected based on previous studies of cyclosporine-A use in a dog model [45]. The mean cyclosporine-A trough concentration in our dogs was well into the therapeutic range in man and higher doses caused severe toxicity in pilot studies. We chose the dosage of ibuprofen based on the maximal dosage for human use. The resulting ibuprofen trough concentrations were within the effective therapeutic range [16]. Larger doses were not tolerated because of gastrointestinal bleeding and systemic side-effects (lassitude, failure to eat, etc.). We based our higher dose of prednisone on doses used in prior clinical studies [30]. We found effectiveness in initial studies at 50 mg/day, and therefore added an additional group treated with 30% of the initial dose, still observing efficacy. It would be interesting to do a full prednisone dose–response trial in a future study.

AF is often a progressive and slowly evolving condition in humans. This needs to be considered carefully in evaluating the results of relatively short-term studies like ours.

5. Conclusions

Atrial tachycardia-induced electrophysiological remodeling and AF promotion are prevented by therapy with prednisone, but not by ibuprofen or cyclosporine-A. Prednisone decreased CRP levels and suppressed atrial tachypacing-induced eNOS up-regulation, compatible with actions mediated by suppression of the inflammatory response and/or oxidative stress. These results contribute
potential new insights into the mechanisms and pharmacological prevention of atrial tachycardia-induced atrial arrhythmogenic remodeling.

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