

Ascorbate Attenuates Atrial Pacing-Induced Peroxynitrite Formation and Electrical Remodeling and Decreases the Incidence of Postoperative Atrial Fibrillation

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Abstract—Atrial fibrillation (AF), the most common chronic arrhythmia, increases the risk of stroke and is an independent predictor of mortality. Available pharmacological treatments have limited efficacy. Once initiated, AF tends to self-perpetuate, owing in part to electrophysiological remodeling in the atria; however, the fundamental mechanisms underlying this process are still unclear. We have recently demonstrated that chronic human AF is associated with increased atrial oxidative stress and peroxynitrite formation; we have now tested the hypothesis that these events participate in both pacing-induced atrial electrophysiological remodeling and in the occurrence of AF following cardiac surgery. In chronically instrumented dogs, we found that rapid (400 min⁻¹) atrial pacing was associated with attenuation of the atrial effective refractory period (ERP). Treatment with ascorbate, an antioxidant and peroxynitrite decomposition catalyst, did not directly modify the ERP, but attenuated the pacing-induced atrial ERP shortening following 24 to 48 hours of pacing. Biochemical studies revealed that pacing was associated with decreased tissue ascorbate levels and increased protein nitration (a biomarker of peroxynitrite formation). Oral ascorbate supplementation attenuated both of these changes. To evaluate the clinical significance of these observations, supplemental ascorbate was given to 43 patients before, and for 5 days following, cardiac bypass graft surgery. Patients receiving ascorbate had a 16.3% incidence of postoperative AF, compared with 34.9% in control subjects. In combination, these studies suggest that oxidative stress underlies early atrial electrophysiological remodeling and offer novel insight into the etiology and potential treatment of an enigmatic and difficult to control arrhythmia. The full text of this article is available at <http://www.circresaha.org>. (*Circ Res.* 2001;89:e32-e38.)

Key Words: atrial fibrillation ■ antioxidant ■ ascorbate ■ oxidative stress ■ cardiac surgery

Atrial fibrillation (AF) is self-perpetuating¹ because of the combined effects of rate-induced electrophysiological and structural remodeling.² The earliest observed change in AF is an abbreviation of the atrial effective refractory period (ERP). The mechanisms by which high-rate activity results in electrophysiological remodeling are poorly understood. AF is also a frequent postoperative complication of cardiac surgery, with a reported incidence of 20% to 50%, increasing the risk of stroke.³ Before arrhythmia onset, patients who experience postoperative AF exhibit increased atrial ectopy, abbreviation of monophasic action potential duration, and increased heart rate.⁴ Evidence from animal models of atrial fibrillation,⁵⁻⁷ as well as our studies of patients with postoperative AF,⁸ supports a prominent role for myocyte calcium overload in initiating the process of atrial electrophysiological remodeling. We have documented both significant electrophysiolog-

ical remodeling^{8,9} and biochemical evidence of oxidative stress in the atrial tissue of patients undergoing Maze surgery to treat permanent AF.¹⁰ Patients undergoing coronary artery bypass graft surgery have increased plasma lipid peroxidation and decreased cardiac glutathione levels following release of the cross clamp, and these changes persist for at least 24 hours following cardiac surgery.¹¹ Similarly, there is direct evidence of increased free radical production in canine hearts subjected to rapid ventricular pacing,¹² and evidence that antioxidants can improve cardiac function in animals with pacing-induced failure.¹³ We have previously demonstrated that peroxynitrite formation may be an important contributor to cardiac myocyte dysfunction in a wide array of settings, including doxorubicin-induced cardiomyopathy,¹⁴ congestive heart failure (rat infarction model),¹⁵ and human atrial fibrillation.¹⁰ We thus inferred a causal relationship between

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increased oxidative stress and the pathological alterations associated with AF and hypothesized that increased oxidative stress may underlie both postoperative AF and the electrophysiological remodeling associated with rapid atrial pacing.

To evaluate this hypothesis, we have examined the effects of supplemental ascorbate (a physiological antioxidant and a potent peroxynitrite decomposition catalyst in biological settings²⁰), on the pacing-induced electrophysiological remodeling in a canine atrial-pacing model. To address the mechanism of these changes, we have evaluated the effects of pacing and ascorbate treatment on tissue ascorbate levels and 3-nitrotyrosine prevalence (as a marker of peroxynitrite formation). Finally, we have begun to assess the impact of supplemental ascorbate treatment on the incidence of postoperative AF in a series of patients undergoing cardiac bypass graft surgery.

Materials and Methods

Canine Rapid Atrial Pacing

Eleven adult male beagle dogs were used for rapid atrial pacing studies. All animal procedures were approved by the Ohio State University Institutional Animal Care and Use Committee. Only dogs with normal baseline cardiac function, as assessed by electrocardiographic and echocardiographic criteria, were included in the study. Animals were anesthetized with butorphanol tartrate (0.1 mg/kg IM) and acepromazine maleate (0.1 mg/kg IM); anesthesia was maintained with halothane 1% to 2%. During anesthesia, two bipolar electrodes (YP 53/10-BP leads, Biotronik) were implanted transvenously in the right atria under fluoroscopic guidance. One electrode was placed in the right midlateral free wall for effective refractory period determination. A second electrode for pacing was placed between the azygos vein and the cranial vena cava. The electrodes were exteriorized and the animals were allowed to recover for 2 to 4 days before initiation of pacing.

All electrophysiological measurements were made in conscious dogs with light butorphanol sedation (0.1 mg/kg IM). ERPs were determined using a train of eight pacing stimuli at a cycle length of 300 ms, followed by an increasingly premature extrastimulus (5-ms decrement) delivered by a programmable stimulator (Medtronic model 5325, Medtronic Inc). The longest extrastimulus not propagated was defined as the ERP. All ERPs were determined at twice the diastolic pacing threshold.

Rapid Pacing Protocol

The right atrium was paced at 400 beats per minute with a modified Prevail 8086 pacemaker (Medtronic Inc). During pacing, the ERP was determined at times 0, 1, 2, 4, 8, 24, and 48 hours. Following the initial pacing period, the animals were allowed to recover for a minimum of 2 days, until the ERP was within 90% of the baseline value. After recovery of the ERP, pacing was reinitiated for an additional 48 hours, with ERP measurements repeated as described for the first pacing period. The study was terminated and the animals were euthanized at the end of the second 48-hour pacing period.

Canine Atrial Pacing Study Design

Animals were randomized to one of two treatment groups. In the first (control) group, animals underwent period 1 pacing, a recovery period, and period 2 pacing without any treatment administered. In the second group, animals underwent period 1 pacing without treatment, a recovery period, and were started on timed-release ascorbic acid 500 mg (CVS Pharmacy) tablets the night previous to initiation of period 2 pacing. Ascorbic acid therapy was continued twice daily throughout period 2. All dogs were fed a standard dry diet (Hill's Science Diet, Canine Original Maintenance), which provides 2.4 mg of ascorbic acid/100 Kcal. Any dog with rapid ventricular response during atrial pacing (defined as sustained 2:1 or

faster atrioventricular conduction), evidence of systemic infection, or loss of pacing because of increased pacing threshold was removed from the study.

Direct Ascorbate Effects

Potential direct effects of ascorbate on atrial electrophysiology were evaluated in a separate series of 5 male beagles. Dogs were anesthetized with propofol induction (6 mg/kg IV, Propofol, Abbott Laboratories) followed by chloralose (100 mg/kg IV, induction, 30 mg/kg/h, IV maintenance, Sigma Chemical). Dogs were ventilated with a Harvard pump (model 613, Harvard Apparatus); tidal volume and respiratory rate were adjusted to maintain PaCO₂ between 35 and 45 mm Hg and PaO₂ over 80 mm Hg. Body temperature was maintained at 37°C to 38°C. Arterial pH was kept between 7.35 and 7.45 and base excess was adjusted to ± 3 with 8.4% sodium bicarbonate. Electrodes were placed as described above. The ERP was determined at baseline and then at 20-minute intervals until the preparation was stable (change in repeat ERP₃₀₀ <10%). Sequential doses of ascorbate (prepared in sterile 0.9% saline) were then administered by rapid injection into the cephalic vein. Doses were calculated based on estimated blood volume (estimated by weight of the animal), and sequential doses were given that were designed to deliver 100, 300, and 1000 μ mol/L increments in plasma ascorbate concentration. Doses were given at 24-minute intervals; the ERP was determined 5 and 20 minutes after each dose at basic cycle lengths of 300 and 150 ms.

Tissue Collection

Atrial tissue was collected from the 11 dogs subjected to rapid atrial pacing at the end of the second 48-hour rapid atrial-pacing period. At the time of euthanasia, atrial tissue was rapidly collected, blotted dry, and immersed in liquid nitrogen; these tissues were then stored frozen at -80°C until use for ascorbate and 3-nitrotyrosine content measurements.

Atrial Tissue Ascorbate Content

Atrial tissues (50 to 75 mg) were homogenized in ice-cold 10% MPA buffer (10% weight per volume). A portion of the resulting homogenate was then centrifuged at 6000g for 10 minutes. The resulting protein-free supernatant was then diluted 4-fold in water and assayed for ascorbate concentration using a specific and sensitive capillary electrophoresis assay method. Free-zone capillary electrophoresis was conducted with a P/ACE 5510 system (Beckman Instruments Inc) using an uncoated fused-silica capillary (57 cm \times 75 μ m). Electrophoretic separation was performed at 25 kV, 25°C, 100 mmol/L sodium borate/50 mmol/L SDS run buffer, pH 8.5, using UV detection at 254 nm. Standard curves for the separation of ascorbic acid were generated using spiked cardiac tissue homogenates. Migration time for ascorbic acid was 5.4 ± 0.2 minutes. Concentration response was linear throughout the range investigated and intraday and interday assay variability was 4% and 4.8%, respectively. Mass limit of detection for ascorbic acid in cardiac homogenates was approximately 30 fmol on column (2- μ mol concentration, 18-nL injection).

Evaluation of 3-Nitrotyrosine Content in Atrial Tissue by Dot-Blot Analysis

Tissue homogenate was resuspended and diluted in Tris buffer (50 mmol/L, 0.01% Tween, pH 7.6). The resulting solution was assayed for protein content (bicinchoninic acid protein assay) and adjusted to a final concentration of 0.5 mg/mL. Protein (50 μ g/well) was then applied to a vacuum-assisted dot-blot apparatus (96-well template, Bio-Rad Laboratories, nitrocellulose membrane). All samples were analyzed in triplicate and standard curves were constructed daily on the same membrane using known standards of nitrated albumin as a reference. Nitration was detected using a polyclonal rabbit anti-3 nitrotyrosine antibody (1:2000; Upstate Biotechnology, Lake Placid, NY), and a secondary antibody (goat anti-rabbit, 1:10 000), and visualized using an alkaline phosphate-streptavidin reporter system. Stained blots were digitally scanned with an HP

TABLE 1. Baseline Patient Characteristics

	Ascorbate (n=43)	Control (n=43)	P Value
Age	61.6±12.2	61.8±11.2	0.96
Gender (M/F)	36/7	36/7	1.0
LVEF	50.4±10.9	48.4±11.2	0.40
LA size, mm	36.8±0.7	41.6±0.6	0.08
LA area, cm ²	23.0±4.1	19.7±3.7	0.10
Hypertensive	27 (63%)	38 (88%)	0.01
Diabetes mellitus	9 (21%)	17 (39%)	0.06
Prior AF	0 (0%)	5 (12%)	0.03
Prior MI	25 (60%)	26 (61%)	0.93
Preoperative β -blocker use	37 (86%)	31 (76%)	0.22
Postoperative β -blocker use	36 (84%)	30 (70%)	0.13

LVEF indicates left ventricular ejection fraction; LA, left atrium; MI, myocardial infarction.

Values represent mean±SD.

ScanJet 6200c (Hewlett-Packard) using research-based software (Image Pro Plus 4.0, Media Cybernetics). The optical density of 3-nitrotyrosine staining intensity was determined for each dot (triplicates per sample). Intrasample variability (coefficient of variation for identical sample analyzed in triplicate) was <7%, and standards were found to be linear throughout the range investigated.

Postoperative AF Study Design

A series of 50 consecutive patients scheduled for primary coronary artery bypass graft surgery were given 2 g ascorbic acid (extended release) the night before surgery, followed by 500-mg doses twice daily for the 5 days after surgery. These patients were all over 18 years old, had normal renal function, and were in sinus rhythm before surgery. Patients were excluded if they were in atrial fibrillation. Seven patients did not receive the preoperative dose of ascorbic acid and were, therefore, omitted from subsequent analysis. An age- and gender-matched control group (not receiving ascorbic acid) was retrospectively selected from the patients undergoing the same surgical procedure during the 6 weeks after the ascorbate-treated group. Clinical characteristics of the patient populations are described in Table 1. The study was approved by the Institutional Review Board of the Cleveland Clinic Foundation.

All patients were monitored continuously via telemetry until discharge. Electrocardiograms were routinely recorded on the day of surgery and then on postoperative days 1, 2, and 5. The endpoint of the study was the detection of AF or flutter recorded on 12-lead ECG or telemetry strips.

Statistical Analysis

Canine ERP data were evaluated using a repeated-measures model to assess the effects of time and treatment on the ERP at each cycle length (SAS version 8.1). When the model revealed significant effects of time, treatment, or the time-by-treatment interaction terms, comparisons were made between the two treatment groups at different time points. Tissue biochemical studies (ascorbate levels and nitration prevalence) were statistically compared by analysis of variance, followed by Newman-Keuls post hoc tests.

Clinical data were evaluated using SPSS 9.0 software, using either univariate or multivariate models, as described.

Unless otherwise noted, all data are expressed as mean±SEM. Statistical significance was defined as $P<0.05$ for all comparisons. Graphics were prepared with Origin 6.0 (OriginLab Software Inc).

Results

Canine Rapid-Atrial Pacing Protocol

Eleven dogs (mean weight 10.7±0.7 kg) completed the rapid-atrial pacing protocol. Five dogs were randomized to

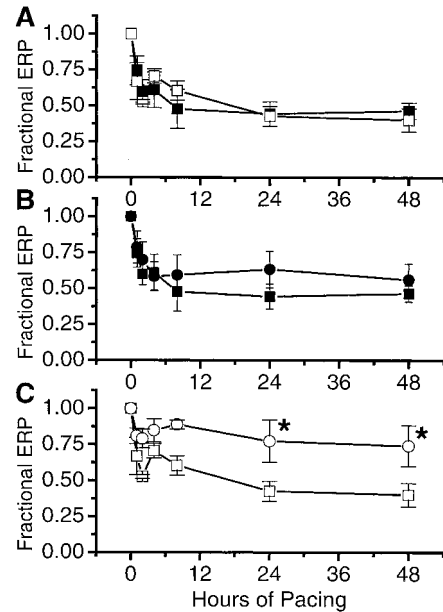


Figure 1. Ascorbate attenuates electrophysiological remodeling in the rapidly paced canine atria. A, Pacing reproducibly abbreviates canine atrial effective refractory period (ERP). ERPs for each animal (300 ms cycle length) were normalized to individual baseline values. Mean±SEM normalized ERP values for group 1 animals (no ascorbate) are shown for first (■) and second (□) pacing periods, demonstrating the reproducibility of the protocol. B, Experimental groups were similar during first pacing period. Mean±SEM normalized ERP values for the first pacing period are plotted for group 1 (■) and group 2 (●). The experimental values were not significantly different at any time point. C, Ascorbate treatment attenuates reduction in ERPs. Mean±SEM normalized ERP values for the second pacing period are plotted for group 1 (no ascorbate, □) and group 2 (ascorbate treated, ○). *Significant difference from control group at indicated time points ($P<0.03$).

group 1 (no treatment either period) and 6 were randomized to group 2 (no treatment in period 1, ascorbic acid treatment in period 2). There was no significant difference between groups with respect to baseline ERP values before either pacing period 1 (124 ± 9 ms versus 120 ± 15 ms, group 1 versus group 2) or pacing period 2 (115 ± 12 ms versus 113 ± 10 ms, group 1 versus group 2). As expected, rapid atrial pacing caused the ERP to shorten as a function of pacing duration ($P<0.005$) (Figure 1A). There was no difference in response between groups 1 and 2 during the initial pacing period (Figure 1B, $P>0.55$). There was also no difference in response between periods 1 and 2 in the control group of dogs (group 1, Figure 1A). Thus, the ERP response to atrial pacing was highly reproducible in this model system.

The effect of ascorbic acid treatment on ERP responses (comparing responses between groups during period 2) was evaluated by fitting a mixed model to the data with an autoregressive correlation structure. Ascorbic acid treatment significantly attenuated the pacing-induced reduction in ERPs (Figure 1C). Both pacing duration ($P<0.007$) and treatment ($P<0.043$) significantly affected the ERPs. Post hoc modeling indicated that by 8 hours the difference between groups approached significance ($P=0.053$), and that the differences reached statistical significance at 24 and 48 hours ($P<0.03$).

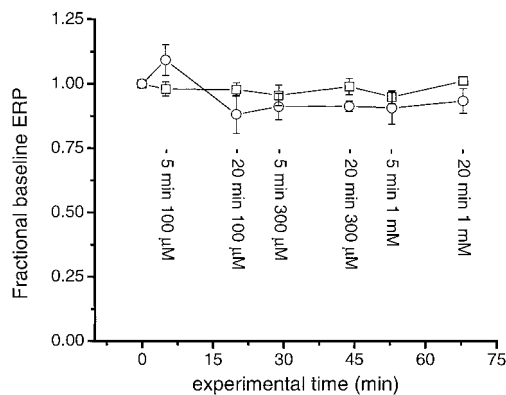


Figure 2. Ascorbate has no acute, direct effect on canine atrial ERPs at relevant concentrations. Mean \pm SEM normalized ERP values are plotted for 5 animals acutely treated with intravenous infusion of ascorbate. ERP measurements were made after stabilization of the ERP at cycle lengths of 300 ms (\square) and 150 ms (\circ). None of ERP values were significantly different from baseline.

This is the first demonstration that an antioxidant can modulate the atrial electrophysiological remodeling process.

Direct Effects of Ascorbate

To evaluate the possibility that attenuation of the ERP changes was mediated by a direct electrophysiological (rather than antioxidant) effect of ascorbate, a series of experiments were performed to evaluate the effects of intravenous ascorbate supplementation at pacing cycle lengths of 300 and 150 ms (400 min^{-1}). Figure 2 documents the results of this study. Analysis of these data (repeated-measures ANOVA) found

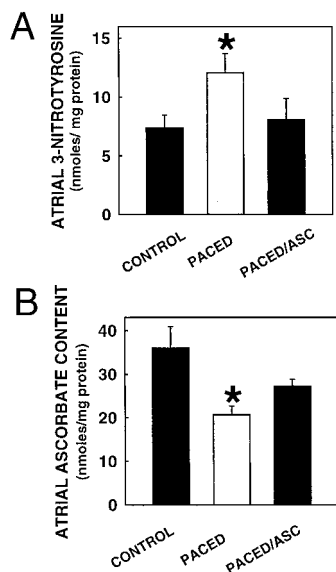


Figure 3. 3-Nitrotyrosine and ascorbate content of paced canine atria. A, Supplemental ascorbate prevents rapid atrial pacing-mediated increases in atrial 3-nitrotyrosine. Mean \pm SEM data for atrial 3-nitrotyrosine content for the three treatment groups. *Significant difference from the control group ($P<0.05$). B, Supplemental ascorbate prevents rapid atrial pacing-induced reduction in atrial ascorbate content. Mean \pm SEM atrial ascorbate content determined via capillary electrophoresis assay method. *Significant difference from the control group ($P<0.05$).

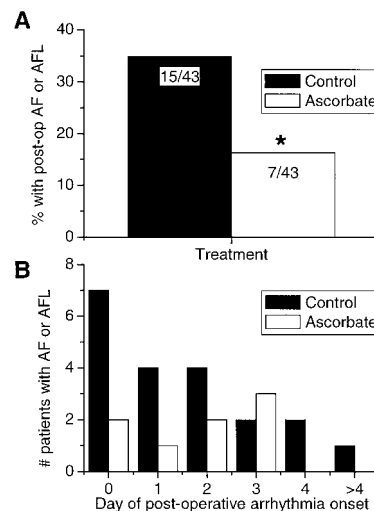


Figure 4. Ascorbate treatment is associated with a lower incidence of postoperative atrial fibrillation. A, Incidence of postoperative AF in patients treated with ascorbate (white column) versus those untreated (black column). B, Time course of first occurrence of postoperative AF in patients treated with ascorbate (white columns) versus those untreated (black columns).

no significant time- or dose-dependent change in ERPs at any dose of ascorbate, at either cycle length. Thus, ascorbate-mediated attenuation of changes in ERPs following longer pacing periods is unlikely to be caused by the direct electrophysiological effects of the compound.

Atrial Tissue Ascorbate and 3-Nitrotyrosine Content

Summary data documenting atrial tissue content of 3-nitrotyrosine (measured with a dot-blot assay) and tissue ascorbate (measured with a capillary electrophoresis assay) are presented in Figures 3A and 3B, respectively. Atrial pacing resulted in a significant increase in atrial 3-nitrotyrosine content relative to atrial tissue from nonpaced controls. Importantly, the pacing-induced increase in 3-nitrotyrosine content was prevented by ascorbate supplementation (Figure 3A). Atrial pacing was also associated with decreased tissue levels of ascorbate relative to nonpaced controls (Figure 3B); this change was similarly prevented by ascorbate supplementation.

Postoperative AF Study

To test the hypothesis that perioperative oxidative stress has a significant role in the etiology of postoperative AF, prophylactic supplemental ascorbate was given to a series of first-time bypass patients on the evening before surgery, and as soon thereafter as it was possible for oral dosing to resume. For the 43 patients receiving both preoperative and postoperative ascorbate, treatment was initiated 1.6 ± 1.1 days after surgery, and patients received ascorbate for a mean of 3.4 ± 2.0 days. The overall incidence of postoperative AF or flutter in the ascorbate-treated group was 16.3% (Figure 4A), significantly lower than the incidence of atrial arrhythmias in the control group, 34.9%.

Table 2 documents the results of a univariate analysis of risk factors for postoperative AF. Ascorbate treatment had a

TABLE 2. Significant Univariate Predictors of Postoperative AF

Variable	–	+	P Value
Ascorbate	15/43 (34.9%)	7/43 (16.3%)	0.048
Postoperative β -blocker use	12/20 (60%)	10/66 (15.2%)	<0.001
Postoperative ACEI	18/59 (30.5%)	2/25 (8.0%)	0.028
Preoperative digoxin	18/80 (22.5%)	3/4 (75%)	0.047
Postoperative pressors	10/63 (15.9%)	10/19 (52.6%)	0.001
Preoperative AF history	19/80 (23.8%)	3/5 (60%)	0.107
Peripheral vascular disease	13/65 (20.0%)	9/21 (42.9%)	0.037
Age, years	59.5 \pm 11.4	68.0 \pm 10.1	0.003
LVEF, %	51.0 \pm 9.4	44.5 \pm 13.9	0.051
LA diameter, cm	3.76 \pm 0.62	4.56 \pm 0.55	0.006

ACEI indicates angiotensin-converting enzyme inhibitor; LVEF, left ventricular ejection fraction; LA, left atrium.

Values represent mean \pm SD.

significant effect ($P=0.048$) when analyzed as a univariate predictor. With respect to other pharmacological parameters, postoperative β -blockers and angiotensin-converting enzyme inhibitors also conferred significant benefit with respect to arrhythmia prevention. As expected, age, left atrial size, and ejection fraction were also significant predictors. Age and postoperative β -blocker use were the strongest independent predictors of postoperative AF. Figure 4B shows the time course of arrhythmia onset in the control and ascorbate-treated groups. It is interesting to note that the greatest difference was in the early occurrence period.

Multivariate analysis was performed using a four-element model (Table 3). This analysis demonstrates that β -blocker use has the most protective effect (84% risk reduction, $P=0.007$) and suggests that ascorbate usage alone (66% risk reduction, $P=0.09$) would also have a clear beneficial effect in an adequately powered study.

Discussion

Electrophysiological remodeling is a critical early step in the perpetuation of AF.¹⁶ By better understanding this process we may gain insight into pharmacological approaches that can prevent arrhythmia. The fundamental mechanisms underlying electrophysiological remodeling during AF have been elusive. Using the goat model, Wijffels et al¹⁷ evaluated the roles of neurohumoral changes, ischemia, atrial stretch, and high-rate electrical activity on the early electrophysiological remodeling process and concluded that it was fundamentally the high-rate activity, rather than the other factors listed, that underlies the remodeling process. Whereas the study identified high-rate activity as an important factor, it did not

TABLE 3. Multivariate Logistic Regression: Independent Predictors of Postoperative AF

	Odds Ratio	95% CI	P Value
Age	1.10	1.03, 1.16	0.0035
LVEF	0.96	0.90, 1.01	0.11
Ascorbate use	0.335	0.095, 1.185	0.09
Postoperative β -blocker use	0.156	0.041, 0.599	0.007

LVEF indicates left ventricular ejection fraction.

provide clear mechanistic insight into the effects of rapid pacing or provide direction for future pharmacological interventions.

We have postulated that calcium overload mediated oxidative stress, and concomitant alterations in cellular redox state may have an important role in the genesis of atrial arrhythmias.^{18,19} We have also recently demonstrated, for the first time, that human AF is associated with cardiac production of peroxynitrite (ONOO⁻).¹⁰ This free radical is formed via the diffusion rate-limited reaction of nitric oxide and superoxide anion and is known to oxidize cellular lipids, proteins, and DNA and to promote cardiac cell death via necrosis and/or apoptosis. Of particular biological importance is the unique and avid capacity of ONOO⁻ to cause nitration of tyrosine residues, both protein-bound and free, forming the modified amino acid residue 3-nitrotyrosine. Thus, 3-nitrotyrosine serves as a stable biomarker of ONOO⁻ formation in vivo, and the nitration reactions of ONOO⁻ can elicit potent effects on protein structure and function. Because an antioxidant system specific to ONOO⁻ has not yet been identified (relative to superoxide dismutase for O₂⁻, catalase for H₂O₂), many in vitro studies have suggested that the glutathione system is likely the central antioxidant controller of ONOO⁻ in vivo. However, ascorbate is a far more potent ONOO⁻ scavenger than glutathione when the more physiological reactivities are considered (particularly the avid interactions of ONOO⁻ with CO₂).²⁰

In the present study, we tested the hypotheses (1) that rapid atrial pacing would result in increased atrial oxidative stress, and (2) that ascorbate could prevent the effects of oxidative stress triggered either by the high-rate activity of atrial pacing or by the increased sympathetic tone and ischemia/reperfusion injury following cardiac surgery. We reasoned that ascorbate, by minimizing peroxynitrite-mediated injury,²⁰ might attenuate or eliminate the atrial electrophysiological remodeling associated with these processes. Our results support this novel hypothesis.

Figure 1 clearly demonstrates that supplemental ascorbate can attenuate the reduction in atrial effective refractory period following 24 to 48 hours of rapid atrial pacing. This is a novel finding that could, in principle, be mediated either by a direct electrophysiological effect of ascorbate on atrial ion channels or indirectly by its antioxidant activity. Figure 2 suggests that a direct effect of ascorbate on atrial ion channels is unlikely; no change in atrial ERPs was observed at relevant concentrations (0.1 to 1.0 mmol/L) or pacing rates (paced cycle lengths of 300 or 150 ms). In contrast, our data support an antioxidant and/or peroxynitrite decomposition catalyst-based effect of ascorbate. Figure 3 demonstrates both that atrial peroxynitrite formation is increased during rapid atrial pacing, and that atrial ascorbate levels are reduced following rapid atrial pacing. We have further demonstrated that supplemental ascorbate can prevent both the atrial tissue ascorbate depletion and the increased 3-nitrotyrosine formation in this canine model (Figure 3). Thus, the beneficial effects of supplemental ascorbate seem likely to be related to either the reduction in peroxynitrite accumulation or to the preservation of intracellular ascorbate levels (with concomitant beneficial effects on cellular redox state).

Recent studies suggest an important relationship between plasma ascorbate levels and cardiovascular events. Plasma ascorbate was inversely related with the incidence of stroke (both ischemic and hemorrhagic, $P=0.002$) and cerebral infarction in a large, prospective cohort study.²¹ AF is an important risk factor for stroke. In another study, there was a similar inverse relationship between plasma ascorbate and all-cause mortality and cardiovascular mortality.²² Plasma and tissue ascorbate levels are likely closely related. Whereas the above-mentioned studies focused on the effects of plasma levels of ascorbate on long-term events, our present study has focused on acute events with clearly demarcated sources of oxidant stress. In this study, we have, for the first time, demonstrated that the electrophysiological remodeling that accompanies rapid atrial pacing is characterized by a decrement in the atrial tissue levels of ascorbate and an accumulation of peroxynitrite, and that supplemental ascorbate can blunt these effects.

In the pilot clinical study reported here, we have begun to examine the potential utility of ascorbate supplementation in modifying the occurrence of postoperative AF. Because of the retrospective nature of the study, the patient groups were not ideally matched with respect to all risk factors for AF, and in particular, the incidence of diabetes, hypertension, and prior AF was increased in the control group relative to the ascorbate treated group (Table 1). However, the baseline incidence of postoperative AF in the control population is very similar to our internal historical control incidence (32%). Thus, our study suggests that ascorbate supplementation may be associated with a reduced incidence of postoperative AF in cardiac surgery patients (Figure 4). This is a well-tolerated treatment, with no apparent side effects. To better evaluate its efficacy, we have initiated a fully powered, randomized, double-blinded, placebo-controlled study to determine the extent to which ascorbate treatment can modify the occurrence of postoperative arrhythmias and other comorbidities.

The hypothesis that oxidative stress underlies atrial electrophysiological remodeling is consistent with an analysis of the other agents that have been documented to attenuate either pacing-induced remodeling or postoperative AF. Calcium channel blockers can directly minimize calcium overload by channel blockade and have direct antioxidant effects. It is interesting to note that the rank-order antioxidant potency of these drugs is mibefradil > verapamil > diltiazem.²³ Mibefradil was recently shown to be more effective than diltiazem in preventing electrophysiological remodeling in a canine rapid atrial-pacing model.⁷ Although this effect was attributed to the importance of T-type calcium channel blockade, it is conceivable that its antioxidant efficacy may also contribute to the beneficial effect of mibefradil.

The usual pharmacological approach to the prevention of postoperative AF is to use drugs such as β -blockers, amiodarone, or sotalol²⁴; note that the latter two compounds also have significant β -blocking activity. In our pilot study, postoperative β -blockers were found to be quite effective in reducing the incidence of postoperative AF (Table 3). Circulating levels of norepinephrine are increased in patients who experience postoperative AF²⁵ and would be expected to increase calcium influx (via the L-type Ca^{2+} channel). We

previously observed that, on average, the patients who experienced postoperative AF had greater preoperative calcium current densities than those who did not.⁸ It is therefore conceivable that β -blocker efficacy is owing in part to their role in minimizing calcium overload. These agents also help to slow sinus rate. More widespread use of β -blockers is limited by their negative chronotropic side effects and by the potential hemodynamic instability of the patient.

The perioperative period is characterized both by acute ischemic/reperfusion injury and by delayed inflammatory responses that together result in a net depletion of plasma antioxidants.¹¹ Rapid pacing causes an increase in cytosolic calcium levels. Calcium overload alters the efficiency of the mitochondrial respiratory chain, leading to increased intramyocyte production of radicals.²⁶ In vitro experiments, exogenous ascorbate has been shown to decrease mitochondrial free radical production and improve recovery from ischemic injury.²⁷

Advanced age is the main predictor both for postoperative AF and for AF in the general population. Aging hearts are more susceptible to ischemic/reperfusion injury²⁸ and have altered mitochondrial electron transport properties that lead to increased oxidant production.²⁹ In our study, advanced age was the strongest univariate and multivariate predictor of the occurrence of postoperative AF.

In sum, the results presented here provide the basis for considering a paradigm shift in understanding the etiology of AF. We propose that increased oxidative stress is a primary mechanism by which calcium overload (in response to sympathetic stimulation, ischemia/reperfusion injury, or high-rate pacing) is translated into altered atrial electrical activity. Key elements in cardiac excitation-contraction coupling are documented to be sensitive to oxidative stress. These include the L-type calcium channel,³⁰ the ryanodine receptor (calcium-release channel in the sarcoplasmic reticulum),³¹ and the Ca^{2+} -ATPase in the sarcoplasmic reticulum (SERCA2a).³² This study does not delineate which of these targets is/are most protected by ascorbate treatment or which reactive oxygen or nitrogen species are involved in these events. However, our findings do suggest that peroxynitrite and/or protein nitration may be important mediators of this process. The conclusion that oxidative stress underlies the early electrophysiological remodeling associated with AF provides important insights into one of the mechanisms responsible for initiation of the arrhythmia. It further suggests that specific, peroxynitrite-targeted antioxidant treatments may provide significant benefit in preventing AF, particularly in settings of acute oxidative stress (cardiac surgery, postinfarction, etc). It will be of great interest to determine if this treatment is associated with beneficial effects on morbidity and mortality.

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Ascorbate Attenuates Atrial Pacing-Induced Peroxynitrite Formation and Electrical Remodeling and Decreases the Incidence of Postoperative Atrial Fibrillation

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