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Cardiac Surgery

Myocardial Redox State Predicts In-Hospital Clinical Outcome After Cardiac Surgery

Effects of Short-Term Pre-Operative Statin Treatment

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Objectives The purpose of this study was to evaluate the role of the myocardial redox state in the development of in-hospital complications after cardiac surgery and the effect of statins on the myocardial redox state.

Background Statins improve clinical outcome after cardiac surgery, but it is unclear whether they exert their effects by modifying the myocardial redox state.

Methods We quantified myocardial superoxide anion (O_2^-) and peroxynitrite ($ONOO^-$) and their enzymatic sources in samples of the right atrial appendage (RAA) from 303 patients undergoing cardiac surgery who were followed up until discharge, and in 42 patients who were randomized to receive 3-day treatment with atorvastatin 40 mg/d or placebo before surgery. The mechanisms by which atorvastatin modifies myocardial redox state were investigated in 26 RAA samples that were exposed to atorvastatin ex vivo.

Results Atrial O_2^- (derived mainly from nicotinamide adenine dinucleotide phosphate [NADPH] oxidases) and $ONOO^-$ were independently associated with increased risk of atrial fibrillation, the need for post-operative inotropic support, and the length of hospital stay. Pre-operative atorvastatin treatment suppressed atrial NADPH oxidase activity and myocardial O_2^- and $ONOO^-$ production. Ex vivo incubation of RAA samples with atorvastatin induced a mevalonate-reversible and Rac1-mediated inhibition of NADPH oxidase.

Conclusions There is a strong independent association between myocardial O_2^-/$ONOO^-$ and in-hospital complications after cardiac surgery. Both myocardial O_2^- and $ONOO^-$ are reduced by pre-operative statin treatment, through a Rac1-mediated suppression of NADPH oxidase activity. These findings suggest that inhibition of myocardial NADPH oxidases may contribute to the beneficial effect of statins in patients undergoing cardiac surgery. (Effects of Atorvastatin on Endothelial Function, Vascular and Myocardial Redox State in High Cardiovascular Risk Patients; NCT01013103) (J Am Coll Cardiol 2012;59:60–70) © 2012 by the American College of Cardiology Foundation

Cardiac surgery is associated with a high risk of post-operative complications such as atrial fibrillation (AF)—in ~30% of patients—heart failure, and stroke (1) that pro-

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long hospitalization and have a major impact on the clinical outcome of these patients (1,2). Increased atrial nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity and altered myocardial redox state have been proposed as possible mechanisms involved in the development and maintenance of AF (3–5). Although myocardial superoxide (O_2^-) has been found to be higher in patients who have post-operative AF (4), it is unclear whether other pro-oxidant molecules such as peroxynitrite ($ONOO^-$), produced by the reaction of O_2^- with myocardial nitric oxide, may play any additional role. Moreover, the impact of myocardial redox state on other post-operative complications is unknown.

Recent evidence suggests that perioperative treatment with statins reduces the risk of AF after cardiac surgery and improves the post-operative outcome of these patients (6,7). We have recently shown that pre-operative treatment with statins reduces vascular oxidative stress by decreasing the activity of NADPH oxidase in human vein grafts (8). Statins reduce myocardial oxidative stress in experimental animal models (9) and prevent atrial electrical remodeling leading to AF (10). However, clinical data implying an effect of statins on myocardial redox state in humans are scanty and limited to a small observational study (11).

In the present study, we examined the role of atrial O_2^- and $ONOO^-$ in the prediction of post-operative complications after cardiac surgery, and evaluated the contribution of individual sources of O_2^- in the development of these complications. We then explored the hypothesis that pre-operative treatment with statins has a beneficial effect on myocardial redox state in these patients and investigated the molecular mechanisms underlying this effect.

Methods

Study population and design. The studies were approved by the institutional ethics committee, and all subjects gave written informed consent.

Prospective study. The study population consisted of 303 patients undergoing cardiac surgery (Table 1). Patients were recruited pre-operatively, and exclusion criteria were any inflammatory, infective, liver, renal disease, or malignancy. Patients receiving nonsteroidal anti-inflammatory drugs, dietary supplements (such as folates), or antioxidant vitamins were excluded. Patients in sinus rhythm (SR) who were taking any antiarrhythmic medication, other than beta-blockers, were also excluded.

Blood samples were obtained the morning before surgery after 8 h of fasting, whereas samples of right atrial appendages (RAA) were obtained at the time of cardiopulmonary bypass and stored at −80°C until assayed. All subjects were followed up prospectively until their discharge from the hospital.

Surface electrocardiography was continuously monitored for the first 48 h after surgery in the intensive care unit. After the patients were transferred to the cardiothoracic ward, heart rhythm was evaluated every 4 h by electrocardiography and whenever a relevant sign or symptom was reported, until their discharge from the hospital. Any episode of AF lasting >30 s was classified as post-operative AF (4,12). Additional clinical data were collected, including length of hospital stay, the need for inotropic support, and the appearance of major cardiovascular events (such as need for redo surgery, acute heart failure, or death) during their hospital stay.

Abbreviations and Acronyms

- **AF** = atrial fibrillation
- **ANOVA** = analysis of variance
- **CABG** = coronary artery bypass graft surgery
- **CI** = confidence interval
- **EuroSCORE** = European System for Cardiac Operative Risk Evaluation
- **HR** = hazard ratio
- **LDL** = low-density lipoprotein
- **LVEF** = left ventricular ejection fraction
- **MDA** = malonyldialdehyde
- **NADPH** = nicotinamide adenine dinucleotide phosphate
- **O_2^-** = superoxide anion
- **$ONOO^-$** = peroxynitrite
- **RAA** = right atrial appendage
- **RLU** = relative light units
- **SR** = sinus rhythm

Effects of pre-operative treatment with atorvastatin on myocardial redox state. Forty-two statin-naïve patients undergoing elective coronary artery bypass graft surgery (CABG) were assigned randomly (using block randomization) to receive either atorvastatin 40 mg/day or placebo tablet of similar size, shape, and color for 3 days pre-operatively, in a double-blind fashion. Serum lipid levels were measured at baseline and on the morning before surgery, and the results were made available to the researchers at the end of the study. At the time of surgery, a sample of the RAA was obtained. In addition to the exclusion criteria listed, patients were excluded if they had known intolerance to statins or had received statin treatment during the last 3 months.

Ex vivo effects of atorvastatin on myocardial sources of reactive oxygen species production. For the ex vivo experiments, RAA samples from 26 patients in SR undergoing CABG were obtained. Exclusion criteria were as described above. Using a pre-cooled Polytron homogenizer (PT2100 Kinematika, Lucerne, Switzerland), 40 to 70 mg snap-frozen RAA tissue (stored at −80°C) was homogenized for 30 s in 800 μl ice-cold Krebs-Hepes buffer containing a protease inhibitor cocktail (Roche Applied Science, Indianapolis, Indiana). Atrial homogenates were then spun at 13,000 rpm at 4°C, after which the supernatant was used for the experiment. Each sample was split into 3 aliquots, and incubated for 60 min at 37°C in a water bath in the presence of atorvastatin 20 μmol/l, atorvastatin 20 μmol/l plus mevalonate 200 μmol/l, or buffer alone (control). Then the samples were frozen at −80°C until luminometry experiments were performed (see the following text). The Rac1 activity, defined by the guanosine triphosphate-Rac1 to total Rac1 ratio, was evaluated by a commercially available affinity precipitation assay (Millipore, Temecula, California).

Measurement of myocardial O_2^-/$ONOO^-$. The O_2^- production was measured in homogenized RAA tissue by lucigenin-enhanced chemiluminescence, as described previously (3,4). Further measurements were taken after application

Table 1 Patient Demographics and Clinical Characteristics

	Study 1 (Prospective)	Study 2 (Oral Atorvastatin)		Study 3 Ex Vivo Atorvastatin
		Placebo	Atorvastatin	
Participants	303	21	21	26
Male/female	248/55	20/1	18/3*	23/3
Age, yrs	66.74 ± 9.1	67.38 ± 9.1	66.1 ± 9.3*	69.5 ± 8.1
CABG/AVR	284/19	21/0	21/0*	26/0
Hypertension	208 (69)	16 (76)	18 (90)*	17 (65)
Diabetes mellitus	87 (29)	9 (43)	7 (33)*	6 (23)
Dyslipidemia	171 (56)	—	—	20 (77)
Smoking				
Active smokers	76 (25)	7 (33)	8 (38)†	6 (23)
Ex-smokers	132 (44)	10 (48)	9 (43)†	14 (54)
BMI, kg/m^2	27.6 ± 4.4	27.7 ± 3.6	27.5 ± 3.3†	26.0 ± 5.0
Urea, mg/dl	44.4 ± 20.3	43.7 ± 10.6	41.7 ± 15.1†	50.69 ± 22.4
Creatinine, mg/dl	1.07 ± 0.29	1.06 ± 0.16	1.02 ± 0.19*	1.11 ± 0.37
Cholesterol, mg/dl	181 ± 42	187 ± 43	184 ± 47*	169 ± 26
LDL, mg/dl	115 ± 39	109 ± 32	113 ± 34*	103 ± 23
HDL, mg/dl	38.6 ± 9.6	37.8 ± 8.0	38.7 ± 9.5*	35.9 ± 6.4
TG, mg/dl	123 [94–156]	193 [90–284]	157 [106–271]*	122 [98–149]
EuroSCORE	4.31 ± 2.8	3.39 ± 2.9	3.56 ± 2.9*	4.53 ± 2.1
Chronic AF	22 (7.3)	3 (14)	3 (14)*	–
LVEF, %	50.4 ± 11.9	52.0 ± 10.4	50.1 ± 14.6*	58.8 ± 4.1
LA diameter, mm	40.7 ± 9.2†	38.0 ± 8.9	39.2 ± 9.0*†	41.6 ± 6.9
Time on bypass, min	101.9 ± 69.3	114.5 ± 49.9	116.3 ± 37.1*†	98.0 ± 36.9
Medications,				
Aspirin or clopidogrel	225 (75)	16 (80)	17 (81)*	20 (77)
Statin	212 (70)	—	—	18 (69)
Beta-blocker	185 (61)	13 (62)	13 (62)*	12 (46)
CCB	63 (21)	5 (24)	5 (24)*	5 (19)
ACEi or ARB	190 (63)	12 (57)	17 (80)*	17 (65)
Diuretics	112 (37)	8 (39)	10 (47)*	11 (42)

Values are n, mean ± SD, n (%), or median [25th to 75th percentile]. *Nonsignificant versus placebo. †Measurement available for only 226 patients.
ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin-receptor blocker; AVR = aortic valve replacement; BMI = body mass index; CABG = coronary artery bypass graft surgery; CCB = calcium-channel blocker; EuroSCORE = European System for Cardiac Operative Risk Evaluation; HDL = high-density lipoprotein; LA = left atrium; LDL = low-density lipoprotein; LVEF = left ventricular ejection fraction; TG = triglycerides.

of 100 μmol/l NADPH, as an index of stimulated NADPH oxidase activity. The NADPH oxidase inhibitor apocynin (1 mmol/l) was then added, and the apocynin-inhibitable fraction was determined. The contribution of mitochondrial oxidases to the overall O_2^- signal was evaluated by the signal inhibitable by rotenone (100 μmol/l).

To determine myocardial $ONOO^-$ production, we evaluated the luminol (100 mmol/l) chemiluminescence inhibitable by urate (1 mmol/l), as described previously (13).

Measurements of plasma malonyldialdehyde levels. Plasma malonyldialdehyde (MDA) levels (a marker of systemic lipid peroxidation) were measured by using a fluorometric technique previously described (8,14).

Measurements of serum lipid levels. Serum lipid levels were measured by using a chromatographic enzymatic method in the Technicon automatic analyzer RA-1000 (Dade-Behring, Deerfield, Illinois). These results became available to the researchers at the end of the study, to maintain its blinded design.

Statistical analysis. All continuous variables were tested for normal distribution by visual inspection of the data and by using the Kolmokorov-Smirnov test. Normally distributed variables are expressed as mean ± SD (unless otherwise indicated) whereas nonnormally distributed variables are given as median [25th to 75th percentile].

Comparisons of normally distributed variables between groups were performed by using unpaired *t* tests or 1-way analysis of variance (ANOVA) for multiple comparisons followed by Bonferroni post-hoc correction, as appropriate. Comparisons of non-normally distributed variables between groups were performed by using the Mann-Whitney *U* test or the Kruskal-Wallis 1-way ANOVA as appropriate. When the Kruskal-Wallis test was significant, then the Mann-Whitney *U* test was used for individual comparisons, followed by Bonferroni post-hoc correction.

For comparison of the effect of treatment on variables (e.g., for changes of lipid levels in the randomized study), 2-way ANOVA for repeated measurements with treatment-by-time

interaction was used. In the ex vivo experiments (where tissue aliquots from the same patient were exposed to 3 interventions), when a significant p value was obtained using 1-way ANOVA for repeated measures, the effect of treatment was examined by paired *t* tests, using the actual (if normally distributed) or log-transformed values (if non-normally distributed), followed by Bonferoni post-hoc correction. Dichotomous variables were compared between groups by using the chi-square test.

Power calculations for study 1 showed that with 280 patients in SR undergoing CABG, we would be able to detect a true hazard ratio 1.87 for post-operative AF between the 2 extreme tertiles of NADPH-stimulated O_2^-, with power 0.9 and $\alpha = 0.05$. For study 2, we estimated that 42 patients would allow us to detect a 10% difference of the log (NADPH-stimulated O_2^-) between the 2 groups, with power 90% and $\alpha = 0.05$. For study 3, we estimated that by using 3 paired samples we would be able to detect a 30% difference of Rac1 activation between control and treated samples, with power 90% and $\alpha = 0.05$. Pearson's *r* or Spearman's rho simple correlation coefficients were estimated, as appropriate.

To evaluate the association between measures of myocardial redox state (basal O_2^-, NADPH-stimulated O_2^-, apocynin-inhibitable O_2^-, rotenone-inhibitable O_2^-, and $ONOO^-$) and post-operative outcome (i.e., AF and length of hospitalization), the study population was divided into tertiles of the redox measurements, and comparisons between Kaplan-Meier curves, defined by these tertiles (as well as by using the continuous variables), were performed by using Cox regression. In these models, redox measurements (as tertiles) and variables (e.g., age, sex, hypertension, diabetes mellitus, dyslipidemia, smoking, body mass index, EuroSCORE [European System for Cardiac Operative Risk Evaluation], left ventricular ejection fraction (LVEF), pre-existing AF, or medication such as beta-blockers and statins) that showed an association ($p < 0.10$ by using appropriate univariate Cox models) with post-operative complications were used as covariates.

To evaluate the association of myocardial redox state with the duration of inotropic support, we performed multiple linear regression, in which the actual duration of inotropic support (in days) was used as dependent (continuous) variable; whereas as independent variables, we used measures of myocardial redox state (as stated) as well as the patients' characteristics (age, sex, hypertension, diabetes, hypercholesterolemia, smoking, body mass index, medication, and EuroSCORE) that showed a relevant simple association with the dependent variable at the level of $p < 0.10$.

All statistical analyses were performed by using SPSS version 18.0 (SPSS, Chicago, Illinois), and p values were considered significant if < 0.05.

Results

The demographic and clinical characteristics of the participants are presented in Table 1. The patients in the prospective study were hospitalized for a median of 8 days (range 5 to 28 days). During follow-up, 3 patients died, 203 patients required post-operative inotropic support (138 for 1 day, 38 for 2 days, and 27 for 3 or more days), and 80 had post-operative AF; no redo operations were required.

Post-operative atrial fibrillation. To identify the clinical and biochemical parameters associated with the new onset of AF in the post-operative period in patients in SR (n = 281), we first screened the potential covariates by using univariate Cox regression. We found that the risk of post-operative AF was increased with age (hazard ratio [HR]: 1.051, 95% confidence interval [CI]: 1.024 to 1.079, p = 0.0001) and EuroSCORE (HR: 1.094, 95% CI: 1.056 to 1.134, p = 0.0001) and reduced by the use of beta-blockers (HR: 0.469, 95% CI: 0.299 to 0.733). Left atrial size was also correlated with the development of post-operative AF (HR: 1.055, 95% CI: 1.018 to 1.094, p = 0.004), but this variable was not included in the multivariable Cox-regression model as it was available for only 209 patients. There was no significant association between prior statin use and post-operative AF among these patients (HR: 1.213, 95% CI: 0.756 to 1.947, p = 0.423); however, most of the patients who were in SR before surgery were receiving treatment with statins (73%). Aortic valve replacement surgery and LVEF were not associated with post-operative AF and were not included in any analysis.

Pre-operative plasma MDA was similar between patients who had post-operative AF (median [25th to 75th percentile]: 1.10 [0.85 to 1.47] μmol/l) and patients who remained in SR (1.09 [0.80 to 1.46] μmol/l, p = NS). However, patients who had post-operative AF had significantly higher basal myocardial O_2^- (84 [59 to 146] vs. 68.3 [48 to 86] relative light units per second per milligram [RLU/s/mg], p = 0.003), NADPH-stimulated O_2^- (31,142 [14,850 to 47,430] RLU/s/mg vs. 14,410 [6,329 to 27,937] RLU/s/mg, p = 0.0001) and $ONOO^-$ production (239 [113 to 484] RLU/s/mg protein vs. 152 [79 to 229] RLU/s/mg protein, p = 0.003). Similarly, the apocynin-inhibitable O_2^- fraction of NADPH-stimulated O_2^- was greater in patients who had postoperative AF (6,655 [4,024 to 13,475] RLU/s/mg protein vs. 3,609 [1,644 to 7,583] RLU/s/mg protein, p = 0.0001). By contrast, no difference in myocardial rotenone-inhibitable O_2^- was observed between groups (3.1 [−7.6 to 25.4] RLU/s/mg protein in patients who had AF vs. 7.4 [0 to 14.8] RLU/s/mg protein in patients who maintained SR after surgery, p = NS).

To investigate the contribution of myocardial redox state to post-operative AF, we plotted the Kaplan-Meier curves for the development of AF by using tertiles of each of these measures and performed Cox regression to compare the tertiles after adjustment for relevant clinical variables. Plasma MDA showed no association with the development of post-operative AF (Fig. 1A); however, basal and NADPH-stimulated O_2^- (Figs. 1B and 1C), and the apocynin-inhibitable fraction of O_2^- (HR: 4.68, 95% CI: 1.32 to 16.66, p = 0.017 for mid vs. lowest tertile; and HR:

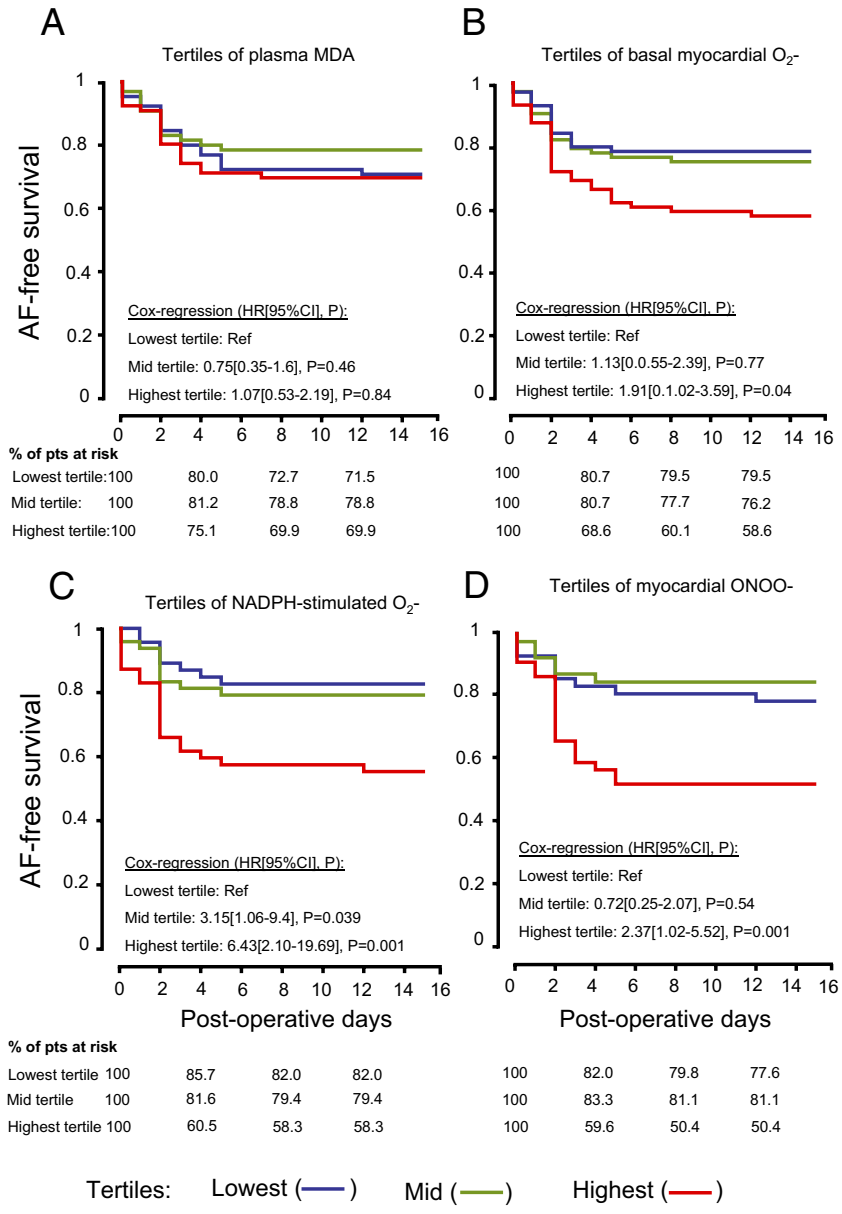

Figure 1 **Systemic Lipid Peroxidation and Redox State in Right Atrium Appendage, as Predictors of Post-Operative Atrial Fibrillation**

(A) Plasma malonyldialdehyde (MDA) was not associated with post-operative atrial fibrillation (AF). **(B)** Right atrial basal and **(C)** nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated superoxide (O_2^-) production, as well as **(D)** peroxynitrite ($ONOO^-$) formation were all associated with increased risk of post-operative AF. Hazard ratio (HR [95% confidence interval (CI)]), p value derived from Cox regression, after adjustment for age, use of beta-blockers, and EuroSCORE. When redox state was used as a continuous variable in each analysis, the respective adjusted p values were 0.526 for MDA, 0.038 for basal myocardial O_2^-, 0.0001 for myocardial NADPH-stimulated O_2^-, and 0.001 for myocardial $ONOO^-$. **Blue lines** = lowest tertile; **green lines** = middle tertile; and **red lines** = highest tertile. EuroSCORE = European System for Cardiac Operative Risk Evaluation; pts = patients.

5.48, 95% CI: 1.63 to 18.48, p = 0.006 for highest vs. lowest tertile) were strongly associated with the development of post-operative AF, even after adjusting for age, use of beta-blockers, and EuroSCORE. Myocardial $ONOO^-$ was also associated with post-operative AF in these patients (Fig. 1D).

Length of hospitalization. In the univariate Cox regression, the length of hospital stay was associated with age (HR: 0.98, 95% CI: 0.97 to 0.99, p = 0.005), female sex (HR: 0.72, 95% CI: 0.54 to 0.98, p = 0.036), hypertension (HR: 1.37, 95% CI: 1.06 to 1.77, p = 0.016) and diabetes (HR: 0.73, 95% CI: 0.56 to 0.95, p = 0.021), while there was a borderline association with the EuroSCORE (HR: 0.93, 95% CI: 0.88 to 1.02, p = 0.09) and prior statin therapy (HR: 1.24, 95% CI: 0.99 to 1.60, p = 0.08); all these variables were included in the multivariable Cox regression models. Pre-

operative LVEF was also significantly associated with the length of hospitalization (HR: 1.05, 95% CI: 1.01 to 1.94, p = 0.002) and was included in the respective multivariable Cox regression analysis. Left atrial diameter was associated with the length of hospital stay (HR: 0.97, 95% CI: 0.94 to 0.99, p = 0.002), but it was not included in the multivariable Cox regression model because this measurement was only available for 226 patients. Aortic valve replacement was not associated with the length of hospital stay.

To investigate the possible contribution of systemic/myocardial redox state to the length of hospitalization, we first examined the associations between the latter and individual measures of systemic or myocardial redox state. The length of hospital stay was correlated with plasma MDA (rho = 0.231, p = 0.001), basal O_2^- (rho = 0.184, p = 0.008), myocardial NADPH-stimulated O_2^- (rho = 0.273, p = 0.001), apocynin-inhibitable O_2^- (rho = 0.259, p = 0.002), and $ONOO^-$ (rho = 0.180, p = 0.034). There was no association between the length of hospitalization and rotenone-inhibitable O_2^- (rho = 0.04, p = 0.712).

Comparisons of the Kaplan-Meier curves between tertiles of redox measurements by using Cox regression showed that

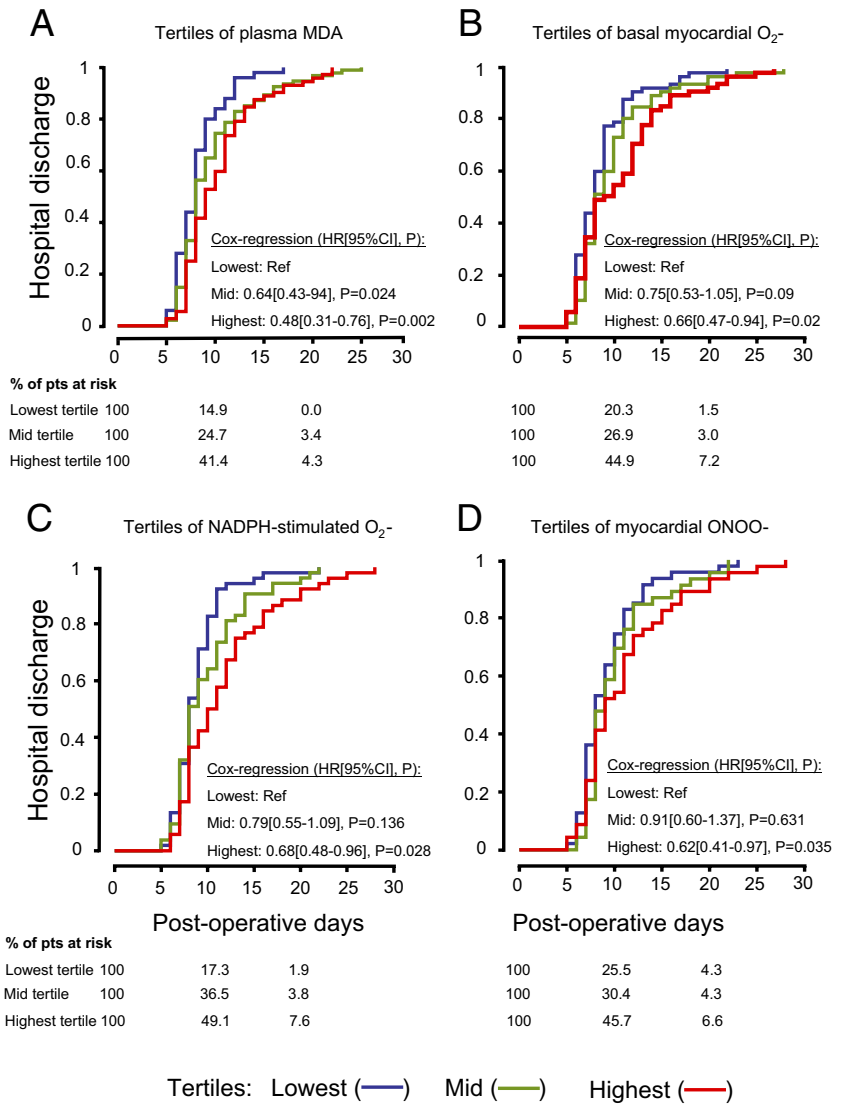

Figure 2 **Systemic Lipid Peroxidation and Redox State in Right Atrium Appendage, as Predictors of the Length of Hospital Stay**

The length of hospital stay was significantly associated with **(A)** plasma malonyldialdehyde (MDA), **(B)** basal myocardial superoxide (O_2^-), and **(C)** nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated O_2^-, while a borderline association was also observed with **(D)** myocardial peroxynitrite ($ONOO^-$). Hazard ratio (HR [95% confidence interval (CI)]), p value derived from Cox regression after adjustment for age, sex, hypertension, diabetes mellitus, use of statins, EuroSCORE, and left ventricular ejection fraction. When redox state was used as a continuous variable in each analysis, the respective adjusted p values were p = 0.041 for MDA, p = 0.013 for basal myocardial O_2^-, p = 0.008 for myocardial NADPH-stimulated O_2^-, and p = 0.05 for myocardial $ONOO^-$. **Blue lines** = lowest tertile; **green lines** = middle tertile; and **red lines** = highest tertile. EuroSCORE = European System for Cardiac Operative Risk Evaluation; pts = patients.

pre-operative plasma MDA (Fig. 2A), basal myocardial O_2^- (Fig. 2B), NADPH-stimulated myocardial O_2^- (Fig. 2C) and its apocynin-inhibitable fraction (HR: 0.593, 95% CI: 0.366 to 0.962, p = 0.034 for highest vs. lowest tertiles of apocynin-inhibitable O_2^-), and $ONOO^-$ (Fig. 2D) were associated with the length of hospitalization after adjustment for age, sex, diabetes, hypertension, use of statins, LVEF, and EuroSCORE. Statin treatment was not a significant predictor of length of hospital stay in any of the Cox-regression models (data not shown).

Importantly, when post-operative AF was included in the Cox-regression models, the association between the length of hospital stay and systemic/myocardial redox state (such as plasma MDA [HR: 0.67, 95% CI: 0.45 to 0.98, p = 0.04 for mid vs. lowest tertiles and HR: 0.48, 95% CI: 0.31 to 0.75, p = 0.001 for highest vs. lowest tertiles of plasma MDA]), NADPH-stimulated myocardial O_2^- generation (HR: 0.67, 95% CI: 0.43 to 0.99, p = 0.048 for the highest vs. lowest tertile of NADPH-stimulated O_2^-), and $ONOO^-$ (HR: 0.63, 95% CI: 0.41 to 0.97, p = 0.04), suggesting that systemic lipid peroxidation (as assessed by MDA), atrial NADPH oxidase activity, and $ONOO^-$ may predict the length of hospitalization, independent of their effect on post-operative AF.

In the whole study population, plasma MDA was weakly correlated with myocardial NADPH-stimulated O_2^- (rho = 0.181, p = 0.029) and basal myocardial O_2^- (rho = 0.155, p = 0.065). There was no association between plasma MDA and myocardial $ONOO^-$ (rho = −0.034, p = 0.701).

Post-operative inotropic support. Age (rho = 0.191, p = 0.004), hypertension (rho = 0.112, p = 0.09), diabetes (rho = 0.115, p = 0.08), EuroSCORE (rho = 0.242, p = 0.001), pre-existing heart failure (rho = 0.270, p = 0.0001), LVEF (rho = −0.207, p = 0.002), chronic AF (rho = 0.271, p = 0.0001), and the time on bypass (rho = 0.143, p = 0.023) were all associated with the duration of inotropic support. In the multivariate analysis, the regression model based on these clinical variables was significantly strengthened when myocardial NADPH-stimulated O_2^- was included (Table 2).

When we classified the patients into 3 groups according to the duration of post-operative inotropic support (i.e., those who required 0 days, 1 day, or ≥2 days of inotropic support), we observed no association between the need for inotropic support and either pre-operative plasma MDA (Fig. 3A) or basal myocardial O_2^- (Fig. 3B). In contrast, the duration of inotropic support was increased in parallel with the increase of myocardial NADPH-stimulated O_2^- (Fig. 3C), and the apocynin-inhibitable O_2^- (rho = 0.250, p = 0.002). Similarly, myocardial $ONOO^-$ increased in parallel with the duration of inotropic support during the post-operative period (Fig. 3D).

Effects of short-term pre-operative atorvastatin treatment on the myocardial redox state. The findings from our prospective study underscore the importance of measures of myocardial redox state as markers of clinical outcome after cardiac surgery. Statin treatment has been associated with improved postoperative outcome (6,7); here, we explored whether short-term pre-operative treatment with atorvastatin 40 mg/day affects the myocardial redox state. After 3 days of treatment, there was a small reduction in serum low-density lipoprotein (LDL) cholesterol in the atorvastatin-treated group (by −13.9 ± 28.3 mg/dl), which was, however, not significantly different from the change observed after placebo (by −4.2 ± 24.1 mg/dl, p = 0.290 for the interaction). Similarly, the changes of high-density lipoprotein (by 3.05 ± 7.1 mg/dl) and triglycerides (by −10.4 ± 57.5 mg/dl) after atorvastatin treatment were not significantly different compared to the changes after placebo

Table 2 Multivariate Predictive Models for Duration of Post-Operative Inotropic Support

Variables	R^2 for Model	Coefficient (Adjusted β, p Value)
Model 1: clinical characteristics	0.218	
Age		0.072, 0.298
Hypertension		0.038, 0.559
Diabetes Mellitus		−0.115, 0.830
EuroSCORE		−0.015, 0.830
Pre-existing heart failure		0.116, 0.080
Chronic atrial fibrillation		0.164, 0.021
Time on bypass		0.355, 0.0001
Model 2: clinical characteristics + NADPH-stimulated O_2^-	0.317	
Age		0.080, 0.326
Hypertension		−0.045, 0.542
Diabetes mellitus		−0.063, 0.404
EuroSCORE		0.018, 0.817
Pre-existing heart failure		0.097, 0.186
Chronic atrial fibrillation		0.121, 0.170
Time on bypass		0.398, 0.0001
NADPH-stimulated O_2^-		0.208, 0.020

EuroSCORE = European System for Cardiac Operative Risk Evaluation; NADPH = nicotinamide adenine dinucleotide phosphate; O_2^- = superoxide anion.

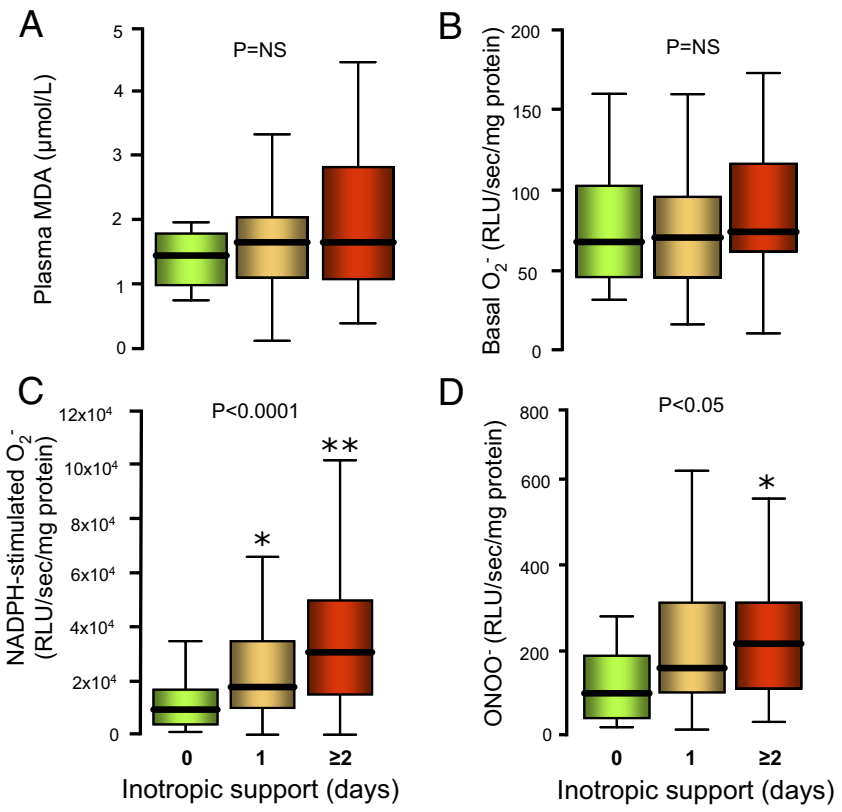

Figure 3 **Associations Between Systemic Lipid Peroxidation/Myocardial Redox State and the Need/Duration of Inotropic Support After Surgery**

The duration of inotropic support in the post-operative period was independent of **(A)** plasma malonyldialdehyde (MDA) or **(B)** basal superoxide (O_2^-) but showed a significant association with **(C)** atrial nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated O_2^- and **(D)** peroxynitrite ($ONOO^-$) production. The p values are derived from Kruskal- Wallis 1-way analysis of variance. *$p < 0.05$ and **$p < 0.01$ versus no use of inotropes after Bonferroni post-hoc correction. Values are expressed as median (25th to 75th percentile). **Green bars** = 0 days; **gold bars** = 1 day; **red bars** = ≥2 days. NS = nonsignificant; RLU = relative light units.

(by 0.7 ± 8.5 mg/dl and 12.5 ± 41.9 mg/dl, respectively; $p = 0.374$ and $p = 0.904$ for the interaction, respectively). However, patients who received atorvastatin showed a significantly lower basal O_2^- and $ONOO^-$ in the RAA (Figs. 4A and 4C), as well as a reduction in the NADPH-stimulated O_2^- (Fig. 4B) and in the apocynin-inhibitable O_2^- ($p < 0.001$; data not shown). Conversely, the rotenone-inhibitable O_2^- was not affected by atorvastatin (p = NS; data not shown). There was no association between the change of LDL and basal O_2^- (rho = −0.055, $p = 0.750$), NADPH-stimulated O_2^- (rho = −0.096, $p = 0.594$), apocynin-inhibitable O_2^- (rho = −0.066, $p = 0.740$), rotenone-inhibitable O_2^- (rho = −0.015, $p = 0.950$), or $ONOO^-$ (rho = −0.088, $p = 0.638$) in the RAA of these patients. These findings support the notion that the effect of atorvastatin on myocardial redox state is independent of LDL lowering.

Effects of ex vivo atorvastatin on myocardial redox state. To further explore the mechanisms by which statins affect the myocardial redox state, we incubated RAA samples from 26 patients with atorvastatin 20 μmol/l for 1 h. Atorvastatin caused a mevalonate-reversible reduction of myocardial O_2^- (derived from NADPH oxidase) and $ONOO^-$ (Figs. 5A and 5B) and the guanosine triphosphate-Rac1 to total Rac1 ratio (Figs. 5C and 5D). Together, these findings suggest that inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase by statins effectively reduces myocardial Rac1 and NADPH oxidase activity, independently of LDL lowering.

Discussion

In the present study, we demonstrate that myocardial RAA O_2^- (derived mainly from NADPH oxidases) and $ONOO^-$ are independent predictors of post-operative complications (i.e., AF and need for inotropic support) and the length of hospital stay among patients undergoing cardiac surgery. We also show that short-term pre-operative treatment with atorvastatin (40 mg/d) reduces myocardial O_2^- and $ONOO^-$ by reducing NADPH oxidase activity, and that ex vivo incubation of human RAA tissue with atorvastatin reduces NADPH oxidase activity and myocardial $O_2^-/ONOO^-$ production by a mevalonate-dependent inhibition of Rac1 activation. These novel findings suggest that inhibition of myocardial $O_2^-/$

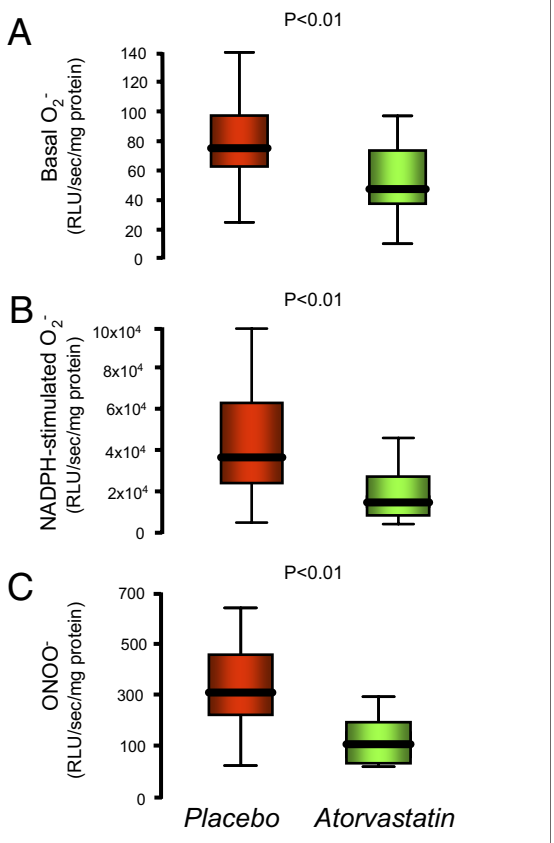

Figure 4 **Effect of Short-Term Pre-Operative Atorvastatin Treatment on Myocardial Redox State**

Pre-operative 3-day treatment with atorvastatin (40 mg/d) reduced **(A)** myocardial basal superoxide (O_2^-) as well as **(B)** nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated O_2^-, and **(C)** peroxynitrite ($ONOO^-$) compared to placebo. Values are expressed as median (25th to 75th percentile). **Red bars** = placebo; **green bars** = atorvastatin. RLU = relative light units.

$ONOO^-$ production (mainly by NADPH oxidases) may contribute to the beneficial effect of statins in patients undergoing cardiac surgery.

Myocardial redox state and prediction of post-operative complications. Atrial fibrillation is the most common complication after cardiac surgery (occurring in ~30% of patients), leading to hemodynamic instability and prolonged hospitalization (1,2). Age and the use of beta-blockers (2) as well as risk scores, such as the EuroSCORE (12), have been consistently associated with the new onset of post-operative AF after CABG surgery. Recent studies have suggested that myocardial O_2^- affects atrial electrical remodeling (15,16) and is associated with an increased risk of post-operative AF after cardiac surgery (4). However, the role of other highly toxic reactive oxygen species such as $ONOO^-$ in the development of post-operative complications is unknown.

In the present study, we show that right atrial O_2^- production (mainly derived from NADPH oxidase) is an independent predictor of post-operative AF in patients undergoing cardiac surgery. We also show for the first time that myocardial $ONOO^-$ may be involved into the pathogenesis of post-operative AF. Myocardial O_2^- and $ONOO^-$ were also associated with the need for post-operative inotropic support, whereas both systemic lipid peroxidation and myocardial NADPH oxidase activity were strong predictors of the length of hospitalization, independent of post-operative AF. In contrast, MDA was not associated with post-operative AF or with measurements of myocardial redox state.

Myocardial redox state as a therapeutic target of statin treatment. Recent studies have suggested that pre-operative treatment with statins reduces the risk of post-operative AF and improves the overall clinical outcome after cardiac surgery (6,7). However, despite previous reports indicating that statins reduce myocardial NADPH oxidase activity in experimental animal models (17), the clinical data are limited (11). In the present study, statin treatment prior to surgery was associated with shorter hospital stay (but not with post-operative AF); however, this association did not hold when included in Cox regression analyses. It should be noted that most of SR patients were receiving statins at the time of surgery (73%), and 33% of them were taking comparatively low doses. To explore the effect of high-dose statin treatment on myocardial redox state, we performed a randomized placebo-controlled study, in which treatment with atorvastatin 40 mg/d for only 3 days before cardiac surgery reduced myocardial $ONOO^-$ and NADPH oxidase activity. We also showed that ex vivo incubation of human atrial homogenates with atorvastatin results in a mevalonate-reversible reduction in NADPH oxidase activity (partly by suppressing Rac1 activation) and myocardial $O_2^-/ONOO^-$ production, supporting the notion that the effects of statins on myocardial redox are independent of LDL cholesterol lowering. The rapidity of this effect (which was observed after 1-h incubation with atorvastatin) suggests that isoprenoids are rapidly depleted in in vitro tissue preparations. Indeed, in cell culture models, atorvastatin induces significant biological changes (e.g., in intracellular adenosine monophosphate protein kinase levels and endothelial nitric oxide synthase phosphorylation status) as early as after 10 min of incubation (18), whereas 6-h incubation of human saphenous veins with atorvastatin inhibits NADPH-oxidase, through suppression of Rac1-activation (8).

Conclusions

Myocardial O_2^- and $ONOO^-$ are strong independent predictors of in-hospital clinical outcome in patients undergoing cardiac surgery, as atrial production of these oxidant and nitrating species is independently associated with the development of post-operative AF, the need for inotropic support, and the overall length of hospitalization. Our study confirms that NADPH oxidases are the main enzymatic source of atrial O_2^- production in patients in SR undergoing cardiac surgery, and it shows that short-term statin treatment

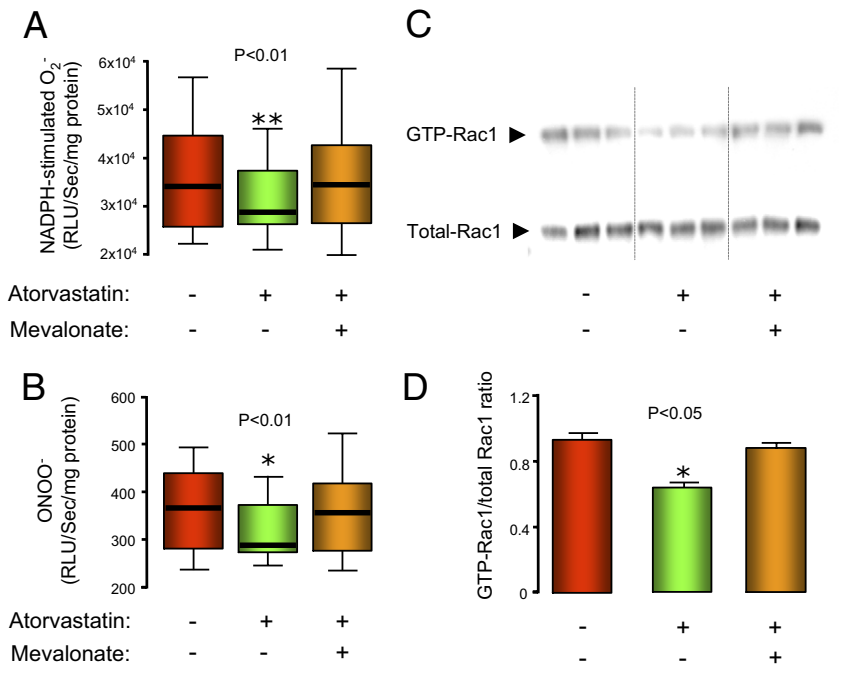

Figure 5 **Direct Effects of Atorvastatin on Human Myocardial Redox State**

Ex vivo incubation of myocardial samples with atorvastatin 20 μmol/l caused a mevalonate-reversible reduction in **(A)** nicotinamide adenine dinucleotide phosphate (NADPH)-stimulated superoxide (O_2^-) and **(B)** $ONOO^-$ production, and **(C, D)** Rac1 activation compared to control. The p values are derived from analysis of variance for repeated measures on the log-transformed **(A, B)** or the actual values **(D)**. *$p < 0.05$ and **$p < 0.01$ versus control (no atorvastatin, no mevalonate), after Bonferoni post-hoc correction. Values are expressed **(A, B)** as median (25th to 75th percentile) or **(D)** as mean ± SEM. GTP = guanosine triphosphate.

reduces myocardial O_2^- and $ONOO^-$ generation mainly by suppressing Rac1 and NADPH oxidase activity. These effects are due to the direct, rapid inhibition of HMG-CoA reductase in the human atrial tissue and are independent of LDL cholesterol lowering. Together, these novel findings suggest that suppressing O_2^- formation from myocardial NADPH oxidases may improve in-hospital outcome for patients undergoing cardiac surgery.

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Key Words: atrial fibrillation ■ cardiac surgery ■ myocardium ■ NADPH oxidase ■ statins.