Characterization of cardiac oxidative stress levels in patients with atrial fibrillation

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Abstract

Atrial fibrillation (AF) is associated with oxidative stress and elevated brain natriuretic peptide (BNP) levels. However, the exact cardiac origin of oxidative stress and its association with BNP levels in AF patients remain unclear. Therefore, we investigated the chamber-specific plasma oxidative stress levels in patients with paroxysmal AF (PAF) and persistent AF (PSAF). Diacron-reactive oxygen metabolite (dROM) levels were measured in patients with PAF (n = 50) and PSAF (n = 35) at different cardiac sites before ablation and in peripheral vein 3 months after ablation. For all sites, dROM levels were higher in PSAF patients than in PAF patients; the levels were the highest in the coronary sinus at 429.0 [interquartile range: 392.0–449.0] vs. 374.0 [357.0–397.8] Carratelli units (P<0.05). dROM levels in the coronary sinus were related to the BNP levels (r = 0.436, P<0.001). Furthermore, the reduction in the peripheral dROM levels was related to that in the peripheral BNP levels in patients with symptomatic improvement (r = 0.473, P <0.001). Cardiac oxidative stress may either be a cause or consequence of prolonged AF, and cardiac oxidative stress levels correlated with BNP levels, though a possible source of oxidative stress in AF patients may be systemic circulation.

Key words: atrial fibrillation, oxidative stress, brain natriuretic peptide, ablation

Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered clinically, and its incidence has dramatically increased owing to an aging population. AF is associated with a high risk of stroke, hospitalization, diminished quality of life, and significant mortality [1]. Although limited, the understanding of the pathophysiology of AF has considerably improved [2]. In particular, an imbalance between reactive oxygen species generation and antioxidant reserves (i.e., oxidative stress) is now considered the major underlying pathophysiological change in AF, and oxidative stress and inflammation may initiate and perpetuate the condition [3-6]. Oxidant and inflammatory pathways can be targets for therapeutic intervention for AF, though clinical trials using general antioxidants have not achieved the expected therapeutic results [7,8].

Recent reports indicate that rapid atrial pacing increases myocardial peroxynitrite formation and vulnerability to AF, which can be reversed by antioxidants such as ascorbate [9] and statins [10]. Furthermore, oxidative stress marker levels are elevated in AF patients [11,12]. Oxidative stress is known to promote inflammation and cause the structural and electrical remodeling of the atrium [13-15], and to alter the structure and function of membrane phospholipids, proteins, and mitochondrial DNA, all of which support myocardial remodeling and cardiac functional failure [16]. AF patients with normal ventricular systolic function, as assessed by echocardiography, also have elevated brain natriuretic peptide (BNP) levels [17-19] and elevated BNP levels could be a useful marker of subsequent thromboembolic events in patients with AF during oral anticoagulant therapy [20]. However, the exact source of oxidative stress and its relation to BNP levels in AF

patients remain unclear. Therefore, we investigated chamber-specific plasma oxidative stress levels in patients with paroxysmal AF (PAF) and persistent AF (PSAF) who underwent radiofrequency catheter ablation (RFCA) to further elucidate the pathophysiology of AF.

Methods

Study population

We enrolled 85 AF patients, including 50 PAF patients and 35 PSAF patients, who underwent RFCA at the Shinshu University hospital between December 2007 and April 2013. PAF was defined as AF lasting up to 7 days with spontaneous self-termination. PSAF was difined as AF lasting more than 7 days or requiring termination by at least 1 antiarrhythmic agent. The exclusion criteria were an age of <20 years, a history of coronary artery disease, cardiac surgery, cancer, renal failure and/or chronic alcoholism, active inflammatory status within 90 days, or structural heart disease, including primary valvular heart disease. Written consent was obtained from the patients before enrolment, and the protocol was approved by the Ethics Committee at Shinshu University Graduate School of Medicine. This study was performed in accordance with the Declaration of Helsinki.

Blood sampling

Peripheral blood was drawn from the left cubital vein via a 20-gauge peripheral venous catheter (Surflo®; Terumo Medical Products, New Jersey, USA) immediately before RFCA. Before ablation, a luminal coronary sinus (CS) catheter was used for plasma sampling from (1) the CS and advance transseptal guiding

sheaths, (2) the right ventricle and atrium before transseptal puncture, and (3) the left atrium immediately after transseptal puncture. Plasma was isolated from all samples within an hour of collection and assayed immediately for diacron-reactive oxygen metabolite (dROM), biological antioxidant potential (BAP), and BNP levels. All blood samples were obtained between 9:00 and 10:00 AM in the fasting state to exclude the effects of diurnal and dietary variation on oxidative stress levels.

Biomarker analysis

Plasma levels of dROM and BAP were used as markers of oxidative stress. The dROM and BAP levels represent the total amount of peroxidized metabolites and the serum antioxidant capacity, respectively, and were determined by the Free Radical Elective Evaluator (Diacron International, Grosseto, Italy), using commercial assay kits (Diacron SRL, Grosseto, Italy). The levels of dROM were expressed in Carratelli units (U Carr), with 1 U Carr corresponding to 0.8 mg/l H_2O_2. The normal range of dROM is 250–300 U Carr. BAP measurement is based on the ability of a colored solution, containing a source of ferric (Fe^{3+}) ions bound to a chromogenic substrate (thiocyanate derivative), to decolor when Fe^{3+} ions are reduced to ferrous ions (Fe^{2+}) by the reducing activity of blood samples. This chromatic change was read using a photometer at 505 nm. Preliminary data from nonuremic healthy individuals indicate that the normal BAP level is >2,200 μmol/L.

Peripheral BNP levels were measured by fluorescence enzyme immunoassay immediately after plasma collection using a commercially available kit (TOSOH II, Tosho Corporation, Japan) and an automated immunoassay analyzer (AIA-2000, Tosho Bioscience, Japan) according to the manufacturer's instructions.

Echocardiographic measurements

For the measurement of hemodynamic parameters, two-dimensional cardiac echocardiography was performed at baseline and again at 3 months after RFCA by the same operator, and the results were reviewed by 2 different readers. The left ventricular ejection fraction (LVEF) was assessed according to the Simpson rule. The internal diameters of the left atrium and left ventricle in diastole (LAD and LVDd, respectively), and the E/e′ ratio of early diastolic mitral inflow velocity (E) and early diastolic mitral annular velocity (e′) were also measured.

RFCA

From the left subclavian vein, a multipolar electrode catheter was placed in the coronary sinus vein. An 8-F SL0 sheath was placed through the right femoral vein for transseptal catheterization, an 8.5-F sheath was placed for ICE (Ultra ICE Boston, MA, USA), and a 5-F sheath was placed for the right ventricular pacing electrode catheter. After transseptal puncture, left atrium and pulmonary vein (PV) geometry was mapped with a 3.5-mm Navistar Thermocool D curve (Biosense Webster, Diamond Bar California, USA) under the CARTO system (Biosense Webster) and merged to three-dimensional computed tomography. PV isolation was started from the left superior PVs to the left inferior PVs and right PVs while achieving electrical isolation. A roof line was also isolated. Radiofrequency power output was about 15 or 20 W. After PV isolation, a block line was made at the right isthmus.

Post-ablation follow-up

All study subjects were followed as outpatients and underwent physical examination and 12-lead electrocardiography (ECG) for 3 min at least twice a month. The peripheral dROM, BAP, and BNP levels were measured 3 months after RFCA. The clinical outcome was classified as successful if the patient did not have any documented arrhythmia and remained symptom-free. Patients with successful outcome were termed the "effective RFCA group," while the remaining patients formed the "non-effective RFCA group."

Statistical analysis

Statistical analysis was performed using SPSS Ver.18.0 (SPSS Inc., Chicago, IL, USA). Unless otherwise stated, data are presented as the mean ± standard deviation, if normally distributed, and as the median with the interquartile range (25^{th}–75^{th} percentiles), if non-normally distributed. The unpaired 2-tailed Student's *t*-test was used for normally distributed intergroup comparisons. Non-normally distributed, unpaired data were analyzed using the Mann-Whitney test. Non-normally distributed, paired data were analyzed with a Wilcoxon signed-rank test. Categorical variables were compared using the Chi-square (χ^2) test. Spearman's correlation coefficient test was used to assess relationships between the reduction in dROM, BAP, and BNP levels. For comparisons between multiple groups, we determined the significance of intergroup differences by one-way analysis of variance, followed by the Steel-Dwass' multiple comparison procedure. P values of <0.05 were considered statistically significant. Reductions in dROM, BAP, and BNP levels were defined as the difference between the levels at baseline and 3

months after RFCA.

Results

The characteristics of the PAF (n = 50) and PSAF (n = 35) patient groups at baseline and 3 months are shown in Table 1 and 2, respectively. No intergroup differences were noted in age, sex, blood pressure, body weight, hemoglobin A1c level, or the CHADS2-VASc score. The use of β-blockers, angiotensin converting enzyme (ACE) inhibitors, and angiotensin II receptor blockades (ARBs) was greater in PSAF patients than PAF patients, although the medication and dosage for all patients were maintained for at least 3 months before the study baseline. No intergroup differences were noted in low-density lipoprotein cholesterol levels or the use of statin and antiarrhythmic drugs. During the study, all the patients received anticoagulant therapy, but none received antioxidant supplementation such as vitamins and minerals. Echocardiography revealed no intergroup differences in LVEF and LVDd, although LAD was significantly greater in the PSAF patients than in the PAF patients (P <0.01) (Table 1). These parameters remained unchanged during the study period and no differences in the parameters were noted between the PAF and PSAF patients (Table 2). Nor was there any significant difference in these echocardiographic parameters between the effective RFCA group and the non-effective RFCA group at 3 months (data not shown).

The dROM levels were significantly higher in PSAF patients than in PAF patients at all examined sites (Figure 1A, B). The dROM levels were the highest in the CS of PSAF patients (429.0 U Carr [interquartile range, 392.0–449.0]; P<0.05). The difference in the dROM levels between the study groups was also the greatest

in the CS (PAF patients, 374.0 [357.0–397.8]; P <0.05). However, the BAP levels showed no intergroup differences (Figure 1C, D). The dROM levels at baseline in PAF and PSAF patients were significantly higher than those of age- and comorbidity-matched matched non-AF control (n = 115; age, 64.7 ± 5.9 years; dROM 340.7 [317.2–364.1] U Carr; P <0.05).

Of the patients in the PAF and PSAF groups, 76.0% (n = 38) and 68.6% (n = 24) were classified into the effective RFCA group, respectively. None of the pre-ablation dROM levels at the different sites were predictive of AF recurrence or symptom relief 3 months after RFCA (data not shown). At 3 months, peripheral dROM levels showed a significantly greater reduction in the effective RFCA group than in the non-effective RFCA group (P <0.001, Figure 2A), while no intergroup differences were noted in the reduction of the peripheral BAP level (P = 0.580, Figure 2B). In PAF (n = 28) and PSAF (n = 24) patients who did not undergo RFCA (defined as the non-ablation group), no changes were observed in the dROM and BAP levels during the study period (Figure 2A and 2B, respectively). Peripheral BNP levels were decreased significantly in the effective RFCA group, while no changes were shown in the non-effective RFCA group (Figure 2C).

The baseline levels of peripheral BNP were significantly higher in PSAF patients than in PAF patients (123.8 [64.3–186.7] vs. 52.7 [24.5–89.6] pg/mL, P <0.001; Figure 3A). The dROM levels in the CS (CS-dROM) were positively correlated with the peripheral BNP levels (r = 0.436, P <0.001; Figure 3B). Furthermore, in the effective RFCA group, a significant correlation was observed between the reduction in the peripheral dROM levels and the reduction in the peripheral BNP levels (r = 0.473, P <0.001; Figure 3C), but not between the

reduction in the peripheral BAP levels and that in peripheral BNP levels ($r = –0.082$, $P = 0.505$; Figure 3D).

Discussion

To our knowledge, no previous clinical study has aimed at identifying the exact cardiac source of oxidative stress in AF patients by measuring chamber-specific oxidative stress levels. Previous studies have reported that peripheral oxidative stress markers, including dROM [11,12], were elevated in AF patients and were strongly correlated with an increased risk of AF; however, their cardiac origin and association with other cardiac biomarkers have not been investigated. Our major findings are as follows: (1) At all sites, dROM levels were higher in PSAF patients than in PAF patients, with the dROM levels being the highest in the CS. (2) Similar BAP levels were noted at all sites in both PAF and PSAF patients, and these factors remained unchanged during the follow-up period. (3) The peripheral BNP levels were higher in PSAF patients than in PAF patients, and the CS-dROM levels were positively correlated with the peripheral venous BNP levels. (4) A reduction in the peripheral dROM levels, but not BAP levels, was correlated with a reduction in the peripheral BNP levels in the effective RFCA group.

The site-specific measurements made in this study provide several insights into the cardiac source of oxidative stress in AF patients. CS-dROM levels were elevated in PSAF patients but not in PAF patients, and the dROM levels in PAF patients were similar at all sites, irrespective of the rhythm during blood sampling (e.g., sinus rhythm or AF). These findings suggest that prolonged AF might be associated with cardiac oxidative stress in PSAF patients, although whether the

relationship is causative or resultant remains unclear. However, dROM levels at all sites were significantly higher in PSAF patients than in PAF patients, and dROM levels in the heart were almost equal to those of the peripheral blood in PSAF patients, suggesting that the heart is unlikely to be the only source of oxidative stress in AF patients. Instead, these results suggest that a possible source of oxidative stress in AF patients may be the systemic circulation.

Unlike a previous study [12], the present one did not find that the baseline dROM levels at the different sites could serve as biomarkers for predicting the risk of AF recurrence or symptoms at 3 and 6 months after RFCA (data not shown). A more prolonged study may elucidate the significance of this factor. Additionally, the BAP levels were similar at all sites both in PAF and PSAF patients, indicating that long-lasting AF promoted oxidative stress by enhancing the generation of pro-oxidants, rather than by reducing antioxidant defense. Several reports have suggested that oxidative stress in the left atrial tissue during AF influences the structural remodeling of the atrium [13,14]; however, our study did not show elevated levels of dROM in the left atrium. The main reason for this discrepancy might be sample differences between blood and left atrial tissue. The CS drains the lateral and posterior left atrium regions, the left atrial appendage and, mainly, the left ventricle [20]; hence, the elevation of CS-dROM levels might be secondary to not only the atrium but also the ventricular production of oxidative stress produced by long-lasting AF. Additionally, oxidative stress levels assessed by chamber-specific blood sampling might not directly reflect the oxidative stress levels in the heart itself; however, to our knowledge, no report has been published on the oxidative stress in left ventricle tissue samples in AF patients; therefore, further studies are

warranted.

Oxidative stress and peripheral venous BNP levels are linked with increasing age, hypertension, heart failure, diabetes mellitus, coronary artery disease, and obesity, which are associated with AF [1,22]. The PAF and PSAF groups had differences with respect to the usage of β-blockers, ACE inhibitors, and ARBs but not blood pressure. The different medications affect oxidative stress levels, or the blood pressure may have differed before starting these medications. However, all the patients had been administered these medications on a stable basis for at least 3 months before the study baseline, and the medications and dosages across the patients were not changed during the study. In the present study, no intergroup difference was noted in blood pressure at the time of sampling, and no differences were noted in dROM and BAP levels between patients who were and were not on these medications (data not shown). These drugs may have improved the oxidative stress in PSAF patients; nonetheless, the dROM levels were significantly higher in PSAF patients than in PAF patients at all examined sites. Therefore, it is unlikely that differences in medication usage affected the results of our study.

Gould et al [17] reported that BNP levels at different cardiac sites are elevated in PAF patients with normal ventricular function, with the highest levels in the CS. Further, this group noted an immediate decrease in BNP levels after ablation of the left atrium. BNP in AF patients could originate in the atria, although this has not been confirmed [17,23]. In our study, both dROM and BNP levels at baseline were correlated positively with the LAD (data not shown); however, the reductions in both dROM and BNP levels were not associated with changes in the LAD, since the LAD did not differ during the follow-up period. As observed in our study, Kurosaki

et al [18] showed that BNP level was elevated in patients with symptomatic PAF and PSAF, and that the BNP levels were reduced after RFCA. Of note, however, our results do not imply that the cause-and-effect relationships between dROM and BNP levels are established.

This study has several limitations. First, since we did not measure the dROM levels immediately after RFCA, we were unable to assess the early and direct effects of RFCA on dROM levels. Richter et al [24] showed that markers of oxidative stress and inflammation were significantly up-regulated for 2 days after RFCA for AF secondary to the ensuing cardiac cytolysis; therefore, evaluation of the immediate improvement in dROM levels would not have been possible. Second, we were unable to evaluate the cardiac oxidative stress levels 3 months after RFCA. The peripheral oxidative stress was improved in the effective RFCA group; however, a reduction in the levels of peripheral oxidative stress might not directly reflect a reduction in oxidative stress in the heart. Third, although our results suggest long-lasting AF may be required for the generation of cardiac oxidative stress in PSAF patients, it remains to be clarified whether the latter is a causative or resultant factor. However, in PAF and PSAF patients who did not undergo RFCA, the dROM and BAP levels did not change after 3 months. Fourth, although ambulatory 12-lead ECG was constantly performed during the follow-up period, asymptomatic episodes of AF might have been missed, and we could not completely exclude patients without any change in the incidences of arrhythmia in the effective RFCA group. Fifth, none of the markers measured in this study, including dROM, BAP, and BNP, are cardiac specific. Sixth, oxidative stress levels assessed by chamber-specific blood sampling might not directly reflect the oxidative stress

levels in the heart itself. Finally, the relatively short-term follow-up period may be responsible for the lack of significance in the pre-ablation peripheral dROM level as a predictive marker for successful RFCA, despite amelioration of the parameter in the effective RFCA group. Since it has been recognized that early recurrence of AF, usually defined as arrhythmia recurrence in the first 3 months following ablation, may not always predict recurrent arrhythmia later, the study period of 3 months might not be appropriate for correctly evaluating the association between improvement in oxidative stress and the effectiveness of ablation for AF. Further studies with a larger number of patients and a longer follow-up period might be needed to confirm our results.

Conclusions

Cardiac oxidative stress may be either a cause or consequence of prolonged AF, although the main source of oxidative stress in AF patients might be systemic circulation.

Conflict of Interest: The authors have no conflicts of interest and no sources of financial support to declare.

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Figure Legends

Figure 1. Oxidative stress at different cardiac sites and a peripheral vein before ablation in atrial fibrillation (AF) patients; (A) and (B), individual data points for diacron-reactive oxygen metabolite (dROM) levels at each site in (A) paroxysmal AF (PAF) and (B) persistent AF (PSAF) patients. Dashed lines denote median values; and continuous lines, 25th and 75th percentiles. "*****" indicates that dROM levels were the most elevated at the coronary sinus in PSAF patients compared with other sites (P <0.05). "**†**" indicates that the PSAF group had significantly higher dROM levels at all sites than the PAF group (P <0.05). (C) and (D), individual data points for biological antioxidant potential (BAP) at each site in (C) PAF and (D) PSAF patients. Dashed lines denote mean values; and continuous lines, the standard deviation. LA, left atrium; RA, right atrium; CS, coronary sinus

Figure 2. Effects of radiofrequency catheter ablation (RFCA) on oxidative stress in atrial fibrillation (AF) patients. Dashed lines denote median values; and continuous lines, 25th and 75th percentiles. Individual data points for the reduction in (A) diacron-reactive oxygen metabolite (dROM), (B) biological antioxidant potential (BAP), and (C) changes in peripheral BNP levels in paroxysmal AF (n = 28) and persistent AF (n = 24) patients who did not undergo RFCA (defined as the non-ablation group), in the effective and non-effective RFCA groups.

Figure 3. Brain natriuretic peptide (BNP) levels and oxidative stress in atrial fibrillation (AF) patients. (A) Individual data points for BNP levels among patients with paroxysmal AF (PAF) and persistent AF (PSAF). Dashed lines denote median

values; and continuous lines, 25th and 75th percentiles. (B) Relationship between BNP and diacron-reactive oxygen metabolite (dROM) levels in the coronary sinus (CS-dROM). (C) Relationship between the reduction in BNP and dROM levels, and (D) between the reduction in BNP and biological antioxidant potential (BAP) levels at the peripheral vein in the effective-ablation group.

Table 1. Baseline characteristics of the patients

	PAF (n = 50)	PSAF (n = 35)	*P*
Age (years)	64.4 ± 7.6	62.8 ± 8.4	0.12
Male	45 (90.0%)	33(94.3%)	0.84
Body weight (kg)	68.5 ± 11.2	71.7 ± 12.5	0.64
Medications			
β-blockers	28 (56.0%)	23 (65.7%)	0.62
Statin	7 (14.0%)	5 (14.3%)	0.92
Anticoagulant	50 (100%)	35 (100%)	1.0
ACEIs/ARBs	6/10 (32.0%)	5/16 (60.0%)	<0.05
Echocardiography:			
LAD (mm)	41.6 ± 6.8	48.5 ± 6.4	<0.01
LVDd (mm)	48.0 ± 5.8	50.0 ± 6.0	0.14
LVEF (%)	66.4 ± 8.0	61.7 ± 11.4	0.46
E/e′ ratio	9.6 ± 4.0	9.9 ± 2.9	0.77
BNP (pg/mL)	52.7 [24.5–89.6]	123.8 [64.3–186.7]	<0.001
LDL-C (mg/dL)	117.6 ± 30.2	118.6 ± 32.0	0.58
Hemoglobin A1c (%)	5.2 ± 1.3	5.1 ± 1.4	0.89
CHADS2-VASc score	0.64 ± 0.48	0.86 ± 0.69	0.11

Data are presented as mean ± SD or median and interquartile range [25th–75th percentiles]. ACEIs, angiotensin-converting enzymes inhibitors; ARBs, angiotensin-II-receptor blockers; BNP, brain natriuretic peptide; E/e′ ratio, early diastolic mitral inflow velocity (E) and early diastolic mitral annular velocity (e′); LAD, left atrium diameter; LDL-C, low-density lipoprotein cholesterol; LVDd, left

ventricular diastolic diameter; LVEF, left ventricular ejection fraction; PAF, paroxysmal atrial fibrillation; PSAF, persistent atrial fibrillation

Table 2. Echocardiographic parameters at 3 months

	PAF (n = 50)	PSAF (n = 35)	*P*
Echocardiography:			
LAD (mm)	42.0 ± 6.1	49.0 ± 7.7	<0.01
LVDd (mm)	47.3 ± 8.7	51.9 ± 6.2	0.20
LVEF (%)	69.2 ± 7.6	61.4 ± 16.9	0.56

Data are presented as mean ± SD. LAD, left atrium diameter; LVDd, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; PAF, paroxysmal atrial fibrillation; PSAF, persistent atrial fibrillation