Association of Complex Fractionated Electrograms with Atrial Myocardial Thickness and Fibrosis

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ABSTRACT

Background and Objectives: Although ablation of complex fractionated atrial electrograms (CFAE) in atrial fibrillation (AF) is one of the strategies for atrial substrate modification, the mechanism behind CFAE as an electrophysiological substrate remains unclear. We investigated structural differences between CFAE sites and their matched non-CFAE sites by comparing their histopathologic characteristics in canine AF models.

Methods: Atrial electrograms of four dogs were obtained from the epicardial site. AF was induced through burst atrial pacing at 600 bpm for 30 min. CFAE sites were identified during AF according to patterns visualized on the electrograms, and their matched non-CFAE sites were selected in the adjacent region, within 5 mm of each CFAE site. Tissues were harvested from CFAE sites and their matched non-CFAE sites at various locations in both atria. Histopathologic differences were identified between CFAE and non-CFAE sites.

Results: A total of 24 atrial tissues (12 with CFAE, 12 with non-CFAE) were evaluated. The atrial myocardium was significantly thicker at CFAE sites (1757.5±560.5 μm) than at non-CFAE sites (1279.5±337.2 μm) (p=0.036). At CFAE sites, it was filled with a significantly larger amount of fibrotic tissue than at non-CFAE sites (22.8±6.9% versus 7.2±4.7%, p<0.001). Results were consistent across various tissue locations. The distribution of autonomic nerve innervation was similar between CFAE and non-CFAE sites.

Conclusion: This study provides a better understanding of histological characteristics of CFAE sites, namely a thicker wall and greater amount of fibrosis. These findings may be associated with the development of CFAE and its pathophysiological contribution to AF.

Key Words: • Atrial Fibrillation • Complex Fractionated Electrograms • Myocardium • Radiofrequency Catheter Ablation • Fibrosis
Introduction

Although radiofrequency ablation has been an important treatment strategy for persistent atrial fibrillation (AF), it remains challenging and results are unsatisfactory. Several additive substrate modification strategies have been attempted, to improve the recurrence rate and long-term outcomes of non-paroxysmal AF. Nademanee et al. reported that complex fractionated atrial electrograms (CFAEs) could be ideal target sites for ablation of refractory AF, and CFAE-guided ablation may show a higher success rate and survival benefit than pulmonary vein isolation alone. However, the pathophysiologic mechanism of CFAE has not yet been clarified, and the structural relationship between CFAE sites and AF is also unknown. Since negative results of CFAE ablation have also been reported, characteristics of CFAE sites as an electrophysiological substrate should be more accurately identified and reflected in clinical practice. Therefore, we sought to evaluate the structural differences between CFAE and non-CFAE sites by comparing their histopathologic features.

Materials and Methods

Animal Preparation

The study protocol was approved by the Seoul National University Hospital Institutional Animal Care and Use Committee (approval No. 16-0136-S1A0). A total of four adult mongrel dogs, weighing 20–25 kg, were involved in the present study. Following the standard and approved protocols, all dogs were anesthetized with thiopental (20 mg/kg IV) and intubated with cuffed endotracheal tubes for mechanical respiration followed by gaseous anesthesia (1–2% isoflurane/O₂). All measures were taken to ensure that discomfort, distress, pain, and injury were limited as much as possible. Standard surface electrocardiogram leads were monitored continuously throughout the entire study, and intermittent arterial blood gas analyses with ventilator adjustments were done to correct for any metabolic abnormalities. An electrical heating pad was used to maintain a body temperature of 36–37 ℃. For the electrophysiological study and simultaneous tissue harvesting, a median sternotomy and pericardectomy were performed to access the canine heart directly. The epicardial patch electrodes for electrophysiological studies were fixed to the atrial free walls by simple sutures.

Electrophysiological Study Protocol

Atrial electrograms were amplified and filtered from 0.05 to 500 Hz and were displayed and recorded on a Prucka Cardiolab EP System (GE Medical Systems, Fairfield, CT, USA). Atrial pacing was performed at twice the current threshold. Epicardial bipolar electrograms were recorded using a standard diagnostic catheter with a 2-mm tip electrode, a 1-mm band electrode and a 2-mm distal inter-electrode spacing (Livewire, St. Jude Medical, Minnetonka, MN, USA).

Atrial Fibrillation Induction and CFAE Detection Protocol

AF was induced with 600-bpm burst atrial pacing for 30 min, and AF that was sustained longer than 10 min was included in the evaluation. The CFAE sites were determined according to the patterns on the electrograms. Electromgrams with the following two characteristics simultaneously were considered a CFAE: 1) atrial electrograms that had fractionated electrograms composed of two deflections or more, and/or perturbation of the baseline with continuous deflection of a prolonged activation complex, over a 10-s recording period; 2) atrial electrograms with a very short cycle length (≤120 ms) averaged over a 10-s recording period. A 10-s duration was used for the assessment of the CFAE to avoid nonspecific sites and to confirm consistent fractionation. The matched non-CFAE sites were selected in the adjacent region within 5 mm from each CFAE site.

Histopathologic Examination

Myocardial tissue from the CFAE and non-CFAE sites, detected at various locations of both atria, were harvested. The excised CFAE and non-CFAE site tissues were fixed in 4% formalin for more than 48 h for staining and histopathologic
examination. Sections of the obtained tissues were cut at 2 mm thickness by a microtome, and serial sections were stained with hematoxylin-eosin (HE), Masson’s trichrome (MT), tyrosine hydroxylase (TH), and choline acetyltransferase (ChAT). Digital photographs of microscopic images were taken under 40-, 100-, and 200-fold magnification (BX51TF; Olympus, Tokyo, Japan), and transmitted to the computer for quantitative analyses using image analysis software (ImageJ, National Institutes of Health, USA). Thickness of the atrial myocardium was measured in sections stained with HE at 40-fold magnification. For every section stained by MT, three to five visual fields under 200-fold magnification were randomly chosen to measure the amount of fibrosis in the atrial myocardium and the proportion of the area containing fibrosis was calculated for each section. The distribution of sympathetic or parasympathetic nerve innervation in the atrial myocardium was examined by quantitative analysis of nerve density.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation and were analyzed using the paired t test. All probability values were two-sided, and a p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS statistical package version 19.0 (IBM Corp, Armonk, NY, USA).

Results

Baseline Distribution of CFAE in Various Atrial Tissues

A total of 24 atrial myocardial tissues (12 from CFAE sites and 12 from their matched non-CFAE sites) were evaluated. Table 1 shows the anatomical location and distribution of the tissues used in the analysis. Of the 12 sites, 7 were located in the right atrium (the lateral wall proximally anterior to the anterior right ganglionated plexus [RALW], the mid portion of the sulcus terminalis [ST], and the sinus nodal area [SN]), and five in the left atrium (mid portion of the ligament of Marshall [LOM], posterior wall [LAPW], roof area [LAR], and left pulmonary vein antrum [LPVA]).

### Table 1. Anatomical distribution of CFAE versus non-CFAE sites of four dogs

| Location       | CFAE (n=12) | Non-CFAE (n=12) |
|----------------|-------------|-----------------|
| Right atrium   |             |                 |
| RALW           | 2           | 2               |
| ST             | 3           | 3               |
| SN             | 2           | 2               |
| Left atrium    |             |                 |
| LOM            | 2           | 2               |
| LAPW           | 1           | 1               |
| LAR            | 1           | 1               |
| LPVA           | 1           | 1               |

CFAE, complex fractionated atrial electrograms; LAPW, left atrial posterior wall; LAR, left atrial roof area; LOM, mid portion of ligament of Marshall; LPVA, left pulmonary vein antrum; RALW, lateral wall just anterior area near the anterior right ganglionated plexus; SN, sinus nodal area; ST, mid portion of the sulcus terminalis.

Relationship Between Thickness and Amount of Fibrosis in the Atrial Myocardium According to the CFAE

Table 2 summarizes the histopathologic differences between CFAE and non-CFAE sites. The atrial myocardium was significantly thicker at CFAE sites (1757.5±560.5 μm) than at matched non-CFAE sites (1279.5±337.2 μm) (p=0.036). The same trend was observed in the subgroups of the left and right atria. In particular, the difference was greatest in the SN in the right atrium (CFAE site, 1536.5±114.7 μm; non-CFAE site, 825.5±134.3 μm) and RALW (CFAE site, 1935.9±539.2 μm; non-CFAE site, 1409.3±275.7 μm). There was a pronounced difference in the amount of fibrosis between CFAE and their matched non-CFAE sites. The atrial myocardium at the CFAE sites was filled with significantly larger amounts of fibrotic tissue than matched non-CFAE sites (22.8±6.9% versus 7.2±4.7%, p<0.001) (Figure 1). The same results were obtained at all locations in the left and right atria. The difference was observed to be particularly large in the LAPW (30.3% versus 6.6%).
Table 2. Histopathologic characteristics of harvested tissues according to the presence of CFAE

|                     | Thickness of atrial myocardium (μm) | Fibrosis in atrial myocardium (%) |
|---------------------|--------------------------------------|----------------------------------|
|                     | CF (n=12) | Non-CF (n=12) | P-value | CF (n=12) | Non-CF (n=12) | P-value |
| Total               | 1757.5±560.5 | 1279.5±337.2 | 0.036   | 22.8±6.9  | 7.2±4.7      | < 0.001 |
| Right atrium        | 1678.6±599.3 | 1192.4±293.9 | 0.088   | 23.4±6.5  | 8.2±5.8      | 0.001   |
| RALW                | 1935.9±539.2 | 1409.3±275.7 | 0.529   | 22.1±4.1  | 6.3±0.5      | 0.130   |
| ST                  | 1621.0±821.5 | 1267.4±204.1 | 0.498   | 26.5±7.9  | 10.1±8.3     | 0.061   |
| SN                  | 1536.5±114.7 | 825.5±134.3 | 0.012   | 18.5±2.3  | 6.5±1.3      | 0.134   |
| Left atrium         | 2073.0±253.7 | 1627.7±339.8 | 0.096   | 21.8±8.1  | 5.5±1.5      | 0.006   |
| LOM                 | 1893.6±0.0   | 1367.4±0.0   | -       | 15.9±10.7 | 4.0±1.0      | 0.335   |
| LAPW                | -           | 1395.7±0.0   | -       | 30.3±0.0  | 6.6±0.0      | -       |
| LAR                 | 2252.4±0.0   | 1868.0±0.0   | -       | 24.6±0.0  | 6.9±0.0      | -       |
| LPVA                | -           | 872.8±0.0    | -       | 22.2±0.0  | 6.3±0.0      | -       |

CFAE, complex fractionated atrial electrograms; LAPW, left atrial posterior wall; LAR, left atrial roof area; LOM, mid portion of ligament of Marshall; LPVA, left pulmonary vein antrum; RALW, lateral wall just anterior area near the anterior right ganglionated plexus; SN, sinus nodal area; ST, mid portion of the sulcus terminalis

Figure 1. A representative example of CFAE versus non-CFAE site associated with amount of fibrosis in atrial myocardium
Electrograms and section stained by Masson’s trichrome (magnification x200) of the CFAE site at left atrial roof area (A and B) are compared to those of the non-CFAE site at Bachmann’s bundle (C and D). The proportion of fibrosis area of the myocardium at CFAE site (24.5%) is significantly larger than that of non-CFAE site (6.9%).

CFAE, complex fractionated atrial electrogram; EGM, electrogram
Distribution of Autonomic Nerve Innervation

The distribution of autonomic nerve innervation, which was assessed by the nerve density, did not differ significantly between the CFAE and their matched non-CFAE sites for either the sympathetic (CFAE site, 1128.1±186.5 μm²/mm²; non-CFAE site, 1170.4±187.1 μm²/mm², p=0.570) or parasympathetic nerves (CFAE site, 299.0±106.4 μm²/mm²; non-CFAE site, 373.7±136.8 μm²/mm², p=0.145), regardless of the location from which the atrial tissue was harvested (Table 3).

Table 3. Comparison of autonomic nerve distribution according to the presence of CFAE

|                         | Sympathetic nerve density (μm²/mm²) | Parasympathetic nerve density (μm²/mm²) |
|-------------------------|------------------------------------|----------------------------------------|
|                         | CFAE (n=12)                        | Non-CFAE (n=12)                        | P-value | CFAE (n=12) | Non-CFAE (n=12) | P-value |
| Total                   | 1128.1±186.5                       | 1170.4±187.1                         | 0.570   | 299.0±106.4 | 373.7±136.8     | 0.145   |
| Right atrium            | 1113.3±196.7                       | 1125.5±167.0                        | 0.878   | 295.1±118.3 | 369.1±152.5     | 0.240   |
| RALW                    | 1080.5±63.7                        | 1102.8±259.2                        | -       | 348.1±135.8 | 325.9±86.3      | -       |
| ST                      | 1038.9±152.2                       | 1102.8±77.7                         | -       | 219.4±99.1 | 366.7±227.9     | -       |
| SN                      | 1327.8±385.0                       | 1216.6±102.1                        | -       | 366.7±62.9 | 438.9±7.9       | -       |
| Left atrium             | 1157.8±181.6                       | 1260.0±211.5                        | 0.567   | 316.7±23.6 | 394.4±7.9       | 0.177   |
| LOM                     | 1194.4±7.9                         | 1316.6±23.6                        | -       | -           | -           | -       |
| LAPW                    | 1311.1±0.0                         | 1200.0±0.0                          | -       | 333.3±0.0  | 388.9±0.0       | -       |
| LAR                     | 1244.4±0.0                         | 944.4±0.0                           | -       | -           | -           | -       |
| LPVA                    | 844.4±0.0                          | 1522.2±0.0                          | -       | 300.0±0.0  | 400.0±0.0       | -       |

CFAE, complex fractionated atrial electrograms; LAPW, left atrial posterior wall; LAR, left atrial roof area; LOM, mid portion of ligament of Marshall; LPVA, left pulmonary vein antrum; RALW, lateral wall just anterior area near the anterior right ganglionated plexus; SN, sinus nodal area; ST, mid portion of the sulcus terminalis

Discussion

Major Findings

In the present study, CFAE was observed at various locations throughout the left and right atrium. Compared with matched non-CFAE sites, a thicker atrial myocardium and a larger amount of fibrosis was observed at all CFAE sites, in all locations. However, distribution of the autonomic nervous system did not differ significantly between the CFAE sites and the matched non-CFAE sites.

Histopathologic Features of CFAE Sites

CFAE sites are known to exhibit low voltage and slow conduction. In addition, focal discharge, wave break and fusion, and pivoting activation observed in CFAE are known to contribute to the maintenance of AF through wave propagation. CFAE areas could indicate the re-entry of fibrillation waves or the overlap of other wavelets superimposed in the same area at different times. CFAE sites may also have rotor boundaries or be associated with migrating rotors and wave breaks rather than the center of a stable rotor itself. In our previous study, CFAE sites have been identified as AF substrates different from the AF nest. One computational study suggested that CFAE is more strongly related to the tissue characteristics of the atrial substrate than to the electrical activation system. In other words, CFAE sites are expected to be associated with the underlying heterogeneous architecture of the myocardium. You et al. reported in their pathological study that the myocardium in CFAE sites was
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heterogeneously arranged and had more intercellular substances. However, till date, there has been only one study on the histopathologic characteristics of CFAE sites, and this study included only qualitative analysis. In the present study, we have provided a pathological analysis of CFAE sites and included confirmatory results through the quantitative and paired analysis of CFAE sites and matched non-CFAE sites.

First, we presented thicker atrial myocardium as one of the histological features of CFAE. According to the cardiac computed tomography study by Park et al., thickness of the interatrial septum was associated with the extent of the CFAE area and acute procedural success of catheter ablation. Furthermore, left atrial wall thickness measured by cardiac computed tomography was also found to be more in the CFAE area (3.0±1.0 mm) than in the non-CFAE area (2.2±0.9 mm), which is consistent with our results. These results suggest that atrial myocardial thickness is closely related to the CFAE sites. This also suggests the possibility of heterogeneous tissue organization and arrangement at CFAE sites, which may be an important factor associated with AF and the procedural success of ablating atrial substrates.

Second, we found that a large amount of atrial myocardial fibrosis was identified as another histopathologic characteristic of CFAE. It has already been suggested that fibrosis is an important component of the AF substrate in several human and animal AF model studies. In addition, structural heterogeneities, such as random microstructural alterations, have been reported to be the major pathophysiological mechanisms of CFAE and persistent AF. Particularly in the CFAE sites, increased fibrosis with decreased connexin 43 has been suggested to underlie structural abnormalities. Jacquet et al. suggested in their computational microstructural modeling study, that microfibrosis could also contribute to CFAE genesis. In addition to simple collagen deposition, fibroblast proliferation in the atria has also been shown to affect CFAE generation during AF. Since this study has confirmed that increased atrial fibrosis is one of the microscopic characteristics of CFAE sites, we may now better understand the underlying mechanism of CFAE-guided AF ablation. However, in studies analyzing late gadolinium enhancement cardiac magnetic resonance imaging of left atrial wall composition, it has been reported that normal substrate without fibrosis is even more associated with CFAE than that with fibrotic sites. Although inconsistent, it is clear that fibrosis is closely related to the CFAE sites, which should be clarified in future studies.

The autonomic basis for CFAE formation and effects of the autonomic nervous system on CFAE are well known. However, in terms of autonomic nerve distribution assessed by nerve density, results of the present study did not show any significant difference between CFAE and non-CFAE sites. Since autonomic nerve distribution analysis was limited to nerve density in this study, further work with detailed quantitative analysis including the number and distribution of ganglionated plexus is warranted.

Clinical Implications

Until now, it has been unclear how CFAE sites function as AF substrates, and the mechanism by which CFAE-targeted AF ablation is helpful for AF termination was unknown. In the present study, the histological features of the CFAE site were described through paired comparisons, but this result alone cannot determine the exact mechanism of CFAE development or the clinical significance of CFAE-targeted ablation. However, it is clear that atrial myocardial thickness and the amount of myocardial fibrosis are closely related to the CFAE sites. Given these features, overlapping of myocardial fiber layers, anisotropy, and epicardial–endocardial dissociation may more likely occur at the CFAE sites. In this regard, we can still utilize CFAE as a possible substrate-modification target for AF ablation.

However, the results of clinical studies on CFAE-targeted AF ablation are inconsistent. Nademanee et al. have repeatedly presented clinical benefits of CFAE-targeted ablation of AF through their series of studies. Contradictory to this, it has been reported in STAR AF II, BOCA, and CHASE AF trials that adding CFAE ablation to pulmonary vein isolation had no additional benefit. Results of meta-analyses till date are also controversial. Further studies on the efficacy of CFAE-targeted AF ablation as well as human studies on the pathological characteristics of CFAE sites are warranted.

Study Limitations

Since the present study is a preclinical animal study, the results
of this study are difficult to apply directly to clinical practice. First, inducing AF in normal healthy dogs may be different from clinical AF in human patients. Second, since all of the electrogams were acquired from epicardial electrodes, conducting endocardial mapping may show results that are different from this experiment. Third, because CFAE sites change continuously, interpretation of the results should take into account the recording duration and spatiotemporal stability of the electrogams. Fourth, in some CFAE sites, such as LAPW and LPVA, accurate measurement of wall thickness was difficult owing to limitations of the harvested tissue and was therefore excluded from the analysis. Lastly, when defining CFAE in this study, we used the criteria proposed by Nademanee et al. However, Rostock et al. defined CFAE as fractional potentials exhibiting more than two deflections from the isoelectric line and/or potentials with continuous electrical activity without an isoelectric line. On the other hand, Oral et al. used a definition of electrogams with a cycle length of 120 ms or less than in the coronary sinus, or showing fractionated or continuous electric activity. Since the definition of CFAE is not uniform, the results of the present study cannot be extrapolated to all CFAE-related studies.

Conclusions

A thicker atrial myocardium and a larger amount of fibrosis were identified as the most important histopathologic characteristics of CFAE sites compared to their matched non-CFAE sites. These results may help elucidate the underlying mechanism of CFAE and the implications for catheter ablation.

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