Relationship between Atrial Tissue Remodeling and ECG Features in Atrial Fibrillation

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Summary: The difference in the atrial organizational structure between patients with atrial fibrillation (AF) and those with sinus rhythm was investigated. In order to analyze the rationality in explaining the electrocardiogram (ECG) characteristics of AF with statistics data or tissue remodeling model, and the logical relationship between the hypothesis of pulmonary veins (PV) muscle sleeves and that of multi wavelets in mechanism of AF, we examined the expression of collagen volume fraction of type Ⅰ (CVF-Ⅰ) with picrosirius red staining, connexin 40 (Cx40) by immunohistochemistry, and intercalated disc (ID) using transmission electron microscope in atrial tissue. The results showed that there was significant difference in the expression of CVF-Ⅰ (t=3.827, P<0.01), Cx40 (t=4.21, P<0.01), and groups of the ID that keeping the electrical transmission and atrial electrical coupling synchronization (t=15.116, P<0.001), but no significant difference was found in total IDs (t=0.611, P=0.543) between patients with AF and those with sinus rhythm. The quantitative differences in the tissue remodeling could not explain the ECG characteristics of AF. The number of normal IDs and abnormal distribution are the structural basis to trigger and maintain atrial electrical remodeling, and induce and maintain AF. Such histological reconstruction supports the hypothesis of multi wavelets and can also explain ECG features.
Key words: atrial fibrillation; collagen volume fraction of type Ⅰ; Cx40; intercalated disc; electrocardiogram

Atrial fibrillation (AF) is a common atrial arrhythmia. The unique way to diagnose AF is typical electrocardiogram (ECG), instead of sinus P wave, that represents the fragmented atrial wave in high frequency, irregular rhythm, no uniform amplitude, no fixed interval period, no regular repeat performance. The development of the anisotropic conduction and the interruption of the electrical excited synchronous coupling are the main factors. The mechanism of AF is not clear, and the importance between the hypothesis of PV muscle sleeve and of multi wavelets is still being debated[1]. At present, no published research can explain the characteristics of the AF ECG logically. This study mainly focuses on the relationship between the restructure of atrial tissue and the features of AF ECG.

1 MATERIALS AND METHODS

1.1 Collection of Patients

For observing the difference in tissue structure between AF and sinus rhythm, and analyzing the relationship between the structure difference and electro-remodeling, 34 cases of AF were collected (19 males and 15 females) with the age from 23 to 65 years old (mean 43.02±19.63 years), and the persist time of AF was from 15 months to 13 years. In the control group, 34 cases of sinus rhythm were collected (21 males and 13 females) with the age from 26 to 60 years old (38.34±11.32 years).

1.2 Ethics Statement

All human studies conformed to the National Institutes of Health (NIH) guidelines, and were approved by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, China, and conducted in accordance with the Declaration of Helsinki. All the patients signed the informed consent.

The tissues of the left or right atrial appendage (1 cm×1 cm×2 cm) were obtained during surgery and quickly cut into a small size (3 mm×3 mm×5 mm). Samples were stored at –80°C until use.

1.3 Histological Analysis

Atrial tissues were fixed in 10% neutral buffered formalin and cut with a microtome into sequential slices of 5 μm and subjected to hematoxylin and eosin (HE) staining. In addition, tissues were cut and stained with Sirius red for image analysis. The collected images were quantitated by quantitative morphometry

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using the Image Pro Plus program, and collagen volume fraction of collagen type Ⅰ (CVF-Ⅰ) was analyzed by the image analysis system.

1.4 Immunohistochemistry

The connexin 40 (Cx40) protein expression was detected by immunohistochemistry. Frozen serial sections (4 μm) were treated with 0.3% H_2O_2 in PBS to block endogenous peroxidase activity, followed by blocking in 4% BSA. Primary antibody was specific for Cx40 (1:200 to 1:500 dilution). All sections were stained with biotinylated secondary antibodies and detected using ABC reagents. The Cx40 protein expression was quantified by assessing the percent positive area of total plague for each marker.

1.5 Electron Microscopy

Sections of myocardium on coverslips were fixed in 2.5% glutaraldehyde overnight. After washing three times in the phosphate buffer, the samples were immersed in 1% Osmium tetroxide buffer for 1 h. After being rinsed again, the samples were dehydrated with alcohol and embedded in epoxy resin. Then the samples were examined using a transmission electron microscope. The arrangement of atrial muscle fibers, sarcomere structure, fibrosis degree and intercalated disk (ID) remodeling change were observed by a transmission electron microscope. The number of ID remodeling was viewed every 40 fields per sample and counted according to the number of groups and the number of ID. The ID remodeling was defined as group number of ID and total number of IDs. The distribution of IDs in the adjacent myocardium was compared between sinus rhythm group and AF group.

1.6 Statistical Analysis

All data were first evaluated for normal distribution using the Kolmogorov-Smirnov test. Results were expressed as the means±standard error of means (SEM) when normally distributed. When data did not pass the test for normality, they are presented as median with 25th and 75th percentiles. The significance of differences was estimated by one-way ANOVA, followed by the Holm-Sida test when data were normally diatributed and group variances were equal. When group data were not normally distributed or if group variances were unequal, the Kruskal–Wallis test followed by the Dunn posthoc test was used. *P*-values <0.05 were considered statistically significant. All statistical analyses were performed using SPSS software (version 17.0, SPSS Inc, USA).

2 RESULTS

2.1 Levels of CVF-Ⅰ, Cx40 and ID

The data in table 1 showed the expression of CVF-Ⅰ was increased in the AF group as compared with the sinus rhythm group. Meanwhile, the level of Cx40 and the groups of ID were accordingly decreased in the AF group as compared with those in the sinus rhythm group. There was no significant difference in the total number of IDs.

2.2 Distribution of CVF-Ⅰ

Sirius red staining assay showed that the expression level of CVF-Ⅰ in the AF group was markedly increased as compared with the sinus rhythm group (fig. 1). Simultaneously, the distribution of CVF-

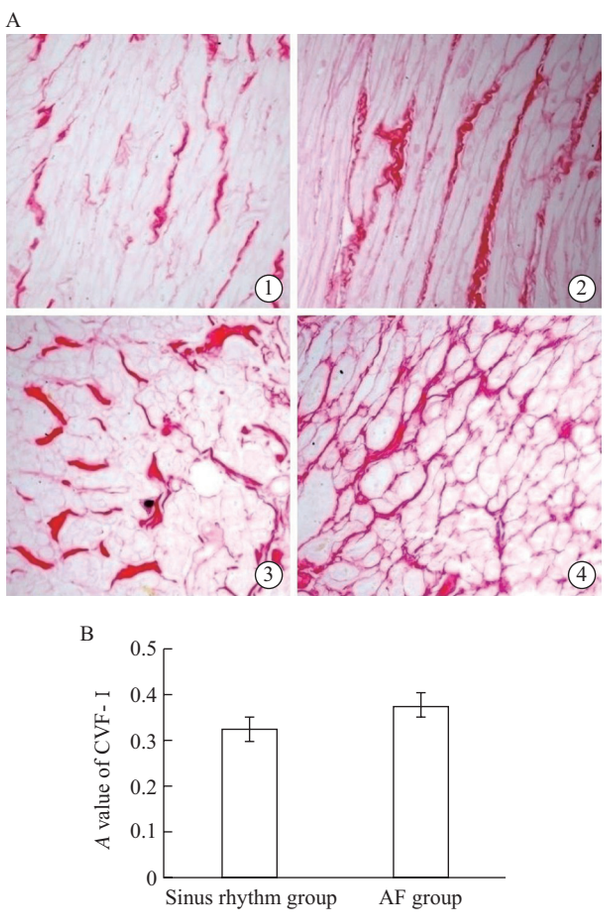

Fig. 1 The distribution features of CVF-Ⅰ
A: Sirius red staining (×200). 1 and 2: longitudinal results; 3 and 4: transverse results. 1 and 3, sinus rhythm group; 2 and 4: AF group. B: The absorbance (*A*) value of CVF-Ⅰ in the AF group was significantly increased as compared with that in the sinus rhythm group (t=3.827, P<0.01).

Table 1 The statistical data of various observation items

Items		Sinus rhythm group	AF group	*t*	*P*
CVF-Ⅰ		0.3239±0.0969	0.3753±0.0914	3.827	<0.01
Cx40		0.3606±0.1040	0.3108±0.0658	4.21	<0.01
IDs	Group number	12.470±1.398	7.2400±1.458	15.116	<0.001
	Total number	112.12±13.061	114.15±14.313	0.611	0.543

Ⅰ in the AF group was specially different from the sinus rhythm group. It was found that there were fewer collagenous fibers between atrial myocytes in the sinus rhythm group. In the AF group, the atrial muscle cells were isolated and packaged by collagenous fiber in the longitudinal and transverse section views.

2.3 Distribution of Cx40

To investigate the roles of Cx40 in AF, we examined the expression of Cx40 in the atrial tissue. As depicted in fig. 2, Cx40 in the AF group was unevenly distributed, displaying as regional aggregation and depletion. The absorbance (*A*) of Cx40 in the AF group was significantly lower than that in the sinus rhythm group.

2.4 Distribution of IDs

To clearly see the microstructure of the atrial tissues, the electron microscopy was performed. The results showed that several IDs were distributed in a line between the parallel myocardial bunches in the sinus rhythm group. In the AF group, dozens of IDs were mussily piled between the several myocardial bunches in different directions. At the same time, the group number and total number of IDs were counted, and there was no significant difference in total IDs between two group, while the groups of IDs were markedly decreased in the AF group as compared with those in the sinus rhythm group (fig. 3), which was similar to the statistical data on the groups of ID and total IDs (table 1). It was suggested there was significant difference in ID distribution between the sinus rhythm and AF.

3 DISCUSSION

AF is characterized by a rapid, irregular and non-repetitive electrical excitation, as well as other features. Our study showed there were the perturbations of CVF-Ⅰ and Cx40 between the AF group and the sinus rhythm group, which was similar to the previous investigations[2, 3], but the perturbations cannot directly explain the features of AF.

The myocardial bundles are isolated and wrapped by fibrous structure of collagen Ⅰ. As a result, the electrical conduction function of adjacent muscle bundles is completely separated from each other. The lateral electrical transfer (side by side), which

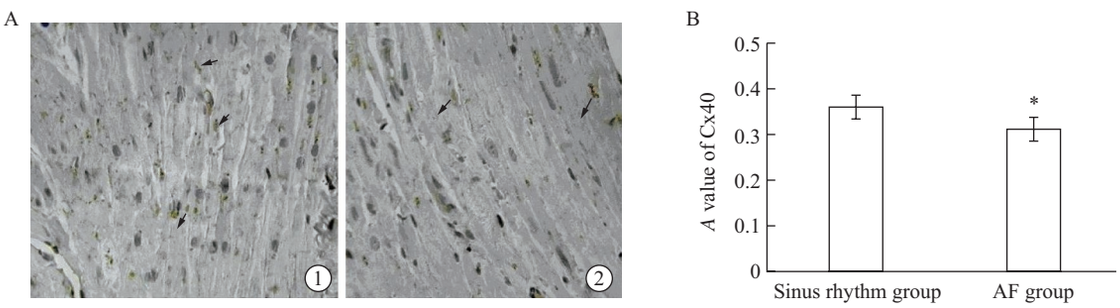

Fig. 2 The distribution of Cx40 in both groups
A: immunohistochemical staining (×200). The color patch size and distribution of Cx40 in the sinus rhythm group (1) were relatively uniform (arrows). In the AF group (2), the distribution of Cx40 was uneven, and the visible range of blank area and the size of the color plaque were significantly enlarged (arrows). B: The absorbance (*A*) value of Cx40 was significantly decreased in the AF group as compared with that in the sinus rhythm group. $^{*}P<0.05$ *vs.* the sinus rhythm group

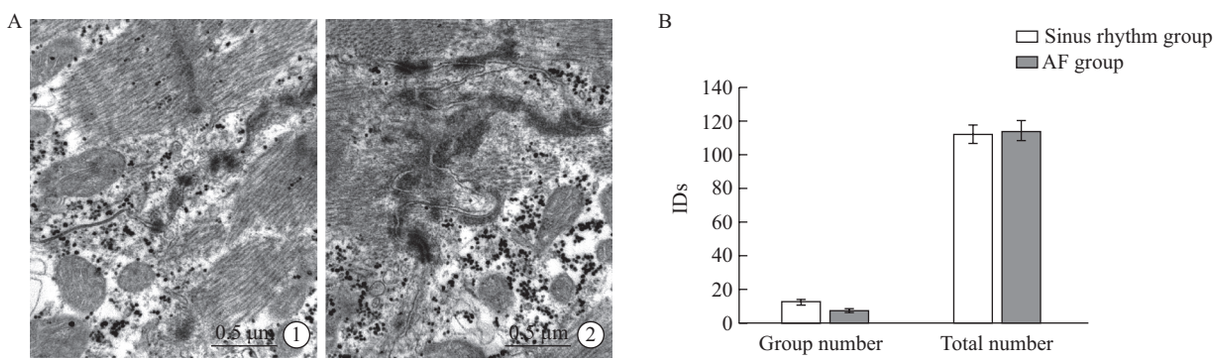

Fig. 3 A: the distribution of IDs in the sinus rhythm group (1) and AF group (2). Scale bar=0.5 μm. B: There was apparently different distribution modes of IDs between the sinus rhythm group and AF group. In the sinus rhythm group, several IDs were distributed in a line between the parallel myocardial bunches, and in the AF group dozens of IDs were mussily piled between the several myocardial bunches in different directions. B: There was significant difference in the group number of IDs (t=15.116, P<0.001), but no significant difference was found in total IDs (t=0.611, P=0.543) between two groups.

maintains the electrical coupling synchronism, is changed.

The down-regulation of Cx40 evidenced by our study was consistent with the previous research[4]. However, the findings have suggested the deterioration of myocardial conduction velocity and atrial electrical excitation expansion, which have contradicted the fast frequency of the AF exciting process. Many studies[5–7] have confirmed a close relationship between ID and Cx40 in atrial muscles. This study showed that the groups of ID were markedly reduced in the AF group as compared with the sinus rhythm group, but total IDs had no statistically significant differences between the sinus rhythm group and the AF group, which indirectly indicated that the expression of Cx40 was not significantly reduced in the AF group. As shown in fig. 3, the re-distribution and abnormal stacking of ID were the main pathological phenomena of AF. A large number of IDs were aggregated between adjacent myocardial bundles, which caused a new big disc group to form, suggesting that the IDs between multiple adjacent muscle bundles may be directly displaced by an extraforce, aggregated, stacked, and fixed by the fibrotic process. As shown in table 1, there was no significant difference in total IDs between the sinus rhythm group and AF group, despite the difference in the groups of IDs. From the findings of this study, the comparison between the sinus rhythm group and AF group revealed that: (1) the double-discs structure of the ID is very firm and not easily destroyed; (2) IDs located on the cell membrane could link the two myocardial cells together and have the function of transmitting electrical excitement; (3) The IDs between the myocardial bundles can move freely in the sinus rhythm group, while in the AF group, the IDs are oriented and fixed in different directions at certain place between the myocardial bundles. Therefore, the electrical signal in sinus rhythm transmitted laterally crossing membrane during the electrical coupling synchronization is changed into a multi-directional focal electrical excitation transmission; (4) The redistributed model of ID is acquired.

The mechanism of AF remains unclear[8], and there are two main kinds of hypotheses[9, 10]: the PV muscle sleeve theory, and the multi wavelets theory. Although the former is high-profile reinforced, the data in this study (fig. 3) supported the latter. The redistribution of ID can explain all ECG futures of AF, such as high frequency, disorder and non-repeatable atrial muscle exciting process, because of the multi-direction electrical transmission and anisotropic electrical conduction.

The comprehensive analysis of the above mentioned factors involving in AF revealed the hypothesis of the multi wavelets can better explain the characteristics of AF than that of PV muscle sleeve (table 2). The hypothesis of the multi wavelets can also explain the effectiveness of PV separation[11–22].

The Cx40 in ID is not regenerative. Once the remodeling of Cx40/ID in AF is ablated, the anisotropic conduction source would be immediately eliminated, and the evoking of AF can be reduced. Therefore, such distribution reconstruction of Cx40/ intercalary disc group was the real ablation target.

To sum up, our findings suggested that the fibrosis has limited the electrical conduction of myocardial bunches, and aggravated the conduction block, which is the main factor interrupting the electrical coupling synchronization within atrium and only plays passive and assistant role in the electrical remodeling of AF. The abnormal staking IDs with electrical conduction, which link and freely slide like button in myocardial cell membrane, were pushed to certain location within the adjacent myocardial bunches by certain force, and fixed by fibrosis. It can trigger the anisotropic conduction between the myocardial bunches in different directions, and forwardly keep the conduction in the atrium. Such remodeling of tissue and electrical re-conduction support the hypotheses of the multi-wavelets within atrium and can explain the ECG

Table 2 Comparison of logic between hypotheses of PV muscle sleeve and multi wavelets

	PV muscle sleeve[8]	Multi wavelets
Evoking source of AF	PV muscle (outside of atrium?)	Atrial myocardium
From source to unit of effect	PV muscle to atrial myocardial cells	Between myocardial cells in atrium
Junction tissue between PV muscle (source) and myocardial cells (effect)	Insulating fibrotic tissues[8] between PV muscle and myocardial fibers	Mussily Cx40, 43/ID
Anisotropic conduction	High[8]	High
Conduction-validity from unit of evoking to unit of effect	Lack of basis of tissue's structure in efficient conduction	High, low-resistance Cx40, 43/ID
High frequency	Dependant on validity of electrical transmission	High
Polytropism	Dependant on validity of electrical transmission	Yes, by mussily IDs
Development of source to responsive structure	Associated with life, inherent[10]	Acquired form
Reentrant exciting	Possible, without evidence	Non
Target site of ablation	PV atrium (atrium?)	Atrium
Target tissue of ablation	Responsive tissue (atrial myocardium)	Source tissue including remodeling disc

features of AF logically.

Conflict of Interest Statement

All authors declare that they have no competing interests.

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