Changes in CD11b and CD18 genes in right atrial appendage of patients with chronic atrial fibrillation

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Abstract: Objective: To explore alteration in expression of cluster of differentiation 11b (CD11b) as well as 18 (CD18) in right atrial appendage (RAA) of patients suffering from chronic atrial fibrillation (CAF). Methods: 43 patients suffering from rheumatic heart disease (RHD) were categorized into 2 categories: twenty-one participants suffering from CAF and twenty-two of those with sinus rhythm. Clinical features as well as blood specimens were acquired prior to operation. RAA specimens were acquired in valve replacement operation. qPCR was utilized to assess expression of CD18 as well as CD11b. Results: Difference in right atrial diameter (RAD), ventricular septal thickness (VST), left ventricular end diastolic dimension (LVEDD), as well as diastolic posterior wall thickness (PWT) was insignificant between the two groups (all p > 0.05). Nevertheless, patients suffering from atrial fibrillation displayed elevated LVEF in comparison with those with sinus rhythm and decreased LAD as well as right ventricular end diastolic dimension (RVEDD) in comparison with those with sinus rhythm (p < 0.001). Expression of CD18 as well as CD11b was promoted in patients suffering from atrial fibrillation in comparison to those with sinus rhythm (p < 0.01 and p < 0.05, respectively). Conclusion: CD18 as well as CD11b are promising to participate in generation as well as maintenance of atrial fibrillation in patients suffering from atrial fibrillation combined with RHD, providing innovative markers to assess myocardial fibrosis.

Keywords: Atrial fibrillation, MF, CD11b, CD18

Introduction

As the most prevalent clinical arrhythmia, AF (atrial fibrillation) is related to RHD (rheumatic heart disease), CHD (coronary heart disease), congenital heart disease, cardiomyopathy, hypertension, pericardial diseases as well as other CVDs (cardiovascular diseases) [1-3]. AF seriously influences the health, attacking 5% of people over 65 years old as well as 7.1% of people over 85 years old [4]. Primary types of AF include paroxysmal, persistent, and permanent. The AF in our study is persistent type. Etiology of AF is complicated. The understanding of exact reactions related to AF generation as well as sustainability is insufficient, since multiple agents are linked with its generation, such as inflammation, neurohormonal stimulation, oxidative stress (OS) as well as autonomic imbalances [5, 6]. Speedy and irregular stimulation brings about inharmonious atrial contraction, frequently causing embolism. Furthermore, it has been proved that inflammation might be essential to participate in AF generation [7, 8].

Interactions between white blood cells and endothelial cells in tissues are modulated via adhesion agents located on the surface of white blood cells as well as endothelial cells [9]. As noncovalently-linked heterodimers of α and β subunits, integrins express on cells surface [10]. In spite of the primary recognition as adhesion agents, integrins modulate various reactions [11]. Consequently, integrins have an effect on multiple biological reactions and participate in hematopoiesis, hemostasis, inflammation as well as immune modulation [12-14]. Integrin CD11b/CD18 (also known as Mac-1 and CR3), are heterodimers of the αM (CD11b) and β2 (CD18) subunits. Previous research has recognized that CD11b/CD18 integrin serves as adhesion agent, which changes initial inflammatory reaction via stimulating focal adhesion kinase (FAK) pathway [15]. Consequently, we
suppose that CD11b/CD18 integrin links with AF and lead to MF (myocardial fibrosis).

Materials and methods

Specimen acquirement and treatment

Our research was approved by Ethics Committee of Harbin Medical University (Harbin, China). Specimens were acquired in conformity with modulations of Fourth Affiliated Hospital of Harbin Medical University. Forty-three patients from 31 to 80 years old suffering from rheumatic heart disease, who received valve replacement, were enrolled. Participants were categorization into 2 groups: 22 with SR (sinus rhythm) as well as 21 suffering from AF. Patients suffering from infective endocarditis, severe pulmonary, renal or hepatic malfunction, hyperthyroidism, malignancies, chronic pulmonary heart disease as well as coronary atherosclerotic heart disease were excluded. One hundred micrograms of myocardial specimens were acquired from right atrium (RA). Connective tissue as well as residual blood was eliminated. Specimens were immediately put into liquid nitrogen for later preparation of molecular as well as biological experiments.

Drugs, reagents, and instruments

Drugs, agents, tools were listed as below: SYBR\textsuperscript{®} Green real-time polymerase chain reaction (PCR) kits (Takara Biotechnology, Shiga, Japan); electrical constant-temperature oscillation sinks (37°C, Shanghai Evergrande Biotechnology, Shanghai, China); a centrifuge (Shanghai Evergrande Biotechnology); a cDNA amplification kit; and a real-time PCR system (ABI 7500, Applied Biosystems, Foster City, CA, USA); TRizol\textsuperscript{®} (Wuhan Boster Biotechnology, Wuhan, China); a CO\textsubscript{2} incubator as well as an inverted phase contrast microscope.

Table 1. Primers and TaqMan probes applied to qPCR

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD11b</strong></td>
<td>5-CCTGGTTCACCTCCTTCCAG-3</td>
<td>180</td>
</tr>
<tr>
<td><strong>CD18</strong></td>
<td>5-TCAGGACTTTACGACCCGC-3</td>
<td>193</td>
</tr>
<tr>
<td><strong>GAPDH</strong></td>
<td>5-TGGCCTTCGTTCTTAC-3</td>
<td>178</td>
</tr>
</tbody>
</table>

GAPDH, CSE, CamkiII, Cx40: 95°C for 5 sec, 55°C for 30 sec, and 72°C for 1 min.

Expression of CD11b and CD18 in atrial myocytes (AM) determined by qRT-PCR

Total RNA from the atrial myocytes was extracted using a standard TRIzol RNA isolation method (Invitrogen) as previously described [16]. The reverse transcription and PCR experiments were performed with the Reverta Ace qPCR RT Kit (TOYOBO FSQ-101) using 0.5 μg of each sample, according to the manufacturer's protocols. The quantitative real-time PCR was conducted in the LightCycler apparatus (Bio-Rad) using the FastStart Universal SYBR Green Master (Roche). The qPCR protocol was as follows: 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 1 min; and then increasing temperatures from 65°C to 95°C at 0.1°C/s. The mRNA level was normalized to GAPDH in the same sample and then compared with the control. Primers as well as probes were displayed in Table 1.

Quantification of FAK via ELISA

ELISA was utilized in order to quantify FAK. The procedures were performed according to the manufacture's instruction. The absorbance (at 450 nm) was measured using a microplate absorbance reader. The amount of FAK was determined by the interpolation from the standard curve.

ECG recording

ECGs were recorded, either from the body surface or the esophagus (ESO), in patients in AF. The ECG was digitized at a sampling rate of 1 kHz with an amplitude resolution of 0.6 μV using 16-bit A/D conversion (equipment supplied by Siemens-Elema AB, Solna, Sweden). The acquisition equipment was connected to an IBM-compatible personal computer and the data were stored on removable disc media.

Statistical analysis

Data were processed with Statistical Package for the Social Sciences 16.0 software (IBM, Armonk, NY, USA). Measurement data were expressed in the form of mean ± SD and differences between two groups were analyzed with Student's t-test. Categorical data were expre-
Table 2. Comparison of age, weight, height, gender, course of disease, smoking, diabetes, alcohol consumption as well as hyperlipidemia between the AF and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
<th>Course of disease (year)</th>
<th>Proportion of male patients (%)</th>
<th>Smoking (%)</th>
<th>Alcohol (%)</th>
<th>Diabetes (%)</th>
<th>Hyperlipidemia (%)</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>22</td>
<td>64.6±5.94</td>
<td>61±7.0</td>
<td>1.75±0.06</td>
<td>5±0.5</td>
<td>53</td>
<td>20</td>
<td>42</td>
<td>24</td>
<td>40</td>
<td>138</td>
<td>80.2</td>
</tr>
<tr>
<td>AF</td>
<td>21</td>
<td>59±1.58</td>
<td>63±8.0</td>
<td>1.72±0.07</td>
<td>6±0.4</td>
<td>55</td>
<td>22</td>
<td>40</td>
<td>20</td>
<td>44</td>
<td>145</td>
<td>76</td>
</tr>
<tr>
<td>P</td>
<td>0.616</td>
<td>0.741</td>
<td>0.917</td>
<td>0.713</td>
<td>0.821</td>
<td>0.5238</td>
<td>0.5238</td>
<td>0.5238</td>
<td>0.5238</td>
<td>0.1667</td>
<td>0.3451</td>
<td></td>
</tr>
</tbody>
</table>

Smoking, diabetes, alcohol consumption as well as hyperlipidemia between both groups.

Table 3. General outcome of ECG

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LVEF (%)</th>
<th>LVEDD (mm)</th>
<th>RVEDD (mm)</th>
<th>VST (mm)</th>
<th>PWT (mm)</th>
<th>LAD (mm)</th>
<th>RAD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>21</td>
<td>57.6±5</td>
<td>43.8±2.86</td>
<td>52.6±1.14</td>
<td>10.74±0.25</td>
<td>9.74±0.19</td>
<td>53±2.12</td>
<td>42.8±1.64</td>
</tr>
<tr>
<td>AF</td>
<td>22</td>
<td>60±3.1</td>
<td>47.4±5.03</td>
<td>30.2±12.87</td>
<td>11.14±1.51</td>
<td>11.22±1.57</td>
<td>39±2.74</td>
<td>42.6±10.76</td>
</tr>
<tr>
<td>P</td>
<td>0.0001</td>
<td>0.2017</td>
<td>0.0179</td>
<td>0.5902</td>
<td>0.1052</td>
<td>0.0001</td>
<td>0.5232</td>
<td></td>
</tr>
</tbody>
</table>

Test outcome of blood FAK

Given CD11b/CD18 integrin serves as adhesion agent which changes initial inflammatory reaction via stimulating focal adhesion kinase (FAK) pathway, we tested FAK levels in the blood. We found that blood FAK level in AF patients was higher than that of SR patients and the difference was significant (p < 0.05)(Table 4).

Discussion

AF serves as the most prevalent arrhythmia clinically. Mural thrombus triggered via AF is able to reinforce the vulnerability to stroke as well as death. It is suggested that AF generation is versatile. Adhesion molecules, as mediators of early inflammatory response, play an inevitable role in the inflammation-related pathogenesis of atrial fibrillation. They can be divided into four categories: selectins, immunoglobulins, integrins, and cadherins. Integrins are classified into CD11a/CD18, CD11b/CD18, CD11c/CD18, and CD11d/CD18, while CD11b/CD18 macrophage differentiation antigens (CR3) and CR3 are members of the integrin family of adhesion molecules. Its main biological activity is the adhesion of cells, neutrophils adhere to vascular endothelial cells, and become adherent cells from blood vessels to inflammation sites. Fernandez-patron C study found that MMP-9 can transform endo-
Zhelin-1 (1~38) into endothelin-1 (1~32), which can increase the expression of CD11b on polymorphonuclear leukocyte surface and help blood vessels. Adhesion of endothelial cells and neutrophils and contributes to the formation of atrial fibrillation [15, 16]. When integrin signaling regulates transduction, the beta cytoplasmic region is required for activation of FAK, and β2 integrin plays an important role in the composition of focal adhesions. Phosphorylation of FAK in fibroblasts requires β2 integrin. The presence of a plasma zone, a chimera containing a transmembrane region of β2, a cytoplasmic region, and an irrelevant extracellular domain, can competitively disrupt integrin

avβ5-mediated cell adhesion and therefore activate focal adhesion kinase (FAK). Increased atrial pressure and various external stimuli signals activate integrins during atrial fibrillation, and increase matrix metalloproteinase activity through focal adhesion kinase pathway, resulting in atrial myocardium fibrosis. FAK pathway is the dominant factor in atrial structural remodeling. The FAK peripheral blood level detected by the ELISA method in this experiment has been reported in the literature that the initiation factor of FAK is due to the aggregation of integrin at the adhesion site, thus indicating that FAK is required to activate and activate integrin [17]. Collagen aggregation has an impact on the whole etiology of atrial activity and brings about local heterogeneity with regard to electrical conduction, causing arrhythmias especially AF [18, 19]. Furthermore, it has been recently

proved that inflammation potentially participates in AF generation as well as sustainability. In this study, we found that patients suffering from atrial fibrillation displayed elevated LVEF in comparison with those with sinus rhythm. CD18 as well as CD11b was promoted in patients suffering from atrial fibrillation in comparison to those with sinus rhythm. CD18 and CD11b are promising to participate in generation as well as maintenance of atrial fibrillation in patients suffering from atrial fibrillation combined with RHD, providing innovative markers to assess myocardial fibrosis.

As an integral protein on the membrane, β2-integrin CD11b/CD18 is located in plasma membrane as well as secondary granules of neutrophils. CD11b/CD18 serves as a dominant adhesion agent binding to ingredients of extracellular matrix intimately [20]. Adhesion agents participate in modulation of initial inflammation reaction. It is reliably demonstrated in our research that β2 integrin CD11b/CD18 is a
Alteration in expression of CD11b and CD18 in RAA of patients with CAF

Findings of our research reveal that CD11b transcription is promoted in patients suffering from AF in comparison to control group (p < 0.05), indicating aberrant myocardial expression of CD11b participates in AF. It is supposed that fibrosis of the atrium is an essential marker in patients suffering from CAF combined RHD. In a word, it is proved in our research that CD11b/CD18 may participate in generation as well as sustainability of fibrillation in patients suffering from CAF combined RHD. It has been revealed previously that FAK is stimulated via integrin aggregation at adhesion region, subsequently, it activates a signaling cascade [21].

Nevertheless, limitations of this research are listed as below: 1) Sample size is relatively limited which should be extended in order to promote power of the outcome; 2) CD11b/CD18 expression is elevated in reactions related to circumstances different from AF; 3) Investigation was carried out in the combination of RHD and persistent AF. Specific transduction signals related to CD11b/CD18 expression require more thorough investigation in the future.

In summary, CD18 as well as CD11b has positive relation with atrial fibrillation in patients suffering from RHD combined with AF.

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Disclosure of conflict of interest

None.

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