Journal of Cardiology (2009) **53**, 127—135

ELSEVIER

JOURNAL of CARDIOLOGY
Official Journal of the Japanese College of Cardiology
www.elsevier.com/locate/jjcc

ORIGINAL ARTICLE

The role of infection in the development of non-valvular atrial fibrillation: Up-regulation of Toll-like receptor 2 expression levels on monocytes

Hitoshi Ichiki (MD)[a], Koji Orihara (MD)[a,*], Shuichi Hamasaki (MD, FJCC)[a], Sanemasa Ishida (MD)[a], Naoya Oketani (MD)[a], Yasuhisa Iriki (MD)[a], Yuichi Ninomiya (MD)[a], Hideki Okui (MD)[a], So Kuwahata (MD)[a], Shoji Fujita (MD)[a], Takehiko Matsushita (MD)[a], Shiro Yoshifuku (MD)[a], Ryutaro Oba (MSci)[b], Hiroyuki Hirai (PhD)[b], Kinya Nagata (PhD)[b], Chuwa Tei (MD, FJCC)[a]

[a] *Department of Cardiovascular, Respiratory & Metabolic Medicine, Graduate School of Medicine, Kagoshima University, 8-35-1, Sakuragaoka, Kagoshima 890-8520, Japan*
[b] *Department of Advanced Medicine and Development, BML, Inc., Saitama, Japan*

Received 31 July 2008; received in revised form 25 September 2008; accepted 30 September 2008
Available online 25 November 2008

KEYWORDS
Non-valvular AF;
Toll-like receptor 2;
C-reactive protein;
Interleukin-6;
Left atrial volume index;
Infectious inflammation

Summary Many studies have suggested that inflammation may participate in the pathogenesis of non-valvular atrial fibrillation (AF). However, it has been unknown by exposure to what the inflammation is caused. Recently, we reported that Toll-like receptor 2 (TLR2) level on monocytes was significantly up-regulated in viral and bacterial infections, but not in non-infectious inflammatory states. Our purpose was to test the hypothesis that expression of TLR2 levels may be up-regulated in patients with non-valvular AF. A total of 48 consecutive patients with non-valvular AF who were hospitalized for catheter ablation were enrolled in this study. TLR2 levels were assayed by using flow-cytometric analysis and compared with volunteers in sinus rhythm (control group, $n = 24$). Additionally, C-reactive protein (CRP) and interleukin-6 (IL-6) levels were assayed, and the left atrial volume indexes (LAVI) in the non-valvular AF group were measured. The results demonstrated that TLR2 levels in the non-valvular AF group were significantly higher than in the control group (median, 4682 vs. 3866 sites/cell; $P < 0.01$). Moreover, non-valvular AF patients had significantly higher IL-6 levels than controls. However, there was no significant difference in CRP levels between the two groups. It was observed in 44 AF patients, in

* Corresponding author. Tel.: +81 99 275 5318; fax: +81 99 265 8447.
E-mail address: orip@po.synapse.ne.jp (K. Orihara).

doi:10.1016/j.jjcc.2008.09.008

whom pulmonary vein isolation was confirmed to be successful, that the LAVI significantly diminished 1 month after ablation (median, 33.6 vs. 29.5 ml/m^2; $P < 0.001$), but not the TLR2 and IL-6 levels. Our results implied that an infectious inflammation may participate in the pathogenesis of non-valvular AF.

Introduction

Atrial fibrillation (AF), the most commonly encountered arrhythmia in clinical practice, is associated with substantial morbidity and mortality. AF is generally categorized into valvular and non-valvular AF/lone AF, depending on the presence of valvular heart disease. Atrial biopsies of non-valvular AF patients provided the evidence that inflammation may participate in the pathogenesis [1], and the reflecting markers in sera have been explored. In this context, it has been controversial whether non-valvular AF patients have higher CRP levels compared with controls in sinus rhythm [2—7]. On the other hand, Toll-like receptor (TLR) family members, which are key regulators of both innate and acquired immune responses, have been studied not only in the immunological response [8—10], but also in the clinical implications [11—13]. In this study, based on our previous finding that TLR2 expression levels on monocytes are up-regulated in infectious diseases [14,15], we tested how the expression level of TLR2 is modulated in patients with non-valvular AF, compared with volunteers in sinus rhythm (control group). In addition, we measured plasma interleukin-6 (IL-6) levels and amino-terminal propeptide of type III procollagen (PIIINP) levels in both groups. PIIINP is a collagen III synthesis marker, and if fibroid degeneration occurs in the atrium of patients with non-valvular AF, we hypothesized that it might be reflected in PIIINP levels. Subsequently, we evaluated TLR2 expression levels and the left atrial volume index (LAVI) before and 1 month after successful catheter ablation in the non-valvular AF group.

Methods

Patient population

Forty-eight patients with non-valvular AF, who underwent catheter ablation for drug-refractory paroxysmal AF in our hospital, were enrolled. This group consisted of 39 males and 9 females, ranging from 30 to 72 years old (mean = 54 years). The patients with non-valvular AF that were indicated for catheter ablation had a left atrial diameter <45 mm. Forty patients were taking medications, including anti-inflammatory drugs, such as angiotensin-converting enzyme inhibitors (ACE-I), angiotensin II receptor blockers (ARB), and statins, and eight patients were not taking medication (Table 1). In the non-valvular AF group, blood samples were taken before and 1 month after catheter ablation. In this study, the ablation was defined to be successful with two criteria: (1) patients were free from palpitations during the first month after ablation; (2) the absence of AF was confirmed by checking the electrocardiogram and 24-h Holter monitor a month after ablation. Consequently, 44 out of 48 AF patients were judged as successful ablation cases. Alternatively, 25 out of 48 AF patients offered blood samples not only from peripherals but also from left atria just before ablation. Volunteers ($n = 24$) in sinus rhythm were confirmed to be infection-free for at least 1 month at the time of blood sampling and did not have a fever within a week from the time the blood sample was drawn. They were categorized as the control group, which consisted of 19 males and 5 females, ranging from 30 to 72 years old (mean = 49 years). The control group was age- and sex-matched with the non-valvular AF group. Sixteen out of 24 volunteers were under medical treatment for hypertension, hyperlipidemia, and diabetes mellitus. Patients were excluded with organic disorders responsible for AF, such as valvular heart disease, congestive heart failure (NYHA class II or greater), recent acute coronary events/revascularization, thyroid disease, and patients with systemic inflammatory diseases including infection, collagen diseases, malignancies, renal failure, and hepatic failure. Informed consent was obtained from all patients and volunteers, which was in accordance with a protocol approved by the Kagoshima University Ethics Committee. Ten milliliters of peripheral blood were taken from each patient with a heparinized blood collecting tube.

Flow-cytometer analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral blood by density gradient centrifugation using Ficoll-Paque plus