Downregulation of Peroxisome Proliferator-activated Receptor-γ Expression in Hypertensive Atrial Fibrillation

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ABSTRACT

Background: Numerous evidence has suggested that either hypertension or atrial fibrillation (AF) is associated with systemic inflammation. Peroxisome proliferator-activated receptor-γ (PPARγ) has been proved to have anti-inflammatory effects and is implicated as a molecular pathway involved in many cardiovascular diseases, such as hypertension. The correlation between PPARγ inflammation and AF is still unknown.

Methods: Using a case-control study design, 57 patients with hypertensive AF (persistent AF: 32, paroxysmal AF: 25) were included into the study groups. A total of 32 age-matched patients with hypertension, but without AF were selected as the control group. The expressions of PPARγ, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) mRNA in monocytes were detected by using a reverse transcription-polymerase chain reaction (RT-PCR). Interleukin-1 (IL-1) was measured by immunoenzymetric methods.

Results: The PPARγ mRNA was markedly decreased in the hypertensive AF group as compared with the hypertensive non-AF group, and it was significantly lower in persistent AF than paroxysmal AF (0.222 ± 0.0702 vs 0.564 ± 0.0436, $P<0.01$). TNF-α mRNA, IL-6 mRNA, and IL-1 were increased in patients with hypertensive AF compared to the non-AF group and it was even higher in persistent AF than in paroxysmal AF (0.721 ± 0.0541 vs 0.530 ± 0.0496, 0.567 ± 0.044 vs 0.457 ± 0.0505, 325.61 ± 88.10 vs 190.65 ± 59.38, respectively, $P<0.01$). TNF-α, IL-6, and IL-1 were in negative correlation with PPARγ, the correlation coefficient was −0.854, −0.769, and −0.702, respectively ($P<0.01$).

Conclusions: In hypertensive patients, increased inflammatory cytokines were associated with increased incidence of AF and atrial remodeling; PPARγ may be involved in the pathogenesis of AF by regulation of inflammation.

Introduction

Atrial fibrillation (AF) and hypertension are common cardiovascular diseases, they are both important risk factors of stroke and death, and are extremely costly public health problems. Pathophysiologic studies have shown that both are related to inflammation.[1,2] Investigations in this field and development of new effective treatment strategies are of great interest.

Hypertension has been shown to be related to chronic inflammation. Many inflammatory markers, such as C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and white blood cell count were proved in association with hypertension and its complications.[1,2] More recently, increasing data show that inflammation may be related to AF.[3,4]

Bruins et al[3] has reported that frequency of AF was increased after cardiac surgery, and changes in levels of CRP, complement-CRP complexes after surgery, and cardiopulmonary bypass (CPB) were responsible for the occurrence of postoperative AF. Thereafter, several studies found that plasma inflammatory cytokines such as high sensitivity C-reactive protein (hs-CRP), CRP, IL-6, and TNF-α were elevated in AF, especially in persistent AF.[3,4,6,7] The association between AF, CRP, IL-6 levels, and increased left atrial diameter (LAD) supports a link between the burden of AF, inflammation, and atrial structural remodeling.[3,4] Furthermore, Frustaci et al reported that inflammatory infiltrates and oxidative damage were uniformly found in multiple biopsy specimens in 12 patients with paroxysmal lone AF refractory to conventional antiarrhythmic treatment.[8] Inflammation may promote the persistence of AF and hypertension, but the mechanism is poorly understood and needs further investigation.

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-dependent transcription factor belonging to the nuclear hormone receptor superfamily.[9] This receptor is a key modulator of lipid and glucose homeostasis, and is predominantly expressed in adipose tissues. PPARγ is also found in many nonadipose tissues including heart, kidneys, spleen, atherosclerotic plaques, macrophages, and

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the smooth muscle and endothelial cells of the vessel wall.[9,10] Published evidence suggest that there is a close relationship between PPARγ and cardiovascular disease. Several studies have shown that PPARγ is expressed in healthy human coronary arteries, aortic vascular smooth muscle (VSM) cells and heart ventricles.[11,12] Babaev et al found that conditional knockout of macrophage PPARγ increases atherosclerosis in C57BL/6 (macrophage-specific PPAR gamma knockout mice) and low-density lipoprotein receptor-deficient mice,[13] Adil et al,[14] realized that cardiac and aortic PPARγ expression is modulated by hypertension and oxidative stress in chronically glucose-fed rats. Abundant evidences suggest that PPARγ agonists exert cardiovascular antioxidant and anti-inflammatory effects.[15,16]

Since AF is closely associated with inflammation, and hypertension is one of the most common conditions associated with AF, it is important to determine whether inflammation and PPARγ play a role in the pathogenesis of AF in patients with hypertension, if so, PPARγ agonists may be useful in the prevention and treatment of AF in such patients. Therefore, in the present study, we hypothesize that there might be a correlation between PPARγ and AF; PPARγ may be involved in the pathogenesis of AF by regulation of inflammation.

Materials and Methods

Study Groups

Using a case-control study design, 57 consecutive patients with hypertensive AF seen in the Atrial Fibrillation Clinic of West China Hospital were included in the study. A total of 32 age-matched patients with hypertension, but without history of AF were included as a control group. Each patient had been receiving antihypertension therapy and blood pressure was well-controlled. The enrollment criteria were patients aged between 45 to 80 years with hypertension and AF, AF was diagnosed by typical clinical symptoms and signs, and electrocardiogram or 24-hour Holter monitor. Exclusion criteria were as follows: valvular heart disease, surgery within 60 days, concurrent infection, heart failure, chronic or ischemic coronary heart disease, acute coronary syndrome within 1 month, diabetes mellitus, autoimmune disease, malignant tumor, hepatitis, taking angiotensin receptor blockers (ARBs) within 60 days or taking a nonsteroid anti-inflammatory drug (NSAID), chronic renal failure, and associated chronic or acute inflammation of the organs. The study was approved by the West China Medical Center and conformed to the Declaration of Helsinki. The protocol of this study was approved by West China Hospital's ethic committee and each patient signed an informed consent.

Data Collection

A detailed medical history, physical examination, biochemical parameters, and 12-lead electrocardiographic recordings (ECG) were performed routinely. Valvular functional and structural abnormalities, left ventricular size, and LAD were measured by transthoracic echocardiography. The LAD was measured in parasternal long axis view. AF duration was determined by the patient's description of a definite and abrupt onset palpitation with subsequent ECG evidence of AF at the time of presentation.

Definitions

Paroxysmal AF is self-terminating within 7 days of a recognized onset. Persistent AF is not terminated spontaneously within 7 days and/or is terminated electrically or pharmacologically.[17] The term nonvalvular AF is restricted to cases in which the rhythm disturbance occurs in the absence of rheumatic mitral valvular disease, a prosthetic heart valve, or a history of mitral valve repair.

Monocyte Preparation

Ten milliliters of overnight fasting blood was collected from an antecubital vein into vacuum containers containing heparin. Human monocytes were isolated and prepared according to the protocol described by Jiang.[18] Experiments were conducted on the same day of blood collection, and all manipulations were carried out under endotoxin-free conditions.[18]

RNA Isolation and Real-Time Semiquantitative RT-PCR

Total RNA from monocytes was extracted using chloroform and precipitated with isopropanol by TRIzolk kit (Invitrogen Corporation, Carlsbad, CA) according to the manufacturer's protocol. The isolated RNA had an OD 260/280 ratio between 1.8 and 2.2 as quantified by densitometry (Smart-Spec 3000, BIO-RAD Laboratories, Hercules, CA) and was electrophoresed in a 1% agarose gel followed by ethidium bromide staining.

One microgram of total RNA was reverse-transcribed into cDNA in a 50 μl polymerase chain reaction (One Step RNA PCR Kit [AMV], Takara Bio Inc., Otsu, Shiga, Japan), which contains 5μl PCR buffer (One Step RNA PCR Buffer, Takara Bio Inc., Otsu, Shiga, Japan) and 5 u/ul reverse transcriptase XL (5u/ul) (u/ul: unit/ul). The PCR reaction was carried out with Ready To Go PCR beading (GE Healthcare Bio-Sciences Corp., Piscataway, NJ).

All samples were analyzed in duplicate. For the amplification of human PPARγ cDNA, 2 primers (5′-GTCTCACA ATGCCATCAG GTT-3′ (upstream) and 5′-TTCAGCTGGTC GATA TCACT-3′ (downstream) were used. The primers used for IL-6 cDNA were 5′-T TCGGTCCA GTTGCCT TCT-3′ (upstream) and 5′-GTACTCATCTGGACAGCTC-3′ (downstream). The oligonucleotide sequences used for TNF-α cDNA were the following: 5′-ACGTCGTAGCAA ACCACCAA—3′ (upstream) and 5′-CTGGGAGTAGATAAG GG ACA-3′ (downstream). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH, as internal

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control) primers were 5′-CCTCAAGATCATCAGCAATG-3′ (upstream) and 5′-CCATCCACAGTCTTCTGGGT-3′ (downstream). The cDNA fragments of human PPARγ (90 bp), IL-6 (120 bp), TNF-α (140 bp), and GAPDH (141 bp) were amplified with the oligonucleotide primers as described previously.

The PCR products were run on 2.0% agarose. Then the products were semiquantified using a phosphoimager (Gel Doc 1000, Bio Rad Laboratories, Hercules, CA; Video Copy Processor, Mitsubishi, North Point, Hong Kong). The cDNAs used for the comparison of gene expression in monocytes of AF and non-AF patients were normalized for the expression concentrations of the housekeeping gene GAPDH.

Biochemical Parameters and IL-1

Fasting blood samples were collected for measurement of white blood cells (WBC), serum glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides. Serum IL-1 was assessed by immunoenzymetric assay using an enzyme-linked immunosorbent assay (ELISA) kit (SenXiong, Shanghai, China) according to the manufacturer's protocol.

Statistical Analysis

Continuous variables are presented as mean ± standard error. Computer-assisted statistical analyses were performed using the 1-way analysis of variance program and linear relation analysis. Correlations between continuous variables were assessed using Spearman's rho. Partial correlation analysis was used to show association between variables to adjust for confounding factors. A value of $P<0.05$ was accepted as statistically significant. All computations were carried out using the SPSS for windows v12.0 (SPSS, Inc., Chicago, IL).

Results

Baseline Characteristics

Of all 57 AF patients, 25 were paroxysmal AF, 33 were persistent AF. The baseline characteristics of AF and non-AF groups are presented in Table 1. The LAD in patients with AF (44.19 mm, 35.32 mm) was larger than that of the non-AF group (29.76 mm, respectively, $P<0.001$). Moreover, the LAD in patients with persistent AF was even larger than patients with paroxysmal AF ($P<0.001$; Figure 1). Age, HDL-C, LDL-C, WBC, systolic blood pressure (SBP), diastolic blood pressure (DBP), and left ventricular diameter (LVD) were not significantly different among these 3 groups.

Expression of PPARγ, TNF-α, IL-6 in Peripheral Blood Monocytes by RT-PCR Assay, and Serum IL-1

As shown in Table 1, patients in both paroxysmal and persistent AF groups had higher expression of TNF-α mRNA, IL-6 mRNA, and level of serum IL-1 than those in the non-AF group. Patients with persistent AF had higher expression of TNF-α mRNA, IL-6 mRNA, and serum IL-1 levels than that in patients with paroxysmal AF (Figure 2). The expression of PPARγ mRNA in peripheral blood monocytes was decreased in the AF group as compared with the non-AF group and the expression of PPARγ mRNA in patients with persistent AF was lower than that of patients with paroxysmal AF.

Correlation of LAD and Expression of TNF-α, IL-6, and Serum IL-1

Correlation analysis was performed by computer-assisted statistical analyses between LAD and the concentrations of TNF-α mRNA and IL-6 mRNA in circulating monocytes and serum IL-1 (Figure 3). After adjustment for confounding factors (age, SBP, DBP, LDL-C, HDL-C, heart rate [HR], WBC), the results showed that LAD was positively correlated with the expressions of TNF-α, IL-6 in monocyte concentration, and the level of serum IL-1 ($r = 0.479, 0.324$, and 0.327, respectively, $P<0.05$; Table 2).

Correlation of PPARγ and Expression of TNF-α, IL-6, and Serum IL-1

Correlation analysis revealed that there existed a significant negative correlation between PPARγ and the expression of TNF-α, IL-6, and serum IL-1 ($r = -0.808, -0.727$, and -0.580, respectively, $P<0.001$; Figure 4, Table 4). The negative correlation still persisted even when age, SBP, DBP, LDL-C, HDL-C, HR, and WBC were taken into account.

Discussion

Conventionally, AF can be clinically classified as valvular AF and nonvalvular AF which is restricted to cases in which the rhythm disturbance occurs in the absence of rheumatic mitral valve disease, a prosthetic heart valve, or mitral valve

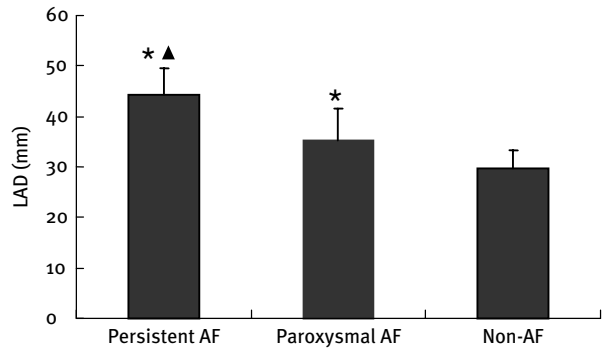

Figure 1. Left atrial diameter in AF group and non-AF group. *Significant variation from non-AF ($P<0.05$). ▲ Significant difference between persistent AF and paroxysmal AF ($P<0.05$)
Abbreviations: AF, atrial fibrillation; LAD, left anterior descending artery. Data are the mean values ± standard error.

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Table 1. Demographic Data (at Baseline) of Patients

Variable	Non-AF (n = 32)	Paroxysmal AF (n = 25)	Persistent AF (n = 32)
Age (year)	61.5 ± 11.5	64.3 ± 9.7	67.3 ± 8.1
Heart rate (bpm)	77.06 ± 11.678	76.44 ± 12.166	72.41 ± 10.815
Systolic blood pressure (mm Hg)	124.0 ± 13.2	125.5 ± 10.0	123.3 ± 14.7
Diastolic blood pressure (mm Hg)	73.3 ± 9.0	73.7 ± 7.3	71.9 ± 6.4
LDL-C (mmol/L)	2.86 ± 1.00	2.71 ± .95	2.30 ± .54
HDL-C (mmol/L)	1.51 ± .29	1.45 ± .33	1.44 ± .46
White blood cells ($\times 10^9$ / L)	6.91 ± 1.20	7.17 ± 1.53	7.06 ± 1.12
Left atrial diameter (mm)	29.76 ± 3.659	35.32 ± 6.336[a]	44.19 ± 5.312[a,b]
Left ventricular diameter (mm)	44.81 ± 3.311	44.52 ± 4.244	45.88 ± 4.943[a]
EF (%)	58.28 ± 9.383	62.60 ± 9.152	57.59 ± 9.618
Antihypertensive agents	16 (30.2%)	15 (28.3%)	22 (41.5%)
ACE inhibitor	16 (47.1%)	8 (23.5%)	10 (29.4%)
Calcium channel blocker	20 (31.7%)	20 (31.7%)	23 (36.5%)
β-Blocker diuretic	5 (15.2%)	5 (20%)	4 (12.5%)

Data are the mean values ±SD.
[a] Significant variation from non-AF ($P<0.05$).
[b] Significant difference between persistent AF and paroxysmal AF ($P<0.05$)
Abbreviations: AF, atrial fibrillation; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.

Table 2. Expression of PPARγ,TNF-α, IL-6 in Peripheral Blood Monocytes, and IL-1 in Serum

Parameter	Non-AF	Paroxysmal AF	Persistent AF
PPARγ	0.650 ± 0.064	0.564 ± 0.043[a]	0.222 ± 0.070[a,b]
TNF-α	0.414 ± 0.040	0.457 ± 0.050[a]	0.567 ± 0.044[a,b]
IL-6	0.483 ± 0.048	0.530 ± 0.049[a]	0.721 ± 0.054[a,b]
IL-1 (ng/ml)	130.7 ± 42.2	190.6 ± 59.3[a]	325.6 ± 88.1[a,b]

Values are expressed as mean ± SD.
[a] Significant variation from non-AF ($P<0.05$).
[b] Significant difference between persistent AF and paroxysmal AF ($P<0.05$)
Abbreviations: AF, atrial fibrillation; IL-1, interleukin-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; PPARγ, peroxisome proliferator-activated receptor-γ; SD, standard deviation.

repair.[13] In fact, the pathophysiology of nonvalvular AF is somewhat different from that of valvular AF, and the former is extremely complex and unclear. It should be emphasized that previous studies did not compare the role of inflammation in valvular AF and nonvalvular AF.[3,4,6,8] This study is the first to report the correlation of nonvalvular AF with elevated expression of markers of systemic inflammation, such as TNF-α mRNA, IL-6 mRNA in peripheral blood monocytes, and serum IL-1.

PPARγ is a modulator of macrophage gene expression and has an anti-inflammation effect. Jiang et al[10] and Jiang et al[18] confirmed that PPARγ did express in human monocytes, and showed that PPARγ agonists (15d-PGJ2 and synthetic PPARγ ligands) could markedly upregulate PPARγ and processing of inflammatory cytokines in activated human macrophages at agonist concentrations similar to those found to be effective for the promotion of adipogenesis. Some genes are negatively regulated by PPARγ including inducible nitric oxide synthase, IL-1, IL-6, TNF-α,[10,18]

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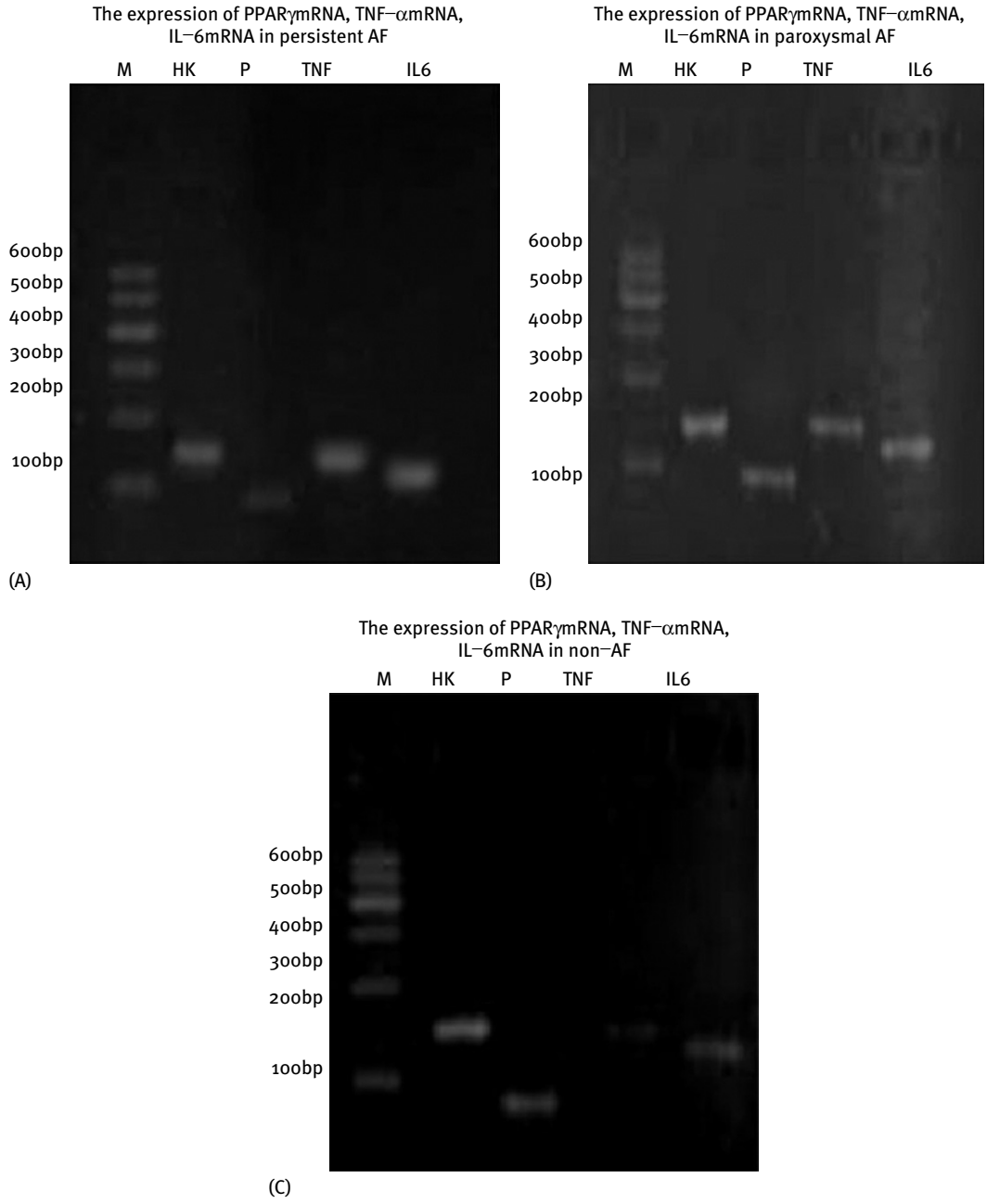

Figure 2. The expression of PPARγ mRNA, TNF-α mRNA, and IL-6 mRNA in peripheral blood monocytes was evaluated by a specific semiquantitative RT-PCR method. The PCR product of PPARγ, TNF-α, IL-6, and GAPDH resulted in 90 bp, 140 bp, 120 bp, and 141 bp fragments, respectively, which indicated an expression of PPARγ, TNF-α, and IL-6 in peripheral blood monocytes. (A) The expression of PPARγ mRNA, TNF-α mRNA, and IL-6 mRNA in persistent AF. (B) The expression of PPARγ mRNA, TNF-α mRNA, and IL-6 mRNA in paroxysmal AF. (C) The expression of PPARγ mRNA, TNF-α mRNA, and IL-6 mRNA in non-AF. Abbreviations: AF, atrial fibrillation; bp, base pair(s); HK, GAPDH (glyceraldehydes 3-phosphate dehydrogenase); IL-6, interleukin-6; M, PCR marker; P, peroxisome proliferator-activated receptor-γ; TNF-α,tumor necrosis factor-α.

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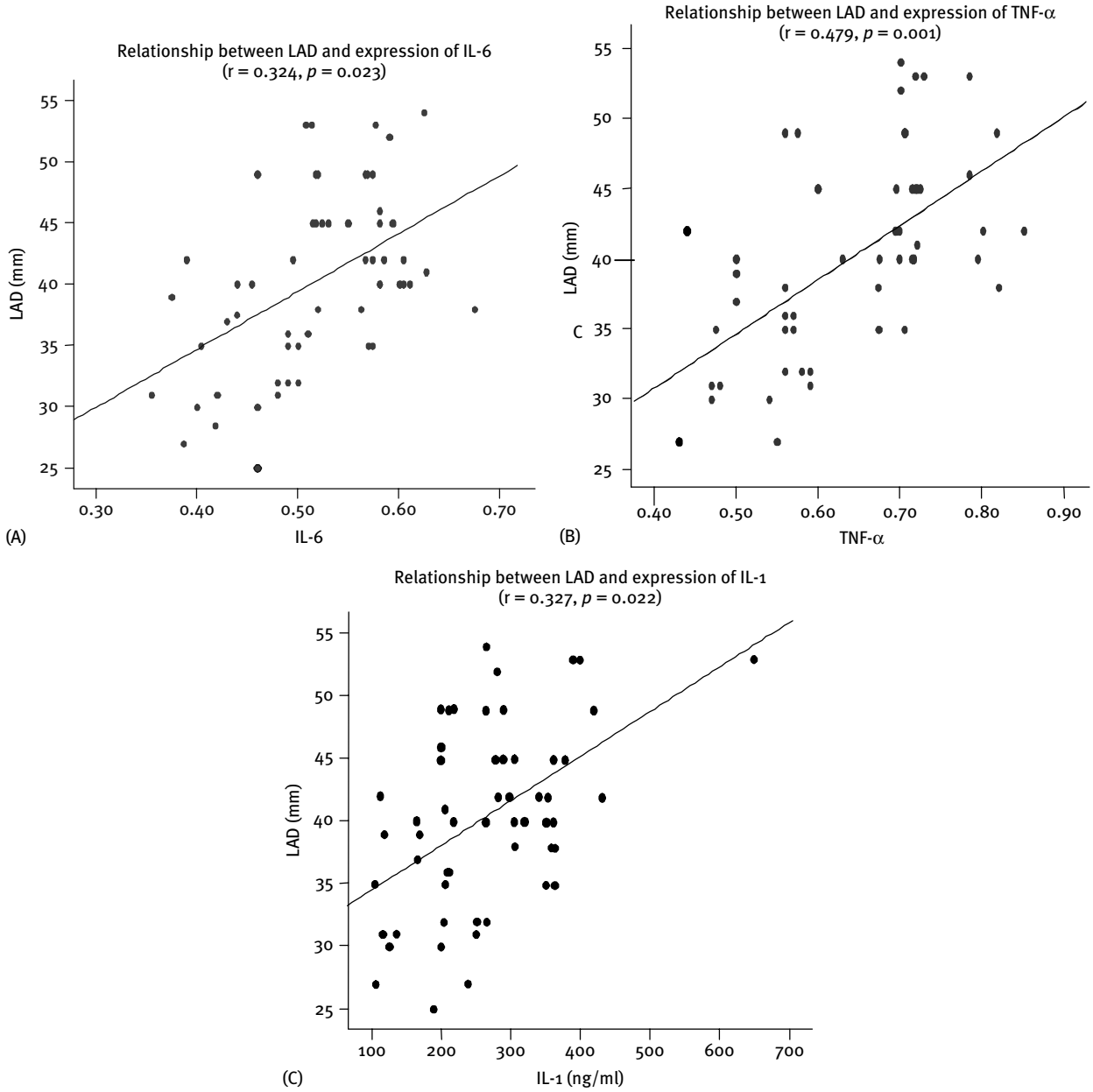

Figure 3. (A) Relationship between LAD and expression of IL-6 ($r = 0.324$, $P = 0.023$). (B) Relationship between LAD and expression of TNF-α ($r = 0.479$, $P = 0.001$). (C) Relationship between LAD and expression of IL-1 ($r = 0.327$, $P = 0.022$). Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; LAD, left atrial diameter; TNF-α, tumor necrosis factor-α.

and several other genes induced by interferon-γ (IFN-γ). There are several pathways involved in these processes, including the formation of nuclear-cytoplasmic shuttling complexes with p65, modulation of p38 mitogen-activated protein kinase activity, and so forth. In a word, this profile of activity suggested a potential anti-inflammatory role of PPARγ in macrophages.

PPARγ plays an anti-inflammation role in a broad spectrum of cardiovascular diseases associated with AF, such as coronary artery disease (CAD), insulin resistance, type 2 diabetes mellitus (DM), and hypertension. The present study is designed to test whether increased inflammation due to decreased PPARγ is involved in the pathogenesis of nonvalvular hypertensive AF. To adjust the

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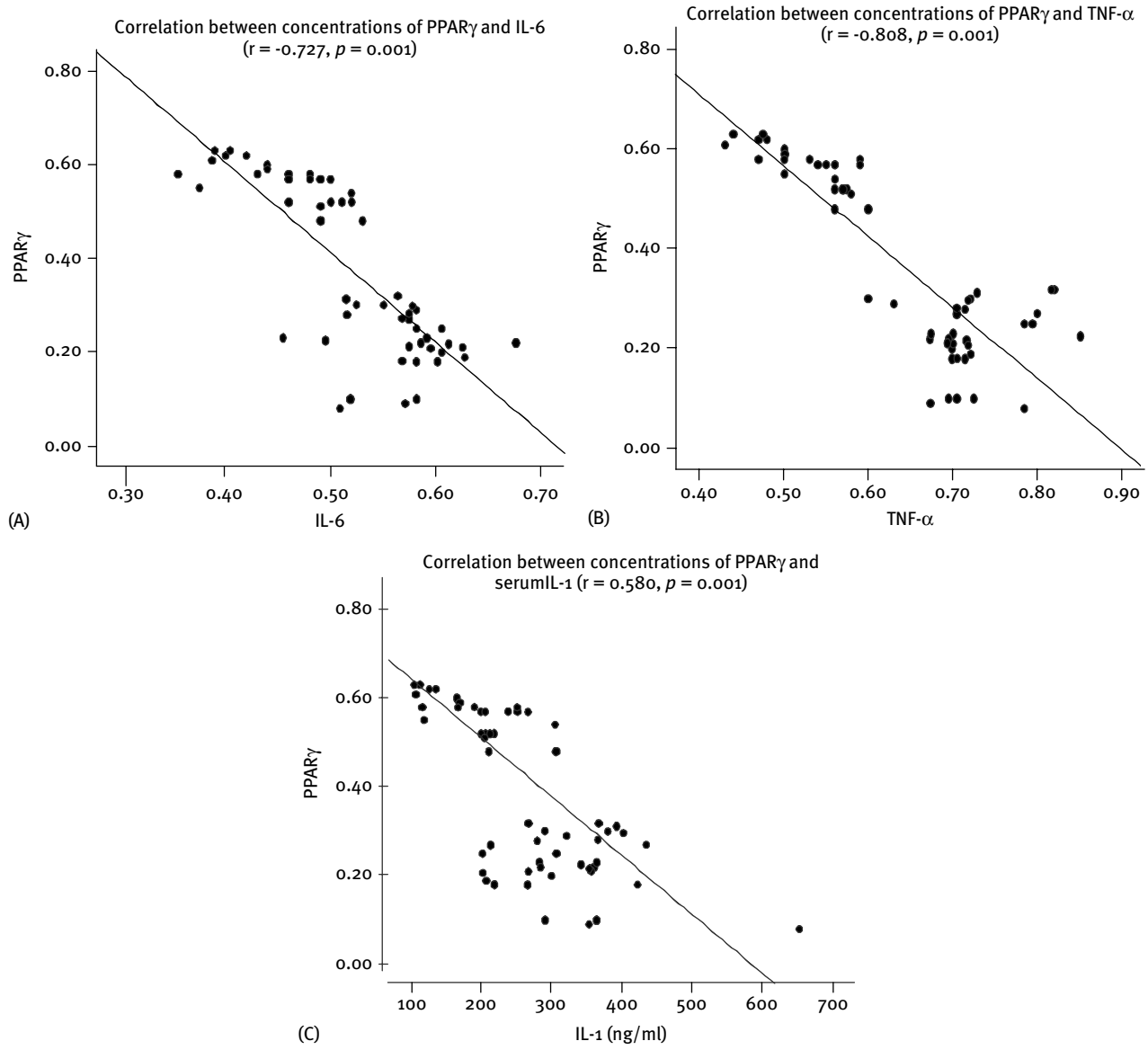

Figure 4. (A) Correlation between concentrations of PPARγ and IL-6 ($r = -0.727$, $P = 0.001$). (B) Correlation between concentrations of PPARγ and TNF-α ($r = -0.808$, $P = 0.001$). (C) Correlation between concentrations of PPARγ and serum IL-1 ($r = 0.580$, $P = 0.001$). Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; PPARγ, peroxisome proliferator-activated receptor-γ; TNF-α, tumor necrosis factor-α .

influence of hypertension, blood pressure in each patient was well-controlled and did not have significant differences statistically in these 3 groups. It is better to compare the changes of inflammation and the expression of PPARγ between a healthy group and a hypertensive AF group.

In this study, we found that the expression of TNF-α mRNA, IL-6 mRNA in peripheral blood monocytes, and the level of serum IL-1 were increased in patients with hypertensive AF compared to the non-AF group. We also found the expression of TNF-α mRNA, IL-6 mRNA in peripheral blood monocytes, and the level of serum IL-1 were even higher in persistent AF than in paroxysmal AF. It is suggested that these cytokines are markers of inflammatory states and may be related to the burden of AF, in other words, it may promote the persistence of AF.

Atrial structural remodeling and fibrosis in AF may occur due to hemodynamic, metabolic, and inflammatory stressors. LAD (an index of atrial structural remodeling)

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is larger in patients with AF than in the non-AF group. We also found that those with persistent AF had obvious enlargement of the left atrium associated with decreased PPARγ and increased inflammatory cytokines. However, since persistent AF, paroxysmal AF, and non-AF groups had similar blood pressure, it seems that the association between inflammation, PPARγ, and AF is independent of systemic hemodynamics.

After adjustment for age, SBP, DBP, LDL-C, HDL-C, HR, and WBC, the expression of TNF-α mRNA, IL-6 mRNA, and the level of serum IL-1 were in positive correlation with LAD. Since increased inflammation cytokines were associated with increased incidence of AF and with larger LAD, it is reasonable to deduce that inflammation could promote persistence of AF through left atrial remodeling and that inflammatory markers may predict the severity of remodeling and the prognosis or treatment of AF. Pathology researches have demonstrated that inflammation contributes to permanent AF through electrical and structural remodeling. Inflammatory infiltrates can result in atrial fibrosis, loss of atrial muscle mass, and deposition of increased amounts of connective tissue between each cell[19,20] (a process known as structural remodeling), which resulted in separation of myocytes from one another and subsequent nonhomogeneity of atrial conduction, dispersion, and shortening of the atrial refractory period (a process known as electrical remodeling). In the present study, it is hard to clarify the cause-result relationship between AF and atrial dilatation, which is a limitation of this study.

Both animal experiments and clinical trials have shown that angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin type 1 receptor blockers (ARBs) appear to be effective in the prevention of AF.[21] Murray et al[22] investigated whether inhibition of endogenous angiotensin II signaling reduces the recurrence rate of AF in patients enrolled in the Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) study. His analysis provides evidence that ACEI use may be beneficial in some patients with AF. It has revealed in the CHARM program[23] that treatment with the angiotensin receptor blocker candesartan, reduced the incidence of AF in a large, broadly-based, population of patients with symptomatic chronic heart failure, which provided the opportunity to prospectively determine the effect of candesartan on the incidence of new AF in this CHF population. Madrid et al[24] found that patients treated with amiodarone plus irbesartan had a lower rate of recurrence of atrial fibrillation than did with amiodarone alone. Therefore, it is interesting to investigate whether ARBs which contain PPARγ-activating potential have superior effects in reducing the development of AF in hypertensive patients.

As we know, mRNA levels of cytokines and PPARγ may not always concur with the protein. It is helpful to identify our findings through investigating the gene and protein expression of cytokines and PPARγ in human atrial biopsies and animal models.

Conclusion

These results indicated that PPARγ as a biomarker may have the potential to inhibit the inflammatory process. Therefore, it may play an important role in the pathogenesis of nonvalvular hypertensive AF by regulation of inflammation.

References

1. Karthikeyan VJ, Lip GY. White blood cell count and hypertension. *J Hum Hypertens*. 2006;20:310–312.
2. Bautista LE, Vera LM, Arenas IA, et al. Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-α) and essential hypertension. *J Hum Hypertens*. 2005;19:149–154.
3. Psychari SN, Apostolou TS, Sinos L, et al. Relation of elevated C-reactive protein and interleukin-6 levels to left atrial size and duration of episodes in patients with atrial fibrillation. *Am J Cardiol*. 2005;95:764–767.
4. Sata N, Hamada N, Horinouchi T, et al. C-reactive protein and atrial fibrillation: is inflammation a consequence or a cause of atrial fibrillation? *Jpn Heart J*. 2004;45:441–445.
5. Bruins P, te Velthuis H, Yazdanbakhsh AP, et al. Activation of the complement system during and after cardiopulmonary bypass surgery: postsurgery activation involves C-reactive protein and is associated with postoperative arrhythmia. *Circulation*. 1997;96:3542–3548.
6. Gedikli O, Dogan A, Altuntas I, et al. Inflammatory markers according to types of atrial fibrillation. *Int J Cardiol*. 2007;120:193–197.
7. Chung MK, Martin DO, Sprecher D, et al. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. *Circulation*. 2001;104:2886–2891.
8. Frustaci A, Chimenti C, Bellocci F, et al. Histological substrate of atrial biopsies in patients with lone atrial fibrillation. *Circulation*. 1997;96:1180–1184.
9. Fajas L, Auboeuf D, Raspe E, et al. The organization, promoter analysis, and expression of the human PPARγ gene. *J Biol Chem*. 1997;272:18779–18789.
10. Ricote M, Huang J, Fajas L, et al. Expression of the peroxisome proliferator-activated receptor-γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. *Proc Natl Acad Sci USA*. 1998;95:7614–7619.
11. Benson S, Wu J, Padmanabhan S, et al. Peroxisome proliferator-activated receptor (PPAR)-γ expression in human vascular smooth muscle cells: inhibition of growth, migration, and c-fos expression by the peroxisome proliferator-activated receptor (PPAR)-γ activator troglitazone. *Am J Hypertens*. 2000;13:74–82.
12. Mehrabi MR, Thalhammer T, Haslmayer P, et al. The peroxisome proliferator-activated receptor gamma (PPARγ) is highly expressed in human heart ventricles. *Biomed Pharmacother*. 2002;56:407–410.
13. Babaev VR, Yancey PG, Ryzhov SV, et al. Conditional knockout of macrophage PPAR[γ] increases atherosclerosis in C57BL/6 and low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2005;25:1647–1653.
14. Midaoui A, Wu LY, Wang R, et al. Modulation of cardiac and aortic peroxisome proliferator-activated receptor-γ expression by oxidative stress in chronically glucose-fed rats. *Am J Hypertens*. 2006;19:407–412.

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DOI:10.1002/clc.20566

15. Vamecq J, Latruffe N. Medical significance of peroxisome proliferator- activated receptors. *Lancet*. 1999;354:141–148.
16. Tao L, Liu HR, Gao E, et al. Antioxidative, antinitrative, and vasculoprotective effects of aperoxisome proliferator-activated receptor agonist in hypercholesterolemia. *Circulation*. 2003;108: 2805–2811.
17. Fuster V, Rydén LE, Cannom DS, et al. ACC/AHA/ESC 2006 Guidelines for the Management of Patients with Atrial Fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the European Society of Cardiology Committee for Practice Guidelines: developed in collaboration with the European Heart Rhythm Association and the Heart Rhythm Society. *Circulation*. 2006;114:257–354.
18. Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature*. 1998;391:82–86.
19. Kostin S, Klein G, Szalay Z, et al. Structural correlate of atrial fibrillation in human patients. *Cardiovasc Res*. 2002;54: 361–379.
20. Schotten U, Ausma J, Stellbrink C, et al. Cellular mechanisms of depressed atrial contractility in patients with chronic atrial fibrillation. *Circulation*. 2001;103:691–698.
21. Healey JS, Baranchuk A, Crystal E, et al. Prevention of atrial fibrillation with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers: a meta-analysis. *J Am Coll Cardiol*. 2005;45:1832–1839.
22. Murray KT, Rottman JN, Arbogast PG, et al. the AFFIRM Investigators. Inhibition of angiotensin II signaling and recurrence of atrial fibrillation in AFFIRM. *Heart Rhythm*. 2004;1(6): 669–675.
23. Ducharme A, Swedberg K, Pfeffer MA, et al. Prevention of atrial fibrillation in patients with symptomatic chronic heart failure by candesartan in the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) program. *Am Heart J*. 2006;152:86–92.
24. Madrid AH, Bueno MG, Rebollo JM, et al. Use of irbesartan to maintain sinus rhythm in patients with long-lasting persistent atrial fibrillation: a prospective and randomized study. *Circulation*. 2002;106:331–336.

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