



Pioglitazone Improves Endothelial Function in Patients with Paroxysmal Atrial Fibrillation and Type 2 Diabetes Mellitus Following Catheter Ablation

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Abstract

In our previous study, pioglitazone, one of the peroxisome proliferator-activated receptor-gamma activators was shown to exert beneficial effects on the clinical outcome of catheter ablation in patients with paroxysmal atrial fibrillation (PAF) and type 2 diabetes mellitus (T2DM). In the current study, we aimed to investigate the influences of pioglitazone treatment on endothelial function in these patients. This study enrolled 150 patients with drug-refractory PAF and T2DM, who were grouped based on whether they received pioglitazone before ablation or not. Then 51 patients treated with pioglitazone and 99 control subjects were followed up after catheter ablation. To determine the effects of pioglitazone on endothelial function, high-frequency ultrasound was performed at baseline and after 12-month following-up. The parameters measured were brachial artery flow-mediated dilation (FMD) and nitrate mediated vasodilation (NMD). Furthermore, serum endothelial nitric oxide synthase (eNOS) level and the quantity of circulating endothelial progenitor cells (EPCs) were also determined. All baseline variables of the two groups were comparable. After 12 months of following-up, FMD improved significantly in the pioglitazone group compared to the control group as well as baseline. Besides, pioglitazone treatment also led to higher circulating EPCs number and serum eNOS level. In conclusion, pioglitazone brings about favorable effects on endothelial function in patients with PAF and T2DM after catheter ablation.

Keywords

PPAR γ agonist, Atrial fibrillation, Catheter ablation, Endothelial function

Introduction

Atrial fibrillation (AF) is the most common type of sustained heart rhythm disorder confronted in clinical practice, which confers a high risk of mortality and morbidity from abnormal hemodynamics and

thromboembolism [1]. The beat-to-beat alteration of flow dynamics in AF has disadvantageous effects on cardiac haemodynamics and is associated with endothelial dysfunction [2]. Endothelial dysfunction may contribute to a prothrombotic state in patients with AF and cardiovascular events [3]. Besides, the presence of diabetes mellitus (DM) augments endothelial dysfunction in AF patients [4].

Pioglitazone, one of peroxisome proliferator-activated receptor-gamma (PPAR γ) agonist, is now widely used in the treatment of type 2 DM (T2DM). Recent clinical studies have shown that pioglitazone improves endothelial function [5-8] as well as ameliorates the quantity and function of endothelial progenitor cells (EPCs) [9,10]. Our previous study indicated that pioglitazone ameliorated the preservation of sinus rhythm (SR) and decreased the reablation rate in patients with paroxysmal AF (PAF) and T2DM after catheter ablation [11]. For those enrolled patients, we further determined the effects of pioglitazone use on the endothelial function as well as the magnitude of EPCs mobilization during pre- and post-ablation period.

Methods

Study design and study populations

The methodology for this clinical trial was previously described in detail [11]. From September 2007 to July 2009, a total of 150 consecutive patients with drug-refractory PAF and T2DM were enrolled in this prospective observational single-centre cohort study. The study protocol was approved by the local ethics committee and informed consent was obtained from all patients. Major exclusion criteria were age >75 years old, unstable angina, acute myocardial infarction, congestive heart failure, pregnancy or severe renal or hepatic dysfunction. Patients were grouped based on whether they received pioglitazone therapy before ablation or not: 51(34%) patients received pioglitazone preablation were in the pioglitazone group and pioglitazone was continued post-ablation (30 mg/day),

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while 99(66%) patients without previous pioglitazone therapy formed the control group, in which patients taking standard therapy except pioglitazone during this study. All enrolled patients were kept free from newly prescribed statin, and angiotensin converting enzyme inhibitor/angiotensin II receptor blocker (ACEI/ARB) during the follow-up.

Mapping and catheter ablation procedure

Electrophysiological study and catheter ablation procedure were performed as we previously described [11]. Briefly, all antiarrhythmic drugs (AADs) except amiodarone were discontinued for at least five half-lives. Oral anticoagulation was ceased 3 days pre-procedure, and full-dose weight-adjusted (1.5 mg/kg daily) subcutaneous low-molecular-weight heparin was used to bridge the procedure until therapeutic oral anticoagulation was achieved following ablation. The presence of atrial thrombus was excluded by transoesophageal echocardiography before ablation. During the procedure, intravenous unfractionated heparin was administered to maintain an activated clotting time between 300 and 350 sec. Circumferential pulmonary vein isolation (CPVI) was performed at the posterior wall 1 cm and at the anterior wall 5 mm away from the angiographically defined PV ostia under the guidance of the CARTO system (Biosense Webster). The endpoint of circumferential PV ablation was PV isolation, which was defined as disappearance of all PV potentials or left atrial (LA)-PV potential dissociation. After the CPVI, if AF was sustained or induced with coronary sinus burst pacing at a cycle length down to 180 ms during the administration of intravenous isoproterenol (1.0 to 3.0 mg/min) and lasted more than 3 min, additional ablation, including linear ablation of the left atrial roof and/or superior vena cava isolation, was conducted. The cavotricuspid isthmus was ablated in patients with a history of atrial flutter (AFL). If the AF did not terminate or was inducible after aforementioned procedures, SR was restored by transthoracic cardioversion. Patients were administered oral anticoagulation (warfarin) (international normalized ratio: 2.0–3.0) for 3 months. AAD was administered in all cases for 1 month after the ablation and was withdrawn 1-month later in cases without AF recurrence, but was continued otherwise.

Following-up

Patients were discharged in SR after 2 days of monitoring. After discharge, all patients underwent following-up at 2 weeks post-procedure and then every month thereafter. At each hospital visit, the patients underwent 12-lead ECG and intensive questioning regarding any arrhythmia-related symptoms. Holter monitoring for 24 h was performed at 2 weeks and 1, 3, 6, 9, and 12 months. An open access to arrhythmia specialists was available subsequently. All patients enrolled in our study received both periodical ECG/Holter recording and ECG/Holter monitoring prompted by symptoms. As early recurrences often settle spontaneously, a 1-month blanking period was observed, during which recurrences were managed medically. After the 1-month blanking period, any episode of symptomatic or asymptomatic atrial tachyarrhythmias [ATa, including AF, atrial tachycardia (AT) and AFL] with ECG and Holter recording that lasted over 30 s was considered as a recurrent event [11].

Endothelial function study

Enrolled patients were asked to fast and discontinued vasoactive medications for 12 h prior to the study. In brief, the patients were instructed to lie quietly in a supine position for 10 min before the testing. All testings were carried out in a temperature-controlled room (20 to 25°). Arterial endothelial and smooth muscle function were evaluated by examining brachial artery responses to endothelium-dependent (flow-mediated) and endothelium-independent [glyceryl trinitrate (GTN)-mediated] stimuli. All images were conducted using a hand-held 10 MHz vascular ultrasound probe (GE Vingmed System 5 ultrasound machine) as previously described [12]. Endothelium-dependent, post ischemic flow mediated vasodilation (FMD) was determined by the maximal brachial artery diameter after 60 sec of reactive hyperemia, compared with baseline vessel diameter.

Endothelium-independent, nitrate mediated vasodilation (NMD) was expressed as the percent change in baseline vessel diameter 3 min after sublingual nitrate administration (0.3 mg glyceryl trinitrate) [12].

Enzyme-Linked Immunosorbent Assay (ELISA) for endothelial nitric oxide synthase (eNOS)

Peripheral blood samples were obtained at baseline at the start of the procedure and at 12 months post-procedure during outpatient following-up. Laboratory personnel who conducted the testing were blinded to patient characteristics. The acquired blood samples were centrifuged at 2500g for 15 min at 4° and stored at -80° for batch ELISA analysis. Serum eNOS level was measured in accordance to company's instructions (R&D Systems, Minneapolis, MN, USA).

Circulating EPCs number

Circulating EPCs were quantified by phenotype in whole blood samples utilizing flow cytometry [13]. The cells were analyzed for phenotypic expression of surface antigens using monoclonal antibodies. The EPCs were defined as CD34⁺/CD133⁺/KDR⁺ cells. EDTA anticoagulant peripheral whole blood (100 µL) was stained with antibodies [anti-CD34-FITC (BioLegend, San Diego, CA, USA), anti-CD133-APC (Miltenyl Biotech, Germany) and anti-KDR (VEGFR2)-PE (Sigma, St Louis, MO, USA)] for 20 minutes in dark chamber at room temperature. Appropriate isotype controls were stained to establish positive stain boundaries. Erythrocytes were lysed (Becton Dickinson, Oxford, UK), and samples were

Table 1: Baseline patient characteristics

	Pioglitazone group (n=51)	Control group (n=99)	P-value
Age (years)	59.6 ± 1.4	58.7 ± 0.9	0.575
BMI (kg/m ²)	23.3 ± 0.3	23.1 ± 0.2	0.657
Male (sex)	27(52.9)	45(45.5)	0.385
Duration of AF (months)	43.6 ± 1.5	40.5 ± 1.1	0.255
CAD	3(5.9)	5(5.1)	0.490
VHD	2(3.9)	4(4.0)	1.000
Cardiomyopathy	1(2.0)	1(1.0)	1.000
Hypertension	32(62.7)	72(72.7)	0.232
Smoking	22(43.1)	39(39.4)	0.839
LAD (mm)	43.9 ± 0.7	43.7 ± 0.4	0.834
LVEF	0.56 ± 0.0	0.57 ± 0.0	0.601
Statins	7(13.7)	12(12.1)	0.780
ACEI/ARB	29(56.9)	45(45.5)	0.186
Amiodarone	19(37.3)	31(31.3)	0.465
Sotalol	2(3.9)	2(2.0)	0.605
Beta-blocker	18(35.3)	37(37.4)	0.802
CCB	18(35.3)	28(28.3)	0.378
Propafenone	12(23.5)	16(16.2)	0.273
FPG (mmol/L)	6.6 ± 0.2	6.8 ± 0.1	0.260
FPG (follow-up) (mmol/L)	6.2 ± 0.1	6.2 ± 0.1	0.928
HbA1c (%)	6.2 ± 0.1	6.4 ± 0.1	0.225
HbA1c (follow-up) (%)	6.2 ± 0.1	6.3 ± 0.1	0.331
hsCRP (mg/L)	2.1 ± 0.2	2.5 ± 0.2	0.074
Sulfonylurea	25(49.0)	61(61.6)	0.140
Biguanide	18(35.3)	23(23.2)	0.116
a-GI	10(19.6)	15(15.2)	0.488
Insulin	2(3.9)	2(2.0)	0.605
FMD(%)	4.2 ± 0.1	4.0 ± 0.1	0.088
NMD(%)	22.2 ± 0.6	23.1 ± 0.4	0.173
EPCs (cells/10 ⁵ events)	3.1 ± 0.1	3.3 ± 0.1	0.234
eNOS (pg/ml)	18.4 ± 0.4	18.7 ± 0.4	0.537

Values are reported as mean + SEM or n (%).

BMI: Body Mass Index, CAD, Coronary Artery Disease, VHD: Valvular Heart Disease, CCB: Calcium Channel Blocker, Including Amlodipine, Felodipine, Nifedipine, Diltiazem and Verapamil, FPG: Fasting Plasma Glucose, HbA1c: Haemoglobin A1c, a-GI: Alpha-Glucosidase Inhibitor, FMD: Flow Mediated Vasodilation, NDM: Nitrate Mediated Vasodilation, EPC: Endothelial Progenitor Cells, eNOS: Endothelial Nitric Oxide Synthase, FPG and HbA1c at follow-up: at 12 months after first ablation.

centrifuged at 1500 g for 5 minutes at room temperature. Acquisition was performed by technicians blinded to subject identity using a FACS Valieur analyzer (Becton Dickison). Analysis was conducted by gating lymphocytes and monocytes on the basis of light-scattering properties. We acquired 100 000 events per gate. The quantity of EPCs was measured based on the percentage of CD34⁺CD133⁺KDR⁺ triple positive leucocytes and expressed as the number of cells per 10⁵ mononuclear cells.

Statistical analysis

All continuous variables are expressed as mean ± SEM and categorical variables as proportions. Between-group comparisons were performed using the t-test or χ2 test as appropriate. A P-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software (version 18; SPSS Inc, Chicago, IL).

Results

Baseline measurements and outcome after primary ablation

The baseline characteristics of the study population are shown in table 1. All baseline variables of the two groups were statistically similar. A total of 150 patients with PAF and T2DM were enrolled in this study. After 12 months of following-up, 46 patients (90.2%, maintaining SR subgroup) from pioglitazone group and 74 patients (74.7%, maintaining SR subgroup) from the control group had no ATa recurrence without AADs therapy (P = 0.025) as we previous described [11].

Changes in endothelial function after pioglitazone treatment

FMD and NMD were not statistically significant between the two groups at baseline (Table 1). At 12 months after catheter ablation, FMD was markedly increased in the pioglitazone group compared with control group (Table 2, Figure 1). Further subgroup analysis showed that in patients with successful maintaining SR following catheter ablation, pioglitazone therapy also gave rise to better FMD compared with control group (Table 2, Figure 1). Moreover, catheter ablation with successful maintaining SR rather than ATa recurrence led to obvious higher FMD compared with baseline in both pioglitazone and control group (Table 3, Figure 2). However, NMD did not differ between the two groups during following-up (22.4 ± 0.7% vs 23.0 ± 0.4%, P = 0.429).

Serum eNOS level after pioglitazone treatment

There was no marked difference in endothelial function marker eNOS at baseline between the two groups (Table 1). However, serum

eNOS concentration was markedly higher in the pioglitazone group compared with the control group during the following-up (Table 2, Figure 3). Further subgroup analysis indicated that in patients with successful maintaining SR following ablation, pioglitazone therapy also resulted into increased serum eNOS level compared with the control group (Table 2, Figure 3). Besides, compared with baseline, serum eNOS level was obviously higher in patients with successful maintaining SR instead of ATa recurrence following catheter ablation in both pioglitazone and control group (Table 3, Figure 4).

Effects of the pioglitazone treatment on circulating EPCs number

The number of circulating EPCs (CD133⁺CD34⁺KDR⁺) between the two groups was not significantly different before the start of therapy (Table 1). Pioglitazone treatment significantly increased the quantity of circulating EPCs compared with control group (Table 2, Figure 5). Further subgroup analysis showed that in patients with successful maintaining SR after ablation, pioglitazone therapy also brought about higher circulating EPCs number compared with control group (Table 2, Figure 5). Moreover, in both pioglitazone and control group, circulating EPCs level at 12-month following-up was significantly augmented compared with baseline level in patients with successful maintaining SR, but not with ATa recurrence following ablation (Table 3, Figure 6).

Discussion

The most notable findings of this study in patients with PAF and T2DM after catheter ablation were: (a) Pioglitazone treatment improved FMD; (b) Pioglitazone therapy led to a higher eNOS level; (c) Pioglitazone treatment augmented circulating EPCs number.

Accumulating evidences have indicated that AF patients have impaired endothelial function [2-4]. It is shown that patients with AF have diminished endothelial-dependent FMD and that this reduction is reversed by restoring SR through electrical cardioversion [14,15] as well as catheter ablation [16,17]. EPCs, as a specific group of cells generated from bone marrow, are suggested to play a key role in

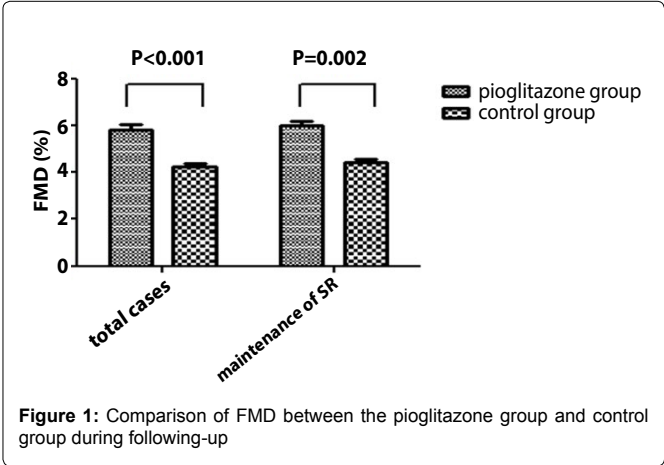


Figure 1: Comparison of FMD between the pioglitazone group and control group during following-up

Table 2: Endothelial function after pioglitazone treatment

	Pioglitazone		Control	
	Total cases	SR	Total cases	SR
FMD (%)	5.8 ± 0.2	6.0 ± 0.2	4.3 ± 0.1	4.4 ± 0.1
eNOS(pg/ml)	22.0 ± 0.6	22.8 ± 0.7	19.8 ± 0.3	20.3 ± 0.4
EPC (cells/10 ⁵ events)	3.9 ± 0.1	4.0 ± 0.1	3.5 ± 0.1	3.6 ± 0.1

Values are reported as mean + SEM.

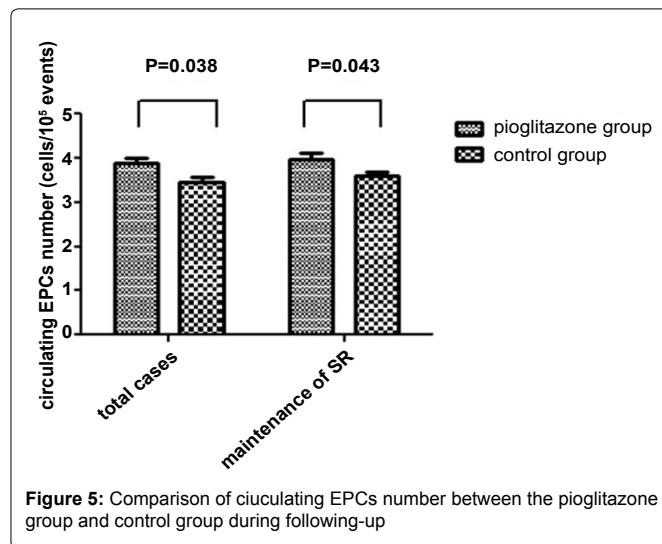
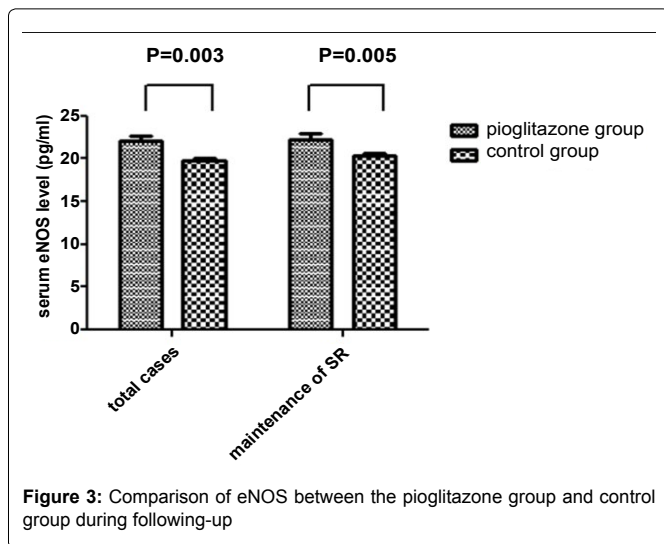
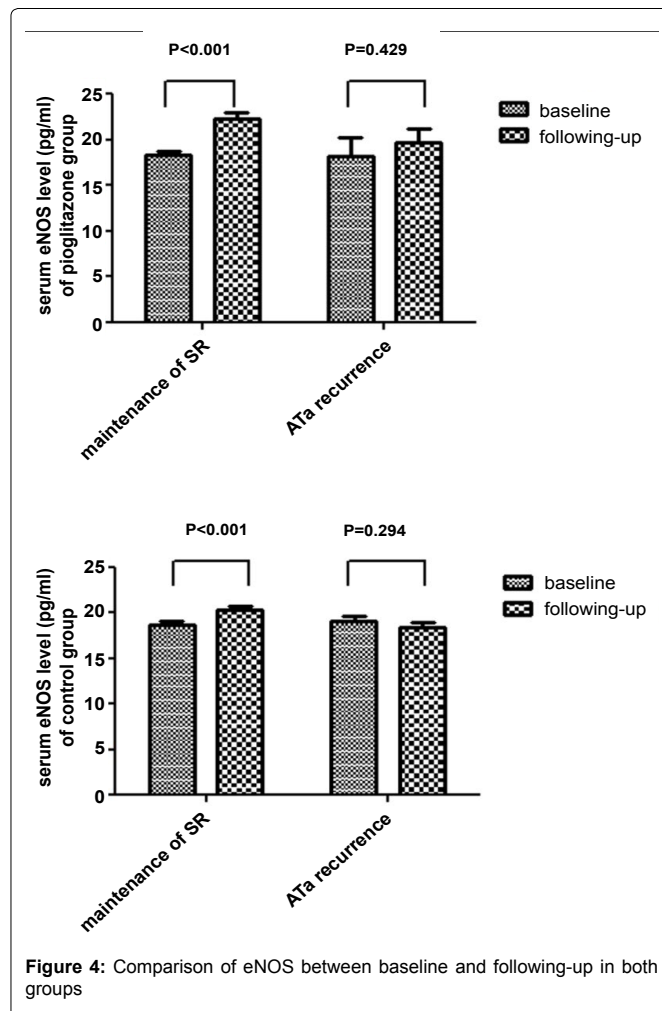
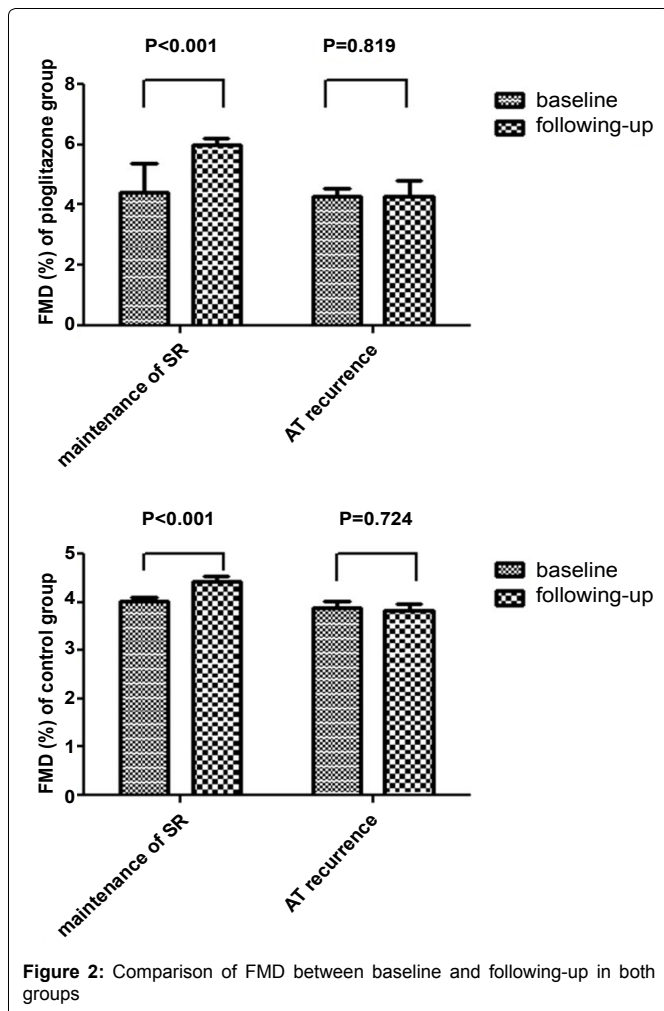
SR: Sinus Rhythm

Table 3: Endothelial function between baseline and following-up

	Pioglitazone				Control			
	SR		ATa		SR		ATa	
	Baseline	Following-up	Baseline	Following-up	Baseline	Following-up	Baseline	Following-up
FMD (%)	4.4 ± 1.0	6.0 ± 0.2	4.3 ± 0.3	4.3 ± 0.5	4.0 ± 0.1	4.4 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
eNOS (pg/ml)	18.4 ± 0.4	22.3 ± 0.7	18.2 ± 2.0	19.7 ± 1.5	18.6 ± 0.4	20.3 ± 0.4	19.0 ± 0.6	18.4 ± 0.5
EPC (cells/10 ⁵ events)	3.2 ± 0.1	4.0 ± 0.1	3.0 ± 0.3	3.0 ± 0.2	3.3 ± 0.1	3.6 ± 0.1	3.4 ± 0.1	3.1 ± 0.2

Values are reported as mean + SEM

SR: Sinus Rhythm, ATa: Atrial Tachyarrhythmias



maintaining vascular integrity and in injury repair. They are thought to contribute to reendothelialization, neovascularization, and normalization of endothelial function and restoration of blood flow at the site of injury [18]. It also appears to be widely accepted that eNOS with normal function maintains vascular homeostasis by producing nitric oxide (NO). In the present study, our results indicated that FMD, circulating EPCs as well as serum eNOS level were ameliorated in patients with PAF and T2DM after successful catheter ablation. Several mechanisms may be responsible for AF induced endothelial dysfunction. First of all, laminar shear stress plays a key role in the process of eNOS activation in normal physiology, the beat-to-beat variation of AF induces a turbulent flow that may weaken arterial vessel distension and responsiveness. Secondly, disorganized atrial contraction induced by AF, markedly reduces eNOS expression and

nitric oxide (NO) production in the atrial endocardium, which leads to endothelial dysfunction [19]. Finally, AF induces atrial inflammatory and oxidative stress that may generate a systemic noxious effect on endothelial cells. CRP, a biomarker of inflammation, directly inhibits endothelial cell NO production via destabilizing eNOS [20]. And ST-elevation myocardial infarction (STEMI) patient with a high C-reactive protein (CRP) peak value has compromised ability to stimulate eNOS-dependent NO production [21]. CRP may also serve to impair EPCs function and promote EPCs apoptosis through receptor for advanced glycation end products (RAGE) [22].

DM has been reported as one of the main risk factors for AF. Previous study identified that DM led to greater atrial arrhythmogenicity, longer intra-atrial activation time, and increased

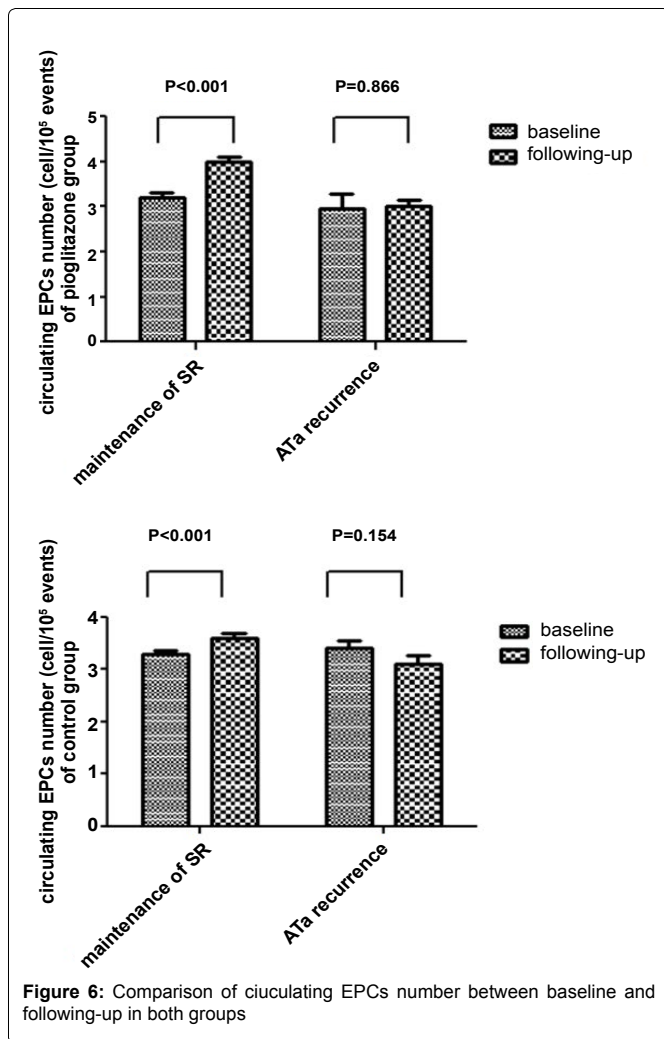


Figure 6: Comparison of circulating EPCs number between baseline and following-up in both groups

atrial fibrotic deposition in the animal model, which could be a major substrate for diabetes-related AF [23]. And patients with DM or an abnormal glucose metabolism had a lower atrial voltage and longer atrial activation time compared to those with a normal glucose metabolism [24]. Furthermore, endothelial dysfunction is greater in diabetes with AF [4]. There is a growing body of evidence elucidating the mechanisms underlying the development of endothelial dysfunction in T2DM. T2DM induced biochemical disturbances (dyslipoproteinemia, oxidative stress and inflammation) uncouple mitochondrial function in endothelial cells, generating reactive oxygen species (ROS) by increasing advanced glycation endproducts (AGEs), protein kinase C (PKC) and N-acetylglucosamine (glcNAC), impairing eNOS activity and inducing endothelial dysfunction [25].

There is increasing evidence that PPAR γ activation plays an important role in the regulation of the vascular endothelial function. Both troglitazone and pioglitazone increased brachial artery FMD in T2DM patients without macrovascular disease [26-28]. In line with previous study, we also found that pioglitazone improved the FMD in patients with PAF and T2DM in the present study. Besides, our results indicated that pioglitazone treatment could increase the quantity of circulating EPCs after catheter ablation. And a reduced quantity and function of EPCs have been related to endothelial dysfunction as well as increased risk cardiovascular morbidity and mortality [18]. Several studies indicates that AF as well as DM is associated with impaired quantity and function of EPCs [29,30]. Likewise, pioglitazone was also shown to increase the circulating number of EPCs in patients with coronary artery disease [9] as well as exert beneficial effects in vitro function of EPCs isolated from impaired glucose tolerance (IGT) subjects [10].

Regarding the favorable effect of pioglitazone on the FMD and circulating EPCs number in patient with PAF and T2DM after catheter ablation, its up-regulating effect on eNOS is supposed to be mentioned.

In the present study, serum eNOS level was obviously increased with pioglitazone treatment. Recent studies have also suggested a potential regulatory role of PPAR γ agonists on eNOS expression or activation in the vascular endothelium or EPCs [6,31,32]. PPAR γ activation by rosiglitazone stimulation attenuated the apoptosis and dysfunction of EPCs induced by AGEs via up-regulation of protein kinase B (PKB) and eNOS signaling [31]. Likewise, Pioglitazone induced PPAR γ activation up-regulated PKB and eNOS phosphorylation, resulting in the amelioration of vascular endothelial dysfunction and enhancement of blood flow recovery after tissue ischaemia in the diabetic mouse [32]. And pioglitazone abrogated the hypertensive effect of cyclosporine (CSA) via ameliorating detrimental changes in vascular eNOS/NO pathway caused by CSA [6]. Further study indicated that PPAR γ -mediated activation of eNOS might involve HSP90 and adiponectin signalling mechanism [7].

It was reported that successful reversion of AF and maintenance of SR by catheter ablation or electrical cardioversion would lead to a decrease endothelial dysfunction [14-17]. So more cases with successful maintenance of SR following ablation might result in better endothelial function in the pioglitazone group of the present study. Accordingly, we further analysed the difference of endothelial function in patients with successful maintaining SR between the two groups. And the result also showed that pioglitazone markedly improved the endothelial function compared with the control group, which suggested that pioglitazone might possess the ability to enhance the beneficial effect of successful catheter ablation on endothelial function.

In the present study, patient characteristics (Table 1) show that there is tendency towards lower hsCRP levels in the pioglitazone group. And pioglitazone treatment for a period of time before ablation in these patients might account for the discrepant tendency of hsCRP. Likewise, another study also demonstrated that pioglitazone attenuated inflammation in patients with IGT or T2DM in a glucose-lowering-independent manner [33].

Study Limitation

At first, the following-up of 12 months might be too short and a longer period might be necessary to show the effects of the drugs on endothelial function. Secondly, the effect of various drugs (ACEI/ARB and statins) on endothelial function could not be fully controlled in the present study, as all subjects were not on the same drug therapy regimen. However, there were no marked differences in baseline medication regimens between the 2 groups. Thirdly, due to the limited quantity and nonrandomization of our study, a much larger, randomized and multi-centred trial is required to testify our conclusions.

Conclusions

In summary, our data indicates that pioglitazone improves endothelial function in patients with PAF and T2DM after catheter ablation, and our findings may also provide a potential rationale for clinical PPAR γ agonists treatment in some particular conditions where the improvement of endothelial function should be taken into account.

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