Beneficial Effects of Pioglitazone on Retardation of Persistent Atrial Fibrillation Progression in Diabetes Mellitus Patients

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SUMMARY

This study aimed to explore the effects of pioglitazone treatment on progression from persistent atrial fibrillation (AF) to permanent atrial fibrillation in diabetes mellitus (DM) patients and to investigate the possible mechanisms involved in those effects.

A total of 146 diabetes mellitus (DM) patients with firstly identified persistent AF were selected. Seventy patients were randomized into the pioglitazone (30 mg/day) group and 76 into the placebo group. Pro-collagen type I carboxy-terminal peptide (PICP), advanced glycation end products (AGEs), and angiotensin II were assayed and left atrial diameter (LA diameter) was measured at the first presence of persistent AF, and at 6 and 14 months of follow-up. The time point of identification of permanent AF and the incidence of permanent AF in the patients were all recorded.

Thirty-seven (49%) of the 76 patients in the placebo group and 21 (30%) of the 70 patients in the pioglitazone group progressed to permanent AF ($P = 0.028$). No significant differences existed in the follow-up time (20.5 ± 3.97 months for pioglitazone group versus 20.9 ± 4.14 months for placebo group) between the two groups ($P = 0.535$). In the pioglitazone group, no significant change was found in angiotensin II level. The PICP level did not change significantly at 6-months of follow-up, but decreased significantly at 14-months of follow-up ($P = 0.032$). The AGE ($P = 0.037$ at 6-month follow-up, $P < 0.035$ at 14-month follow-up) level was significantly lower at both 6 and 14-months of follow-up.

By lowering the PICP level, pioglitazone treatment may decrease the incidence of permanent AF in DM patients with persistent AF, which may be associated with the suppressing effect of pioglitazone on AGEs. (Int Heart J 2014; 55: 499-505)

Key words: Type I carboxy-terminal peptide, Left atrial diameter, Atrial dilation, Angiotensin II, Advanced glycation end products, Atrial fibrosis, Atrial structural remodeling, Cardioversion, Arrhythmia, Clinical trial

Many pathophysiologic mechanisms are involved in the progression from persistent to permanent atrial fibrillation (AF) in diabetes mellitus (DM) patients.¹⁾ Among them, atrial fibrosis has been proven to be an important mechanism. Fibrosis can induce atrial fractionated potentials and conduction delay by accelerating the formation of anchoring points for reentry circuits and the forward propagation of wavelets. Collagen fibers play an important role in atrial fibrosis and atrial structural remodeling.²⁾ During synthesis of the fibril forming collagen type I in atria, the serum pro-collagen type I carboxy-terminal peptide (PICP) is cleaved from the pro-collagen and can be detected in the blood. A previous study has linked serum concentrations of PICP to the prevalence or incidence of atrial fibrillation (AF).³⁾

By suppressing the production of collagen fibers and promoting their degradation, the newly developed upstream therapy (statin and renin-angiotensin system inhibitors) has become a promising strategy to prevent AF progression.⁴⁻⁶⁾ Nevertheless, the effects of upstream therapy are still controversial.

Some studies did not find any effects of upstream therapies by renin-angiotensin system inhibitors or statins on AF recurrence.⁷⁾

In clinical practice, pioglitazone has become a standard therapy for DM. By activating peroxisome proliferator-activated receptor-gamma (PPAR-gamma), pioglitazone acquires several pleiotropic properties such as inhibiting inflammatory and oxidant processes,⁸⁻¹²⁾ which may contribute to attenuating atrial fibrosis in the setting of AF. Therefore, it has attracted much attention as a new upstream therapy in recent studies on DM patients with AF. Gu, *et al* have indicated that the chance of success in catheter ablation can be significantly enhanced by pioglitazone in DM patients with paroxysmal AF.¹³⁾ However, whether the pioglitazone treatment is beneficial to persistent AF patients in AF progression has not yet been investigated.

Thus, in the present study, we attempted to further examine the controversial upstream therapy by exploring the effects of pioglitazone treatment on progression from persistent to

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permanent AF in DM patients and investigating the possible mechanisms and implications underlying these effects.

METHODS

Study population: This was a prospective, randomized, placebo-controlled, double-blind trial carried out in Beijing Friendship Hospital Affiliated to the Capital Medical University. Inclusion criteria were a history of DM and the first presence of persistent AF defined as AF lasting beyond 7 days or demanding electrical or chemical cardioversion for termination.¹⁴⁾ Exclusion criteria were liver or renal dysfunction; history of hyperthyroidism, pulmonary fibrosis, cirrhosis or scleroderma; current treatment with pioglitazone, an angiotensin converting enzyme inhibitor (ACEI), or angiotensin II receptor blocker (ARB); history of acute coronary syndrome (ACS); congestive heart failure; pregnancy; or history of catheter ablation. From February 2012 to July 2013, a total of 264 consecutive DM patients with firstly identified persistent AF were initially referred to our study. Among these 264 patients, 118 were excluded due to current treatment with ACEI/ARB (50 patients), fibrosis disease (18 patients) such as pulmonary fibrosis and cirrhosis, history of acute coronary syndrome (15 patients), and contraindications to pioglitazone (35 patients) including liver dysfunction, renal dysfunction, and congestive heart failure. According to a computer generated randomization list, 146 qualified patients were assigned to treatment with placebo or pioglitazone (30 mg/day) at the first presence of persistent AF. All pills were identical with the same color, size and taste to ensure blindness. During the follow-up, ACEI and ARB were not prescribed to patients. The physicians participating in this study were blinded to the randomized assignment of the patients. The institutional review board of our institution reviewed and approved the study protocol and informed consent forms.

Blood analysis: At the first presence of persistent AF and at 6 and 14-months follow-up, peripheral venous samples were obtained in the morning after overnight fasting. The serum was extracted from these samples and stored at -80°C. PICP level was measured by enzyme linked immunosorbent assay using commercial antisera (Jianchen Biological Institution, China) according to the manufacturers' protocol.¹⁵⁾ Angiotensin II was measured by radioimmunoassay¹⁶⁾ using a kit obtained from Abcam (HK) Ltd. Using Munch's method,¹⁷⁾ fluorescence was used to evaluate advance glycation end products (AGEs) which were expressed as arbitrary units (a.u.). All measures were assessed in duplicate.

Echocardiography analysis: A 2-dimensional echocardiogram was performed to evaluate left ventricular ejection fraction (LVEF) and left atrial diameter (LA diameter) at the first presence of persistent AF, and at 6 and 14-months of follow-up. The analysis was carried out by 2 observers without any knowledge of the clinical data.

Follow-up: Patients were allowed to leave the hospital when sinus rhythm (SR) was sustained for 2 days. All patients were checked every other month thereafter. At each visit, blood pressure, a resting 12-lead ECG, and history of arrhythmia-related symptoms were recorded, and 24-hour Holter monitoring was carried out every month to detect asymptomatic AF. ECG and arrhythmia specialists were always available when any episode of palpitation occurred in our patients. After the confir-

mation of recurrent AF by 12-lead ECG, a continuous infusion of amiodarone was introduced until stable SR was obtained. Electrical cardioversion was conducted when unstable haemodynamics emerged or amiodarone was unable to convert the AF. When stable SR could be maintained in the follow-up, the amiodarone was discontinued. Failed attempts of electrical cardioversion and a medical rhythm control approach to restore sinus rhythm were considered as progression to permanent AF.^{14,18)}

Endpoints: The primary endpoint of this study was the incidence of progression to permanent AF. The secondary endpoint was to identify the significantly changed variables and the correlation of such changes with the incidence of permanent AF.

Statistical analysis: The estimated sample size was based on our estimate of the primary endpoint difference that the incidence of permanent AF is to be lower by 25% in the pioglitazone group than in the placebo group.¹³⁾ With a significance level of 0.05, a total of at least 140 randomized patients (70 in each arm) provided the study with 85% power. Continuous variables are expressed as the mean $\pm$ SD, and categorical variables as a percentage. Continuous variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test. Differences in paired variables between baseline, and 6 and 14-months of follow-up were compared with the paired-*t* test. The independent-samples *t* test was used to compare continuous variables between the pioglitazone and placebo groups; otherwise, the chi-square test was applied. Statistical significance was assumed at $P < 0.05$. Pearson correlation coefficients were used to evaluate the correlation of atrial fibrosis with various parameters. The time to permanent AF was analyzed with Kaplan-Meier statistics and compared with the log-rank test.

RESULTS

Study population: Table I presents the baseline clinical variables of the study population. No differences were found in age, gender, medical therapy, or history of smoking, drinking, coronary artery disease, and valvular heart disease between the placebo and pioglitazone groups. There were also no differences between the study groups with respect to lipid level, HbA1c, systolic blood pressure (SBP), diastolic blood pressure (DBP), LVEF, LA diameter, or serum PICP, AGEs and angiotensin II levels. All patients were followed-up for at least 14 months (range, 14-30 months). The safety of pioglitazone was reliable in general. In our study, mild peripheral edema occurred in 6 patients from the pioglitazone group and in 3 patients from the control group ($P = 0.312$). No acute heart failure was found in any patient.

Primary endpoint: The number of patients that suffered from recurrent AF was 48 (63.2%) in the placebo and 31 (44.3%) in the pioglitazone group ($P = 0.030$). There were 6 AF episodes detected by Holter monitoring in the placebo group and 4 in the pioglitazone group. The primary outcome of progression to permanent AF occurred in 37 of the 76 placebo group patients (49%) and in 21 of the 70 pioglitazone group patients (30%) ($P = 0.028$). No significant difference existed in the follow-up time (20.5 ± 3.97 months for pioglitazone group versus 20.9 ± 4.14 months for placebo group) between the two groups ($P =$

Table I. Clinical Characteristics

	Placebo (n = 76)	Pioglitazone (n = 70)	P
Male gender	50 (65.8%)	52 (74.3%)	0.284
Age (years)	62.25 ± 12.39	60.70 ± 12.25	0.449
Hypertension	23 (30.3%)	20 (28.6%)	0.859
Current smokers	42 (55.3%)	41 (58.6%)	0.739
Alcoholic history	16 (21.1%)	19 (27.1%)	0.441
CAD	23 (30.3%)	20 (28.6%)	0.857
VHD	7 (9.2%)	5 (7.1%)	0.767
CHOL (mmol/L)	5.44 ± 1.61	5.30 ± 0.97	0.541
TG (mmol/L)	2.24 ± 1.77	2.42 ± 1.65	0.615
LDL-C (mmol/L)	2.69 ± 0.73	2.72 ± 0.46	0.706
HDL-C (mmol/L)	1.08 ± 0.38	1.10 ± 0.24	0.732
Amiodarone	15 (19.7%)	13 (17.1%)	0.831
Beta-blockers	29 (38.2%)	29 (41.4%)	0.736
CCB	13 (17.1%)	14 (20%)	0.675
Propafenone	10 (13.2%)	6 (8.6%)	0.435
Statins	26 (34.2%)	22 (31.4%)	0.729
LVEF	0.57 ± 0.04	0.58 ± 0.04	0.494
HbA1c (%)	6.19 ± 1.45	6.41 ± 1.83	0.407
SBP	132 ± 19	131 ± 21	0.877
DBP	76 ± 10	77 ± 12	0.653

CHOL indicates cholesterol; TG, triglycerides; LDL-C, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; LVEF, left ventricular ejection fraction; CCB, calcium channel blocker; CAD, coronary artery disease; VHD, valvular heart disease; HbA1c, haemoglobin A1c; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

Figure 1. Kaplan-Meier permanent AF-free survival curve for pioglitazone and placebo groups. AF indicates atrial fibrillation.

0.535). Kaplan-Meier permanent AF-free survival analysis for 14 months follow-up is presented in Figure 1. Pioglitazone significantly decreased the incidence of permanent AF ($P = 0.031$).

Secondary endpoint: The patients that progressed to permanent AF during the 14 months of follow-up demonstrated a higher baseline LA diameter ($P < 0.01$), PICP ($P < 0.01$), AGE ($P < 0.01$), and angiotensin II ($P < 0.01$) levels than patients free from permanent AF (Figure 2). The lipid level, SBP, DBP, LVEF, and LA diameter did not change in either group. The HbA1c ($P = 0.031$) significantly decreased in the pioglitazone

Figure 2. Baseline mean serum PICP levels, serum AGEs levels, serum angiotensin II levels, and LA diameters in patients who did not develop permanent AF during follow-up (white bars) and in patients who developed permanent AF during follow-up (black bars). AF indicates atrial fibrillation; LA diameter, left atrial diameter; PICP, pro-collagen type I carboxy-terminal peptide; and AGEs, advanced glycation end products.

Table II. Variables at Baseline and at Month 14

	Placebo group (<i>n</i> = 76)			Pioglitazone group (<i>n</i> = 70)		
	Month 0	Month 14	<i>P</i>	Month 0	Month 14	<i>P</i>
CHOL (mmol/L)	5.44 ± 1.61	5.41 ± 1.33	0.677	5.30 ± 0.97	5.33 ± 0.96	0.388
TG (mmol/L)	2.24 ± 1.77	2.19 ± 1.56	0.438	2.42 ± 1.65	2.41 ± 1.62	0.377
LDL-C (mmol/L)	2.69 ± 0.73	2.73 ± 0.84	0.356	2.72 ± 0.46	2.73 ± 0.47	0.686
HDL-C (mmol/L)	1.08 ± 0.38	1.06 ± 0.35	0.252	1.10 ± 0.24	1.08 ± 0.24	0.216
LVEF	0.57 ± 0.04	0.57 ± 0.04	0.862	0.58 ± 0.04	0.57 ± 0.05	0.429
HbA1c (%)	6.19 ± 1.45	6.21 ± 1.54	0.744	6.41 ± 1.83	6.07 ± 2.26	0.031*
SBP	132 ± 19	133 ± 18	0.612	131 ± 21	132 ± 17	0.750
DBP	76 ± 10	75 ± 12	0.447	77 ± 12	76 ± 12	0.431

CHOL indicates cholesterol; TG, triglycerides; LDL-C, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; LVEF, left ventricular ejection fraction; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; and DBP, diastolic blood pressure. * Difference is significant.

Figure 3. Baseline (white bars), 6 month (stripe bars), and 14 month (black bars) mean serum PICP levels, serum angiotensin II levels, LA diameters, and serum AGEs levels in patients randomized to the placebo-treatment group and in patients randomized to the pioglitazone-treatment group. AF indicates atrial fibrillation; LA diameter, left atrial diameter; PICP, pro-collagen type I carboxy-terminal peptide; and AGEs, advanced glycation end products. * Difference is significant.

group after 14 months (Table II). In the placebo group, the serum PICP, AGE and angiotensin II levels remained stable during the 14-month follow-up. In the pioglitazone group, no significant change was found in angiotensin II level. The PICP did not change significantly at 6-months of follow-up, but decreased significantly at 14-months of follow-up ($P = 0.032$). The AGE ($P = 0.037$ at 6-month follow-up, $P < 0.035$ at 14-month follow-up) level had decreased significantly at both

6 and 14 months of follow-up (Figure 3).

We examined the correlation of the change in PICP with the change in AGEs after 14-months of follow-up in the pioglitazone group and found that the change in PICP was significantly correlated with the change in AGEs ($r = 0.567$, $P < 0.01$). The correlation of the change in AGEs with the change in HbA1c was also calculated. The results showed that the change in AGEs was positively correlated with the change in

Figure 4. Correlation of the change in AGEs with the changes in HbA1c and PICP after 14-months of follow-up. PICP indicates pro-collagen type I carboxy-terminal peptide; AGEs, advanced glycation end products; and HbA1c, Haemoglobin A1c.

HbA1c ($r = 0.688$, $P < 0.01$) (Figure 4).

DISCUSSION

Atrial fibrosis has been observed in AF patients and proved to be an important mechanism for AF progression in experimental models.¹⁹⁻²² It has been found to expand with age in animal models and humans.^{23,24} Although many mechanisms such as a reduction of conduction velocity, heterogeneity of the conduction tissue, and reentrant mechanisms have been proposed for AF,²⁵⁻²⁷ the mechanism of AF induced by atrial fibrosis is still under debate. Microscopic cardiac biopsy is the most reliable method to measure the extent of atrial fibrosis, but it cannot be applied on a wide scale because of its invasive nature. Therefore, some noninvasive serum markers such as PICP have gradually gained favor in clinical studies to assess atrial fibrosis.²⁸⁻³⁰ Type I collagen is the major fibrillar collagen in atria.³¹⁻³³ During its synthesis, PICP is separated from procollagen I and released into blood. By measuring PICP, we may indirectly evaluate the extent of atrial collagen synthesis.³⁴ Some studies have shown that the serum PICP level and LA diameter might indirectly demonstrate the extent of left atrial fibrosis and predict post-operative AF even in patients without a history of AF.³ Our results confirmed these findings by demonstrating a higher baseline PICP level and LA diameter in patients that progressed to permanent AF.

AGEs are crystallized proteins which accumulate with aging.³⁵ Non-enzymatic reactions between proteins and sugar residues produce them, and the process is markedly accelerated in the setting of DM.³⁶ AGEs can directly improve protein cross-linking including the synthesis of type I collagen and elastin in the extracellular matrix. In addition, when AGEs interact with AGE receptors (RAGE), the production of matrix metalloproteinases (MMPs) and tissue growth factor-beta were stimulated.³⁷⁻³⁹ All of these actions together lead to atrial fibrosis, resulting in multiple wavelets and reentrant anchor points in atria. A recent clinical study also found that the serum AGE level was higher in AF patients with or without diabetes mellitus.⁴⁰ Another important factor involved in atrial fibrosis is angiotensin II. Some studies have indicated that angiotensin II played an important role in atrial fibrosis in experimental models.⁴¹ By promoting tissue growth factor expression, fibroblast proliferation, trans-differentiation, and the production of colla-

gen I and angiotensin II significantly facilitated the development and progression of atrial fibrosis.^{42,43} It was also demonstrated that blockade of angiotensin II with an ACEI and/or ARB retarded the progression of atrial fibrosis and AF.^{44,45} In present study, we observed higher baseline angiotensin II and AGE levels in patients who had progressed to permanent AF. Furthermore, in the pioglitazone group, a significantly decreased AGE level was identified at 6-months of follow-up, and this occurred prior to the significant elevation of PICP level. Moreover, the change in PICP after 14-months of follow-up was positively correlated with the change in AGEs. All of the findings in our study further confirmed the pathophysiologic roles that angiotensin II and AGEs may play in atrial fibrosis.

Some studies have indicated that pioglitazone prevented age-related atrial structure remodeling in rat models⁴⁶ and attenuated atria fibrosis in rabbits with congestive heart failure.⁴⁷ Consistently, our present study observed significantly decreased PICP levels in the pioglitazone group at 14-months of follow-up. However, no significant change in LA diameter was observed in our study. We speculate that the left atrial dilation caused by atrial fibrosis is a lengthy process and can not be detected during 14 months of follow-up. The mechanisms underlying the inhibitory effects of pioglitazone on atrial fibrosis are uncertain. Anti-inflammation,⁴⁸ anti-oxidation⁴⁹ and inhibiting angiotensin II, AGEs and TGF cascade,⁵⁰ were all proposed as possible mechanisms of this process. Among them, our study mainly focused on two possible therapeutic targets revealed in recent experimental studies of pioglitazone: angiotensin II and AGEs.

A growing body of evidence suggests that pioglitazone might modulate AGEs-AGE receptor (RAGE) axis by inhibiting the effects of AGEs, increasing soluble RAGE, and down-regulating RAGE expression. In this regard, the adverse effects of AGEs on the pancreatic beta cell can be inhibited by pioglitazone.⁵¹ Another study indicated that pioglitazone significantly increased the soluble RAGE level in patients with DM.⁵² Furthermore, oral pioglitazone treatment in hypercholesterolemic rabbits caused significant alleviation of aortic valve calcification by targeted reduction of RAGE activation.⁵³ Nevertheless, the effect of pioglitazone on serum AGE levels has seldom been investigated in a clinical study. By showing a significantly decreased AGE level in the pioglitazone group at 6 and 14-months of follow-up, our study results indicated that pioglitazone may suppress serum AGEs in DM patients with

AF. We also found that the significant decrease in AGEs was positively correlated with the decrease in HbA1c in the pioglitazone group, implying that the antidiabetic effects of pioglitazone may be associated with its inhibition of AGE levels.

Many clinical and experimental studies have reported that pioglitazone inhibited the effect and decreased the level of angiotensin II. Saiki, *et al*⁵⁴⁾ showed that pioglitazone treatment for 16 weeks resulted in a decrease in plasma angiotensin II in DM patients. Subramanian, *et al*⁵⁵⁾ found that pioglitazone retarded angiotensin II-related atherosclerosis progression by activating the PPAR- γ receptors in smooth muscle cells. Furthermore, pioglitazone suppressed angiotensin II-induced atrial fibrosis in an experimental model.⁵⁶⁾ However, in our clinical study, we did not find that the angiotensin II level was significantly decreased in the pioglitazone group. We speculate that the volatility of the angiotensin II level in DM patients, which was influenced by many factors that were not fully assessed in our study such as blood pressure, serum sodium concentration, blood volume and autonomic nervous system, may account for the inconsistency with previous studies.

A recent cohort study indicated that pioglitazone prevented DM patients from incipient AF,⁵⁷⁾ and Gu, *et al* reported that pioglitazone (30 mg/day) decreased the incidence of recurrent AF and reablation in AF patients who underwent ablation.¹³⁾ Similar to these observations, we found that the pioglitazone group demonstrated a significantly lower incidence of recurrent AF, and more importantly, less recurrent AF progressed to permanent AF in the pioglitazone group compared with the placebo group. These findings, together with the suppressing effect of pioglitazone on AGEs and the close correlation between atrial fibrosis and AGEs observed in our study, suggest that the beneficial effects of pioglitazone on retardation of persistent AF progression in DM patients is associated with the inhibitory effects of pioglitazone on serum AGE levels.

Study limitations: First, although accelerated by AF and DM, atrial fibrosis is still a lengthy process. Therefore, the 14 months of follow-up might be too short to show the effects of pioglitazone on atrial structure remodeling such as left atrial dilation. Second, AGEs contain not only fluorescent AGEs but also non-fluorescent AGEs. Since non-fluorescent AGEs were not assayed in the present study, the association between non-fluorescent AGEs and atrial fibrosis was not investigated. Third, as the study enrolled a small number of patients from a single center, a larger randomized trial conducted in multiple centers is needed to consolidate our findings. Finally, pioglitazone possesses several pleiotropic properties, and many other mechanisms of pioglitazone for preventing AF progression such as anti-inflammation were not investigated in our study.

Conclusion: In conclusion, this study shows pioglitazone treatment may decrease the incidence of permanent AF in DM patients with persistent AF by lowering PICP levels, which may be correlated with the inhibitory effects of pioglitazone on AGEs. These findings may expand the AF prevention mechanisms of pioglitazone and improve the outcome of DM patients with persistent AF.

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