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Febuxostat attenuates paroxysmal atrial fibrillation-induced regional endothelial dysfunction

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ABSTRACT

Background: Paroxysmal atrial fibrillation (PAF) can increase thrombogenesis risk, especially in the left atrium (LA). The exact mechanism is still unclear.
Objective: We assessed the effects of PAF on endothelial function, and investigated if febuxostat (FX) can attenuate endothelial dysfunction by inhibition of xanthine oxidase (XO).
Materials and methods: Eighteen male New Zealand white rabbits were divided randomly into sham-operated (S), PAF (P) or FX + pacing (FP) groups. Group P and group FP received rapid atrial pacing (RAP). Group FP was administered febuxostat (FX) for 7 days before RAP. Post-procedure, blood samples were collected from the LA, right atrium (RA) and peripheral circulation. Tissues from the LA and RA were obtained. Endothelial dysfunction (thrombomodulin [TM], von Willebrand factor [VWF], asymmetric dimethylarginine [ADMA]), and indirect thrombin generation (thrombin-antithrombin complex [TAT], prothrombin fragment 1 + 2 [F1.2]) and oxidative stress in atrial tissue (xanthine oxidase [XO], superoxide dismutase [SOD], malondialdehyde [MDA]) were measured using an Enzyme-linked immunosorbent assay. Atrial endothelial expression of TM and VWF was measured by histology/western blotting.
Results and conclusions: Endothelial dysfunction (TM, VWF, ADMA), TAT generation and oxidative stress (XO, SOD, MDA) in group P were more significant compared with that in group S ($p < 0.05$, respectively). In group P, all of these changes occurred to a greater extent in the LA compared with those in the RA or peripheral circulation. In group FP, FX attenuated endothelial dysfunction and reduced TAT levels by inhibition of XO-mediated oxidative stress.
PAF can lead to endothelial dysfunction and TAT generation by XO-mediated oxidative stress. The LA is more susceptible to these effects. FX can attenuate these changes by inhibition XO and XO-mediated oxidative stress.

1. Introduction

Atrial fibrillation (AF) is the most common type of arrhythmia. Thrombogenesis in the left atrium (LA) confers a fivefold increase in stroke risk [1]. The mechanism of thrombogenesis is characterized by Virchow's triad including endothelial dysfunction [2]. Specific reasons underlying endothelial dysfunction during AF, however, remain poorly understood. Investigation of endothelial dysfunction during AF is important to show AF-related thrombogenesis.

Studies have focused mainly focused on relationship between chronic AF and endothelial dysfunction. However, paroxysmal atrial fibrillation (PAF) with a lower burden also increases the risk of thrombogenesis and silent stroke [3,4]. The impact of short-term PAF on endothelial dysfunction has not been studied in detail.

Some researchers have noted hypercoagulability in the LA compared with the right atrium (RA) and in the peripheral circulation during AF. These findings suggest the importance of local contributory factors to thrombogenesis in the LA [5,6]. Whether there are regional differences between the LA, RA and peripheral circulation with regard to extent of endothelial dysfunction is not known.

Xanthine oxidase (XO) plays an important part in oxidative stress, which participates in AF and AF-related thrombogenesis [7–12]. Endothelial dysfunction is critical for thrombogenesis. Some antioxidants can attenuate endothelial dysfunction [13]. While, the role of XO in AF-related endothelial dysfunction has not been studied.

We hypothesize that acute PAF results in local endothelial dysfunction through XO-mediated oxidative stress. Furthermore, we investigated the effects of a specific inhibitor of XO, febuxostat(FX), on endothelial dysfunction in PAF.

2. Material and methods

2.1. Ethics statement

This experimental protocol was approved by the Animal Care and Use Committee of the Chinese PLA General Hospital. All procedures

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were performed in compliance with the National Institutes of Health Guide for The Care and Use of Laboratory Animals published by National Institutes of Health in 1996.

2.2. Experimental animals

Eighteen male New Zealand white rabbits (weighting 2.5–3.5 kg) were purchased from the Experimental Animal Department of the Chinese PLA General Hospital. The rabbits were randomly divided into 3 groups: FX + pacing (FP, $n = 6$), Pacing (P, $n = 6$) or Sham-operated (S, $n = 6$). Prior to operation, rabbits in group FP were administered with FX(10 mg/kg/d; Wanbang Biochemical Pharmaceutical Co., Jiangsu, China) by gavage once a day for 1 week, in order to obtain a steady blood concentration [12]. Rabbits in other groups were administered with equal amount of saline once a day for 1 week. Whereafter, rabbits in group P and FP received rapid atrial pacing (RAP) to simulate PAF just as previously described [11]. Briefly, rabbits were anesthetized with pentobarbital sodium (30 mg/kg, iv), intubated, and ventilated with a ventilator (40 cycles/min, 20 ml/kg). Heart rhythms were monitored using an electrocardiogram. Left thoracic cavity was opened at the third intercostal space and the heart was exposed by a dilator. The pericardium was opened gently. The distal quadripolar pacing electrode (Medtronic Inc., Minneapolis, MN, USA) was attached to the free wall of LA and connected to a programmed stimulator (DW08-DF-5A; Suzhou Dongfang Electronic Instruments Factory, Jiangsu, China). Atrial pacing was performed at 600 beats/min with a 2-ms rectangular pulse width and two times the diastolic threshold for 3 h. Rabbits in group S received identical operation except RAP.

2.3. Electrophysiology studies

Electrophysiology studies were performed before and 3 h after respective procedures to evaluate the impact of pacing on electrophysiological properties of the atrium of rabbits in above 3 groups. The quadripolar pacing electrode was firmly attached to the free wall of the LA. The Jinjiang multi-channel physiology recorder(LEAD-7000, Sichuan Jinjiang Electronic Science and Technology Co., Ltd., Sichuan, China)was used to deliver a 2-fold-threshold current with a pulse width of 2 ms. The atrial effective refractory period (AERP) was measured at the left atrial appendage with 200 ms basic cycle length, with a train of 8 basic stimuli (S1), followed by a premature extra stimulus (S2) in a 5-ms decay. The AERP was defined as the longest S1S2 interval that failed to induce the propagated atrial response [14]. The inducibility of AF was tested by burst pacing 10 times (3-fold threshold current, cycle length 60 ms, duration 10 s)using a stimulator (DF-5A; Suzhou Dongfang Electronic Instruments Plant, Jiangsu, China). AF was defined as a rapid (>500 bpm) irregular atrial rhythm that lasted for at least 1 s [15]. AF inducibility was defined as the percentage of successful induction of AF.

2.4. Samples harvesting

Blood was quickly drawn by a syringe (pre-filled with an appropriate amount of sodium citrate) from the LA, RA and jugular vein (2 ml from each site) after respective procedures. The blood was then centrifuged at 2500 × *g* for 15 min at 4 °C. The supernatant was separated and stored at −80 °C for batch analysis. The rabbits were then sacrificed by an intravenous overdose of sodium pentobarbital (0.5 g). The left and right atrial tissues were collected and rinsed with saline to remove the residual blood and immediately frozen in liquid nitrogen (tissue harvesting was completed within 30 s). Some parts of the tissues were fixed in 4% paraformaldehyde for 48 h and embedded in paraffin for subsequent histologic studies.

2.5. Enzyme-linked immunosorbent assay

Endothelial dysfunction was assessed by measuring the plasma levels of von Willebrand factor (VWF), thrombomodulin (TM) and asymmetric dimethylarginine (ADMA). Indirect thrombin generation was evaluated by measuring the plasma levels of thrombin-antithrombin (TAT) complex and prothrombin fragment 1 + 2 (F1.2). Oxidative stress was examined by measuring the tissue malondialdehyde (MDA) levels, XO and superoxide dismutase (SOD) activity. Enzyme-linked immunosorbent assay (ELISA) using the rabbit ELISA kit (Shanghai Elisa Biotech Co., Ltd. Shanghai, China) was used to detect their levels. All experiments were performed in duplicate or triplicate.

2.6. Histological analysis

Cut the transverse sections (3 μm) of paraffin-embedded tissues from the LA and RA. And the atrial tissues were stained by antibodies of VWF (Abcam, ab778) or TM (Abcam, ab6980).

2.7. Western blotting

Expression of the VWF in the atrial endocardium was evaluated by western blotting. Protein concentrations were determined by the bicinchoninic acid (BCA) assay. Fifty micrograms of the total protein was solubilized in a volume-loading buffer (1% SDS, 30% glycerol, 0.8 M DTT, 1 mM Tris-HCl, pH 6.8, 2% bromophenol-blue) at 95 °C for 5 min. The mixture was then load onto a 10% or 5% polyacrylamide gel. The gel was then incubated with a primary antibody (ab778, Abcam) of VWF, after which the secondary antibody (anti-mouse) was added. The temperature was maintained at 37 °C for 2 h. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the loading control.

2.8. Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0 software (SPSS, Chicago, IL, USA). Values were presented as mean ± SD and categorical variables were expressed as percentages. Normality testing of the date was performed. One-way analysis of variance (ANOVA) was used to compare the endothelial dysfunction, thrombin generation and oxidative stress among the 3 groups. The differences between oxidative stress in the LA and RA of rabbits from group P were compared by paired *t*-test. Differences in endothelial dysfunction and thrombin generation among the LA, RA and peripheral circulation were analyzed by one-way ANOVA. Repeated measures ANOVA was used to compare AF inducibility and AERP before and after stimulation. The chi-square test was used to compare the rate of induction of AF. $P < 0.05$ was considered statistically significant.

3. Results

3.1. AERP and inducibility of AF

The electrocardiograms of a rabbit during PAF and electrophysiology studies are shown in Fig. 1. PAF effects on the AERP and the inducibility of AF are shown in Fig. 2. The sham operation had no effect of the AERP or the inducibility of AF (Fig. 2A and D). PAF reduced the AERP in group P by 12% ($P < 0.01$; Fig. 2B) and in group FP by 10% ($P < 0.01$; Fig. 2C). PAF increased AF inducibility by 2.4-fold in group P ($P < 0.01$; Fig. 2D) and 3.3-fold in group FP ($P < 0.01$; Fig. 2D).

3.2. PAF effects on endothelial dysfunction

Effects of PAF on endothelial dysfunction in the LA are shown in Fig. 3. PAF increased levels of TM ($P < 0.01$; Fig. 3A), VWF ($P < 0.01$; Fig. 3B), ADMA ($P < 0.01$; Fig. 3C), TAT ($P < 0.01$; Fig. 3D) significantly in group

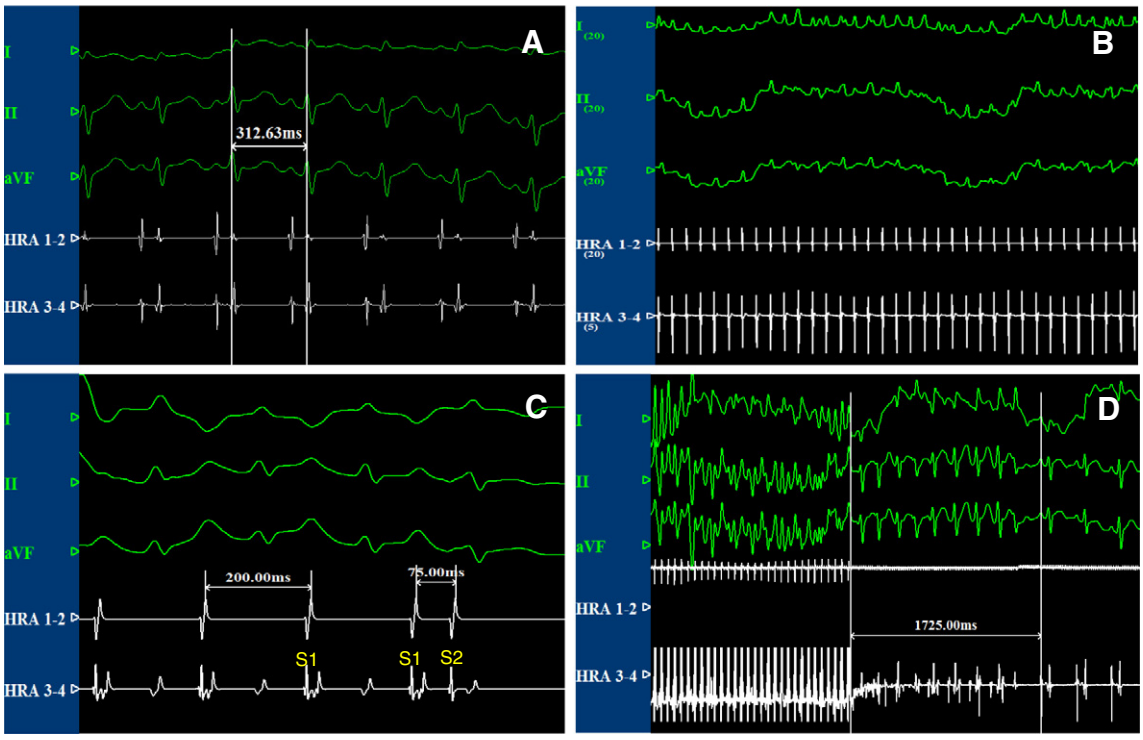

Fig. 1. Electrocardiograms of rabbit. Potential of limb leads and left atrium under normal state (A); Potential of limb leads and left atrium during rapid atrial pacing at 600 bpm (B); AERP measurement: S2 (stimulate interval = 75 ms) was unable to induce propagated atrial response (C); Atrial fibrillation induced by rapid atrial pacing in 1000 bpm (D).

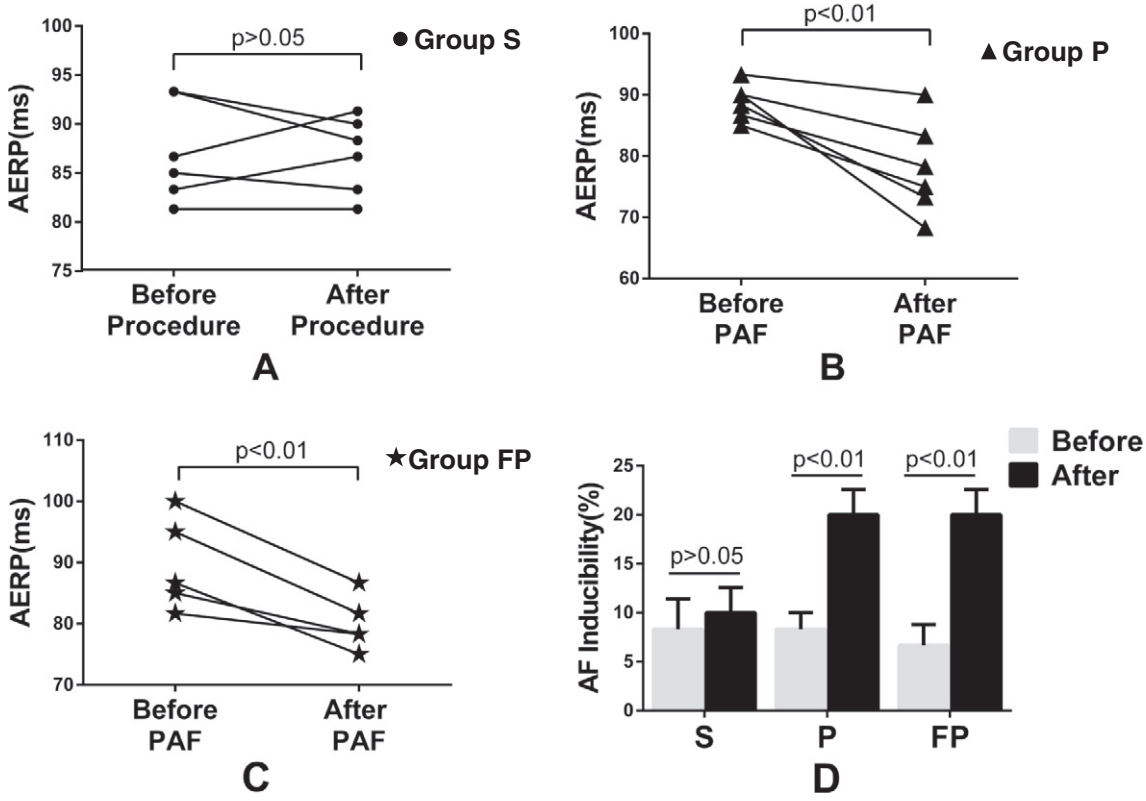

Fig. 2. Effects of PAF or sham operation on the atrial effective response period (AERP) and inducibility of AF. Sham procedure did not affect AERP (A) and AF inducibility (D) in group S. PAF decreased the AERP in group P (B) and group FP (C), while it increased the inducibility of AF in group P and group FP (D). Group S = group Sham operated; Group P = group pacing; Group FP = group FX + pacing; PAF = paroxysmal atrial fibrillation; AF = atrial fibrillation.

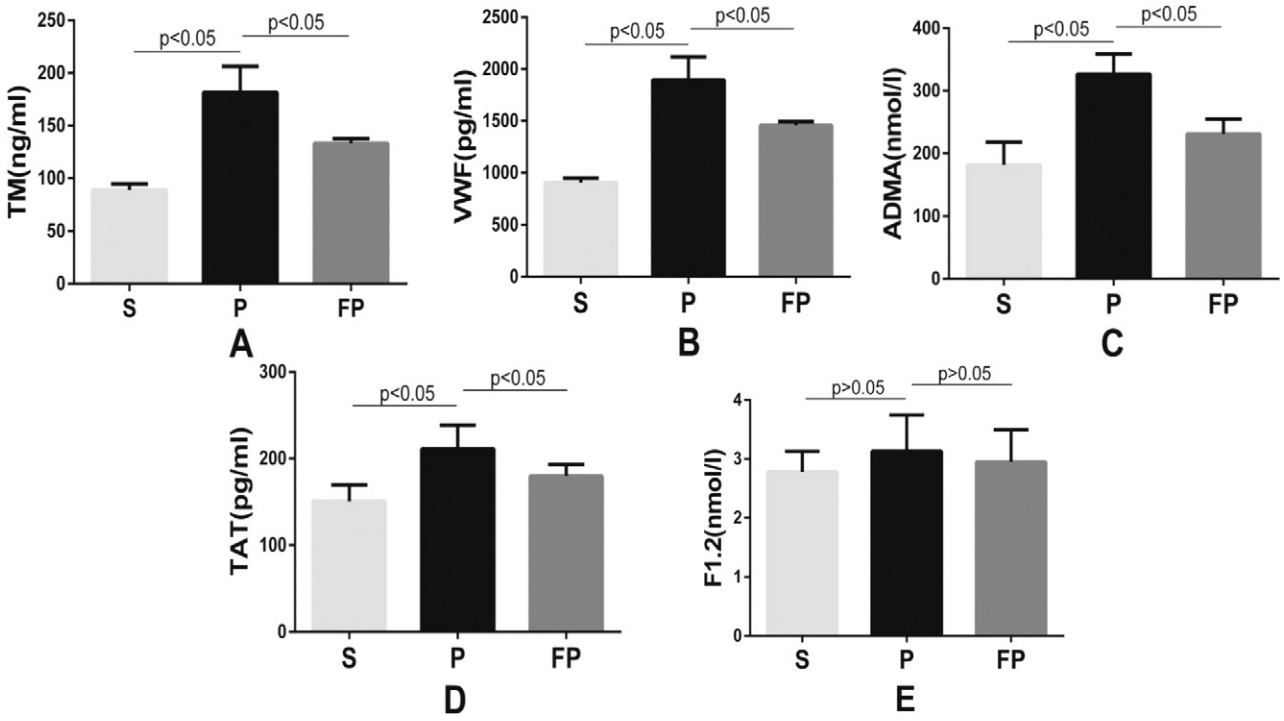

Fig. 3. Effects of PAF and febuxostat (FX) on endothelial dysfunction. PAF increased the plasma levels of TM (A), VWF (B), ADMA (C), TAT (D); FX significantly attenuated these changes in group FP. Level of *F*1.2 failed to show such changes. S = group Sham operated; P = group Pacing; FP = group FX + pacing; TM = thrombomodulin, VWF = von Willebrand factor, ADMA = asymmetric dimethylarginine, TAT = thrombin-antithrombin complex, F1.2 = prothrombin fragment; the other abbreviations are as in Fig. 2.

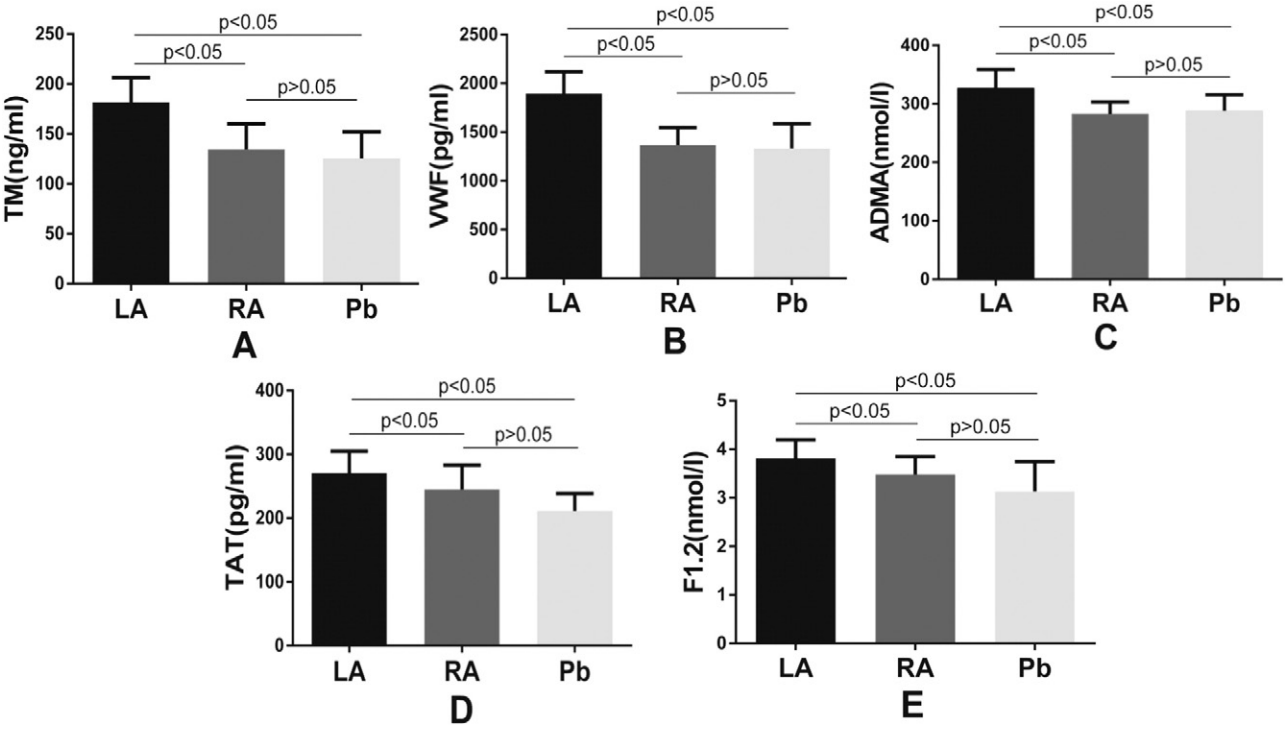

Fig. 4. Local differences of endothelial dysfunction among rabbits in group P. The plasma levels of TM, VWF, ADMA, F1.2 and TAT in the LA were higher than the levels in RA and Pb(A, B, C, D). No significant differences were observed between the levels in the RA and Pb(E). LA = left atrium, RA = right atrium, Pb = peripheral blood; other abbreviations are as in Fig. 3.

P. FX administration attenuated these changes ($P < 0.05$ for all). Level of *F*1.2 failed to show such changes ($P < 0.01$; Fig. 3E).

3.3. Local differences in endothelial dysfunction

In group P, blood in the LA showed higher levels of TM, VWF, ADMA, TAT and F1.2 compared with blood in the RA or blood from the peripheral circulation ($P < 0.05$ for all; Fig. 4). There were no significant differences in levels of TM, VWF, ADMA, TAT or *F*1.2 between blood in the RA or peripheral blood ($P > 0.05$; Fig. 4).

3.4. Immunostaining of TM and VWF in the atrial endothelium

Immunostaining of TM and VWF in the endothelium of the LA and RA is shown in Figs. 5 and 6. The proteins of TM and VWF were stained predominantly in endocardial cells. In group S, staining results for TM and VWF were comparable between the endothelium of the LA and RA (Figs. 5A and B, 6A and B). PAF increased the staining of VWF (Fig. 5C and D) but decreased the staining of TM (Fig. 6C and D); the extents of changes was greater in the LA (Figs. 5C and 6C). However, these changes could be attenuated by FX administration (Figs. 5E and F, 6E and F).

3.5. Western blotting of VWF

Protein expressions of VWF increased in group P (especially in the LA) (Fig. 7). After FX treatment, protein expression of VWF in the LA and RA was decreased dramatically.

3.6. Oxidative stress

Effects of PAF on oxidative stress in tissues are shown in Fig. 7. PAF increased XO activity (Fig. 8A) and MDA levels (Fig. 8B)significantly (especially in the LA) ($P < 0.05$ for all). FX administration could inhibit XO activity (Fig. 8A) and decrease MDA levels ($P < 0.05$ for all; Fig. 8B) significantly. Significant differences in SOD activity among the three groups were not observed (Fig. 8C).

4. Discussion

The present study provides new information on the relationship between oxidative stress and indirect thrombin generation during AF. These results demonstrates that: (i) short-term PAF is associated with increased endothelial dysfunction, TAT generation and oxidative stress; (ii) FX can suppress XO activity and oxidative stress, which attenuates AF-related endothelial dysfunction and TAT generation; (iii) endothelial dysfunction and TAT generation occurs to a much greater extent in the LA compared with that in the RA and peripheral blood; (iv) oxidative stress is more significant in the LA than in the RA during AF.

4.1. The PAF model

We established a PAF model by rapid pacing of LA. It has been demonstrated that RAP can induce electrophysiology remodeling similar to that seen during AF [16]. In present study, RAP shortened AERP and increased the inducibility of AF significantly. Therefore, the present model could well simulate the clinical feature of PAF.

In our study, the PAF model was established through thoracotomy rather than by intervention, which avoids catheter-related thrombogenesis. Moreover, the LA has a shorter AERP than that in the RA, which enables the LA to respond to a higher pacing rate during AF induction.

4.2. Endothelial dysfunction

The mechanism of thrombogenesis in the LA during AF is complex and known commonly as Virchow's triad, including endothelial dysfunction [2]. VWF, TM and ADMA are well-established biomarkers of endothelial dysfunction [17,18].

VWF is released into the circulation upon stimulation of endothelial cells by oxidative stress. High levels of VWF in blood are associated with

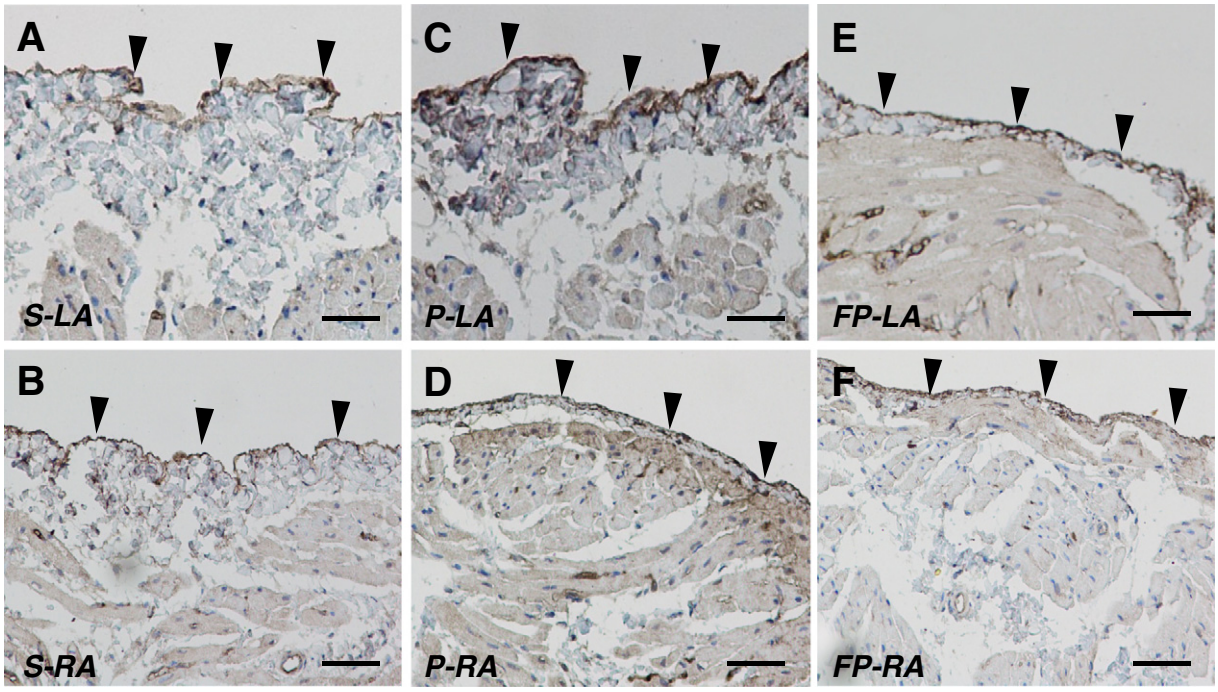

Fig. 5. Immunohistochemistry of VWF in the endothelium. VWF staining results were comparable in the endothelium of the LA (A) and RA (B) in group S. PAF increased VWF staining in the endocardium of the LA (C) and RA (D), especially in the LA (C). FX attenuated these changes of the LA (E) and RA (F). Bar = 50 μm. *S-LA* = left atrium of group Sham operated; *P-LA* = left atrium of group Pacing; *FP-LA* = left atrium of group FX + pacing; *S-RA* = right atrium of group Sham operated; *P-RA* = right atrium of group Pacing; *FP-RA* = right atrium of group FX + pacing; Abbreviations are as in Figs. 2, 3 and 4.

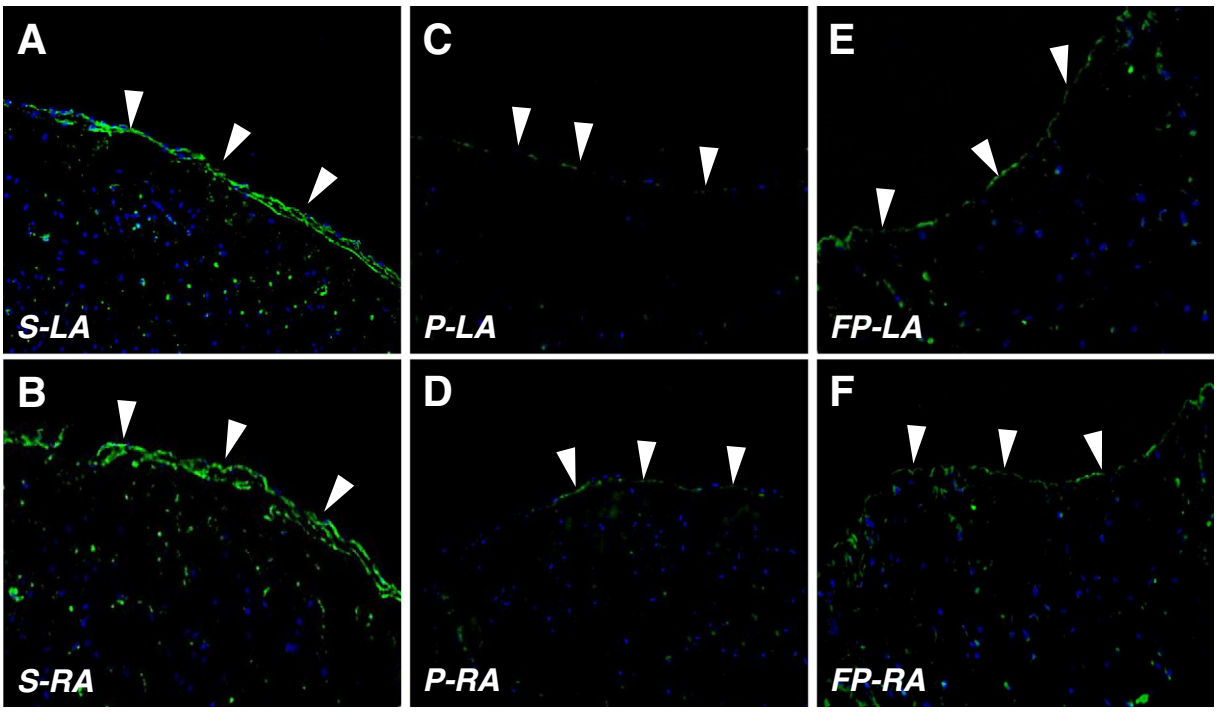

Fig. 6. Immunofluorescence of TM in the endothelium. TM straining results were comparable in the endothelium of the LA (A) and RA (B) in group S. PAF decreased TM staining in the endothelium of the LA (C) and RA (D), especially in the LA (C). FX attenuated these changes of the LA (E) and RA (F). Bar = 50 μm. *S-LA* = left atrium of group Sham operated; *P-LA* = left atrium of group Pacing; *FP-LA* = left atrium of group FX + pacing; *S-RA* = right atrium of group Sham operated; *P-RA* = right atrium of group Pacing; *FP-RA* = right atrium of group FX + pacing; Abbreviations are as in Figs. 2, 3 and 4.

stroke risk in AF [19]. TM is co-localized along with the protein C receptor on the surface of endothelial, and is an important anticoagulant factor. In the case of endothelial dysfunction, localized TM is shed from endothelial cells, leading to a weakened local barrier to anticoagulation. High blood levels of TM and reduced local TM expression could increase the risk of local thrombogenesis [18,20]. ADMA is an endogenous inhibitor of endothelial nitric oxide synthase. A higher level of ADMA is associated with several types of cardiovascular event (including stroke) [21].

We found that AF is associated with increased blood levels of VWF, TM and ADMA, results that are consistent with data from other studies [22,23]. Present results reproved that AF can induces significant endothelial dysfunction. Other studies didn't evaluate local endothelial changes. In our study, histology studies showed increased staining of VWF and decreased staining of TM in group P. These changes leads to impaired anticoagulation barrier and increased risk of thrombogenesis. Also, such changes occurred to a greater extent in the LA, which could explain why thrombogenesis was observed predominantly in the LA.

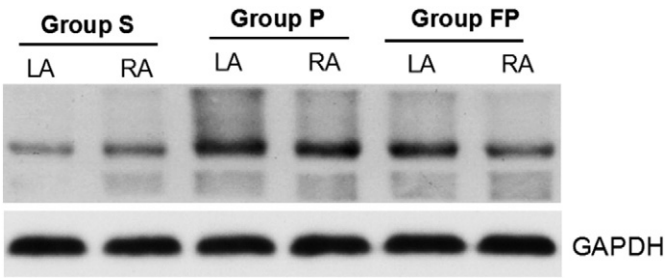

Fig. 7. Tissue expression of VWF in the endothelium of the LA and RA. Western blotting analysis showed increased VWF levels in group P, especially in the LA. FX treatment significantly reduced the upregulation of VWF in both the LA and RA. GAPDH = glyceraldehyde-3-phosphate dehydrogenase; Group S = group Sham operated; Group P = group Pacing; Group FP = group FX + pacing; other abbreviations are as in Figs. 2 and 3.

4.3. Generation of TAT and F1.2

Thrombin generation expressed as elevated TAT and F1.2 is the final stage of thrombogenesis. Previous studies have suggested that AF is associated with increased TAT and F1.2 [24]. And these changes are exactly related to thromboembolic events in AF patients [25]. Hence, intimate relationship exist between high levels of the two markers and AF-related thrombogenesis or thromboembolism. Researches related to AF-induced generation of TAT and F1.2 may illustrate the mechanism of thrombogenesis during AF. Moreover, investigating the generation of TAT and F1.2 regarding different sites can explain (at least in part) the tendency of thrombogenesis in the LA.

TAT and F1.2 are very important indirect biomarkers for assessment of thrombin generation. We obtained blood samples directly from the LA, RA and peripheral circulation, and found elevated level of TAT. These results are consistent with those of other studies, and demonstrate that the LA is more inclined to thrombin generation [10]. Such data explain (at least in part) the tendency of the LA towards thrombogenesis. However, no such changes regarding F1.2 were observed, which may result from the hyposensitivity of *F*1.2.

4.4. Oxidative stress

XO is an important component of oxidative stress. Accumulating evidences suggests that XO activation is associated with several cardiovascular diseases (including stroke and “silent infarction” of the brain) [26,27]. Whether XO activation is involved in AF-related thrombogenesis is not known.

We found increased XO activity and MDA level in the atria of rabbits in group P, which are conforming to local endothelial dysfunction and elevation of TAT. Hence, XO activation and XO-mediated oxidative stress may participate in AF-related endothelial dysfunction and thrombin generation.

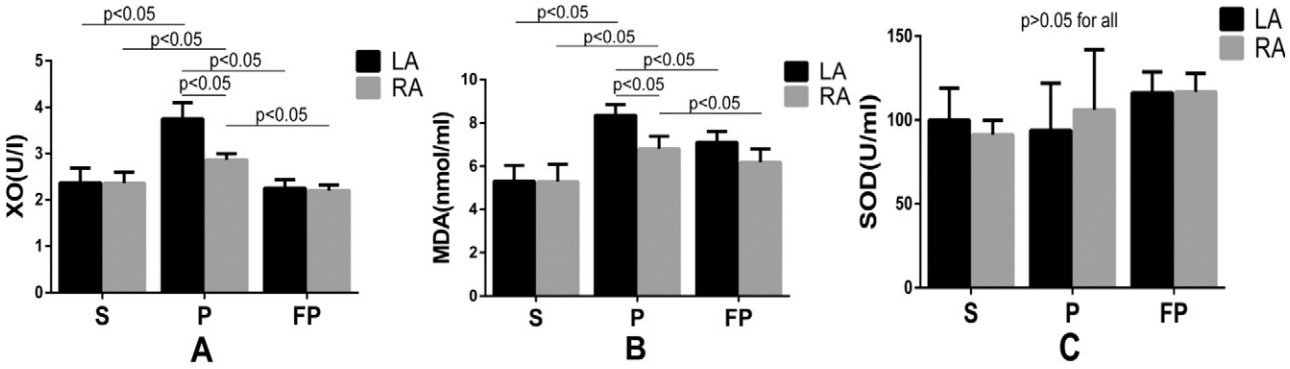

Fig. 8. Effects of PAF and FX on tissue oxidative stress. PAF increased xanthine oxidase activity (A) and methane dicarboxylic aldehyde levels (B) in atrial tissues, especially in the LA (A and B), while administration of FX alleviated these changes (A and B). No significant difference in superoxide dismutase (SOD) activity were observed among the 3 groups (C). Group S = group Sham operated; Group P = group Pacing; Group FP = group FX + pacing; Abbreviations are as in Figs. 3 and 4.

Noteworthy, divergences exist over this issue. In a porcine model of AF, XO activity in the LA was shown to be increased significantly [9]. Administration of the XO inhibitor oxypurinol reduced levels of superoxide anions [9]. However, in another study on AF patients, examination of RA specimens did not demonstrate significant activation of XO, and failed to support the role of XO in AF [28]. This discrepancy may have resulted from XO activity in different tissues. In the porcine model of AF, increased XO activity in the LA was apparent, whereas, in the study on AF patients, only RA tissues were examined. Therefore, some researchers have speculated that the LA is more sensitive to oxidative stress [29]. It has been suggested that oxidative stress can lead to the necrosis or apoptosis of endothelial cells [30]. The difference in the oxidative stress at different sites explains (at least in part) the greater extent of endothelial dysfunction and elevation of TAT and *F*1.2 in the LA.

In group P, we found significantly higher XO activities and MDA levels in the LA compared with those in the RA. These findings are in accordance with the notion of greater extent of endothelial dysfunction and elevation of TAT and F1.2 in LA. In addition, SOD activity was not affected by administration of AF and FX, hence, exclude the impact of SOD. Taken together, LA is more susceptible to oxidative stress, and XO-related oxidative stress may have an intimate relationship with PAF-related local endothelial dysfunction and elevation of TAT and *F*1.2.

4.5. Effects of FX

Previous studies have suggested that conventional XO inhibitors (allopurinol or oxypurinol) can attenuate endothelial dysfunction [31]. Endothelium-combined XO shows potent endothelial activity, may effect endothelial function. However, conventional XO inhibitors affect endothelium-associated XO only slightly [32]. FX is an investigational, potent XO inhibitor. It is more effective in inhibiting endothelium-combined XO compared with conventional XO inhibitors [32]. However, its role in AF-related endothelial dysfunction has not been studied. Dudley and colleagues found increased production of superoxide anions in the LA and left atrial appendage of AF pigs and XO contributed to increased production of superoxide anions [9]. They concluded that increased oxidative stress may contribute to some consequences of AF, such as thrombogenesis [9].

The XO activity observed in rabbits is similar to that of humans. FX administration (10 mg/kg body weight, p.o.) for 7 days before surgery can help to achieve a steady drug concentration in plasma and potentially suppress XO activity [11,12]. We showed, that FX administration suppressed PAF-related XO activation and reduced levels of MDA. Moreover, it alleviated local endothelial dysfunction (increased levels of TM, VWF and ADMA) and elevation of TAT and *F*1.2 induced by PAF. These results suggested that: (i) XO-mediated oxidative stress participates in endothelial dysfunction and elevation of TAT and *F*1.2 in PAF; (ii) FX can alleviate such changes by inhibition of XO.

4.6. Limitations

This study had some limitations. First, we established a PAF model through widely used approach, RAP, which cannot simulate the irregular atrial activation in clinical AF. Second, RAP through thoracotomy may induce inflammation and influence some parameters. Third, we did not regulate the ventricular rate to the normal level by suppression of atrioventricular conduction. Hence, the increased ventricular rate could have influenced some parameters (though it has been reported that rapid ventricular rate dose not affect endothelial function markers) [21]. Fourth, we did not obtain the results for TM using western blotting because of a lack of western blot-specific antibody. Finally, illustration of the exact relationship between oxidative stress and AF-induced endothelial dysfunction and thrombin generation need some molecular-biology studies (which will be done by our research team).

5. Conclusions

AF is the most common type of arrhythmia with increased stroke risk. Illustration of thrombogenesis mechanism can provide better strategy for stroke prevention. Present study explored relationship between PAF and endothelial dysfunction, provided possible approaches for endothelial protection. Four main conclusions can be drawn form the present study. First, PAF plays a important part in endothelial dysfunction. Second, the LA is more vulnerable to endothelial dysfunction, clotting activation and oxidative stress during PAF. Third, XO-mediated oxidative stress may participate in PAF-related endothelial dysfunction and thrombin generation. Fourth, FX can inhibit XO activation and oxidative stress during PAF, and attenuates PAF-induced local endothelial dysfunction and reduces TAT levels.

References

[1] P.A. Wolf, R.D. Abbott, W.B. Kannel, Atrial fibrillation as an independent risk factor for stroke: the Framingham study, Stroke 22 (1991) 983–988, http://dx.doi.org/10.1161/01.STR.22.8.983.
[2] T. Watson, E. Shantsila, G.Y. Lip, Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited, Lancet 373 (9658) (2009) 155–166, http://dx.doi.org/10.1016/S0140-6736(09)60040-4.
[3] M.P. Turakhia, P.D. Ziegler, S.K. Schmitt, Y. Chang, J. Fan, C.T. Than, et al., Atrial fibrillation burden and short-term risk of stroke: a case-crossover analysis of continuously recorded heart rhythm from cardiac electronic implanted devices, Circ. Arrhythm. Electrophysiol. 8 (2015) 1040–1047, http://dx.doi.org/10.1161/CIRCEP. 114.003057.
[4] K. Hahne, G. Monning, A. Samol, Atrial fibrillation and silent stroke: links, risks, and challenges, Vasc. Health Risk Manag. 12 (2016) (2016) 65–74, http://dx.doi.org/10.2147/VHRM.S81807.
[5] G.Y. Lip, J. Zarifis, Coagulation activity is increased in the left atrium of patients with mitral stenosis, JACC 26 (1995) 302–303.
[6] S.R. Willoughby, R.L. Roberts-Thomson, H.S. Lim, C. Schultz, A. Prabhu, D. Sciscio, et al., Atrial platelet reactivity in patients with atrial fibrillation, Heart Rhythm. 7 (2010) 1178–1183, http://dx.doi.org/10.1016/j.hrthm.2010.01.042.

[7] J.H. Wu, R. Marchioli, M.G. Silletta, S. Masson, F.W. Sellke, P. Libby, et al., Oxidative stress biomarkers and incidence of postoperative atrial fibrillation in the omega-3 fatty acids for prevention of postoperative atrial fibrillation(OPERA) trial, J. Am. Heart Assoc. (2015) 4, http://dx.doi.org/10.1161/JAHA.115.001886 (pii:eoo1886).
[8] E.R. Lubbers, N.P. Murphy, P.J. Mohler, Defining the links between oxidative stress-Based biomarkers and postoperative atrial fibrillation, J. Am. Heart Assoc. (2015) 4, http://dx.doi.org/10.1161/JAHA. 115.002110 (pii:e002110).
[9] S.C. Dudley, N.E. Hoch, L.A. McCann, C. Honeycutt, L. Diamandopoulos, T. Fukai, et al., Atrial fibrillation increases production of superoxide by the left atrium and left atrial appendage: role of the NADPH and xanthine oxidases, Circulation 112 (2005) 1266–1273, http://dx.doi.org/10.1161/CIRCULATIONAHA.105.538108.
[10] M.A. Sagor, N. Tabassum, M.A. Potol, M.A. Alam, Xanthine oxidase inhibitor, allopurinol, prevented oxidative stress, fibrosis and myocardial damage in isoproterenol induced aged rats, Oxidative Med. Cell. Longev. 478039 (2015), http://dx.doi.org/10.1155/2015/478039.
[11] P. Korantzopoulos, K.P. Letsas, T. Liu, Xanthine oxidase and uric acid in atrial fibrillation, Front. Physiol. 3 (2012) 150, http://dx.doi.org/10.3389/fphys.2012.00150.
[12] R. Khosravan, B.A. Grabowski, J.T. Wu, N. Joseph-Ridge, L. Vernillet, Parmacokinetics, pharmacodynamics and safety of febuxostat, a non-purine selective inhibitor of xanthine oxidase, in a dose escalation study in healthy subjects, Clin. Pharmacokinet. 45 (2006) 821–841.
[13] P. Francesco, O. Elena, C. Sarah, et al., Deletion of the activated protein-1 transcription factor JunD induces oxidative stress and accelerates age-related endothelial dysfunction, Circulation 127 (11) (2013) 1229–1240, http://dx.doi.org/10.1161/CIRCULATIONAHA.112.00826.
[14] Z. Liang, X.M. Shi, L.F. Liu, X.P. Chen, et al., Renal denervation suppresses atrial fibrillation in a model of renal impairment[J], PLoS One 10 (2015), e0124123.
[15] T. Yamamoto, M. Shimano, Y. Inden, M. Takefuji, S. Yanagisawa, N. Yoshida, et al., Alogliptin, a dipeptidyl peptidase-4 inhibitor, regulates the atrial arrhythmogenic substrate in rabbits, Heart Rhythm. 12 (2015) 1362–1369.
[16] C. Sticherling, H. Oral, J. Horrocks, S.P. Chough, R.L. Baker, M.H. Kim, et al., Effect of digoxin on acute atrial refractoriness, Circulation 102 (2000) 2503–2508.
[17] L. Drabik, P. Wolkow, A. Undas, Denser plasma clot formation and impaired fibrinolysis in paroxysmal and persistent atrial fibrillation while on sinus rhythm: association with thrombin generation, endothelial injury and platelet activation, Thromb. Res. 136 (2015) 408–414.
[18] M.M. Polovina, G.Y. Lip, T.S. Potpara, Endothelial (dys)function in lone atrial fibrillation, Curr. Pharm. Des. 21 (2015) 622–645.
[19] V. Roldan, F. Marin, B. Muina, J.M. Torregrosa, D. Hernandez-Romero, M. Valdes, et al., Plasma von Willebrand factor levels are an independent risk factor for adverse events including mortality and major bleeding in anticoagulated atrial fibrillation patients, J. Am. Coll. Cardiol. 57 (2011) 2496–2504.
[20] T. Yamashita, A. Sekiguchi, Y.K. Iwasaki, K. Sagara, S. Hatano, H. Linuma, et al., Thrombomodulin and tissue factor pathway inhibitor in endocardium of rapidly paced rat atria, Circulation 108 (2003) 2450–2452.
[21] A. Goette, M. Hammwohner, A. Bukowska, F. Scalera, J. Martens-Lobenhoffer, D. Dobrev, et al., The impact of rapid atrial pacing on ADMA and endothelial NOS, Int. J. Cardiol. 154 (2012) 141–146.
[22] N. Wu, S. Tong, Y. Xiang, L. Wu, B. Xu, Y. Zhang, et al., Association of hemostatic markers with atrial fibrillation: a meta-analysis and meta-regression, PLoS One 10 (2015), e0124716, http://dx.doi.org/10.1371/journal pone.0124716 (accessed April 17, 2015).
[23] A. Scridon, N. Girerd, L. Rugeri, E. Nonin-Babary, P. Chevalier, Progressive endothelial damage revealed by multilevel von Willebrand factor plasma concentrations in atrial fibrillation patients, Europace 15 (2013) 1562–1566.
[24] J.G. Akar, W. Jeske, D.J. Wilber, Acute onset human atrial fibrillation is associated with local cardiac platelet activation and endothelial dysfunction, JACC 51 (2008) 1790–1793.
[25] K. Enta, K. Iwade, M. Aosaki, T. Uchida, H. Kasanuki, Predictive value of coagulative molecular markers for thromboembolism in patients with nonvalvular atrial fibrillation: prospective five-year follow-up study, J. Cardiol. 44 (2004) 223–232.
[26] M. Li, W. Hou, X. Zhang, L. Hu, Z. Tang, Hyperuricemia and risk of stroke: a systemic review and meta-analysis of prospective studies, Atherosclerosis 232 (2014) 265–270.
[27] S.H. Heo, S.H. Lee, High levels of serum uric acid are associated with silent brain infarction, J. Neurol. Sci. 297 (2010) 6–10.
[28] Y.M. Kim, T.J. Guzik, Y.H. Zhang, M.H. Zhang, H. Kattach, C. Ratnatunga, et al., A myocardial Nox2 containing NAD(P)H oxidase contributes to oxidative stress in human atrial fibrillation, Circ. Res. 97 (2005) 629–636.
[29] D.R. Van Wagonder, Molecular basis of atrial fibrillation: a dream or a reality? J. Cardiovasc. Electrophysiol. 14 (2003) 667–669.
[30] G.K. Glantzounis, E.C. Tsimoyiannis, A.M. Kappas, D.A. Galaris, Uric acid and oxidative stress, Curr. Pharm. Des. 11 (2005) 4145–4151.
[31] A. Kelkar, A. Kuo, W.H. Frishman, Allopurinol as a cardiovascular drug, Cardiol. Rev. 19 (2011) 265–271.
[32] U.Z. Malik, N.J. Hundley, G. Romero, R. Radi, B.A. Freeman, M.M. Tarpey, et al., Febuxostat inhibition of endothelial-bound XO: implications for targeting vascular ROS production, Free Radic. Biol. Med. 51 (2011) 179–184.