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Full Length Article

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