

Original Article

Therapeutic effects of *panax notoginseng* saponins against atrial fibrillation in rats

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Abstract: *Background:* Atrial fibrillation (AF) is the most serious disorder of atrial electrical activity, and the treatment effect is sub-optimal. This study aimed to investigate the therapeutic effects of *Panax notoginseng* saponins (PNS) against AF in rats. *Methods:* SD rats were randomized into control, model, with 100 mg/kg PNS and 150 mg/kg PNS groups, and 15 rats in each group. The Ach-CaCl₂-induced AF model was established in later 3 groups. From the 4th day, the later 3 groups were treated with 100 and 200 mg/kg PNS by intra-gastrical administration, respectively for 7 days. The duration of AF, atrial effective refractory period (ERP) were measured. The blood biochemical indexes were determined. *Results:* Compared with the model group, the duration of AF in 100 mg/kg PNS and 150 mg/kg PNS groups was remarkably shortened on the 6 th, 8th, and 10 th day and the atrial ERP in 150 mg/kg PNS group was remarkably prolonged (all $p < 0.05$). Compared with model group, in 150 mg/kg PNS group the serum GSH, SOD, and TIMP-2 levels were remarkably increased, respectively (all $p < 0.05$), and the serum MDA, TNF- α , ICAM-1 hs-CRP, and MMP-2 levels were remarkably decreased, respectively (all $p < 0.05$). *Conclusion:* PNS can mitigate the AF in rats. The mechanism may be related to its inhibition of oxidative stress and inflammatory response and regulation of MMP-2/TIMP-2 expression.

Keywords: *Panax notoginseng* saponins, atrial fibrillation, MMP-2, TIMP-2

Introduction

Atrial fibrillation (AF) refers to the loss of regular and orderly atrial electrical activity, which is replaced by rapid and disordered fibrillation. It is the most serious disorder of atrial electrical activity [1]. AF is one of the most common arrhythmia in clinic, and its prevalence is increasing year by year. The prevalence of AF is closely related to the age [2]. AF can cause serious harm to the patients. It leads to disability and fatal complications such as stroke and heart failure, which significantly increases the incidence and mortality of cardiovascular diseases, reduces exercise tolerance and cardiac function, thus seriously affecting the life quality and health of patients [3]. At present, the drug therapy of AF mainly includes ventricular rate control, cardioversion and anticoagulation. Single or combined drug therapy can effecti-

vely prevent and treat the AF [4]. However, the AF has the very complex pathogenesis and is easy to recur, which makes the long-term treatment effect is not ideal [5]. A recent study has been found that, the atrial remodeling plays an important role in the development of AF. Inhibition of atrial remodeling has a beneficial effect on the treatment of AF, and has become a research hotspot in the AF treatment [6]. *Panax notoginseng* is a perennial *Araliaceae* herb, and is a valuable traditional Chinese medicine. *Panax notoginseng* has the effect of activating blood circulation, dissipating blood stasis, and relieving swelling and pain [7]. Saponins are the most effective active ingredients of *Panax notoginseng*. *Panax notoginseng* saponins (PNS) are widely used in the prevention and treatment of cardiovascular diseases, such as atherosclerosis [8], hypoxic pulmonary hypertension [9], myocardial ischemia [10], etc.

In addition, some studies have shown that PNS can prevent the occurrence of abnormal impulse or impulse conduction, reduce the incidence of premature contraction, thus has the anti-arrhythmia effect [11]. There are still some unexplained aspects of the study, in particular whether PNS can prevent the AF. This study investigated the therapeutic effect of PNS against AF in rats and explored the related mechanism.

Materials and methods

Establishment of rat AF model

The rat AF model was established by intravenous administration of CaCl_2 -acetylcholine (Ach) mixture. The SD rats (male; 280-300 g; Shanghai Slake Laboratory Animal Co., Ltd., Shanghai, China) rats were weighed, and were anesthetized by intraperitoneal injection with 3% sodium pentobarbital with dose of 40 mg/kg. Then, the rats were immobilized in supine position on the wooden board. The limbs were disinfected, and were punctured with needle-shaped electrocardiography electrodes. After recording the normal electrocardiogram, the tail hair of rats was scraped clean. After disinfection with 75% ethanol, the tail vein back-flow was prevented. The CaCl_2 -Ach mixture (CaCl_2 , 10 mg/ml; Ach, 66 $\mu\text{g}/\text{ml}$) was injected into the tail vein, with dose of 1 ml/kg. The electrocardiogram of rats was recorded. The injection was performed once per day, for continuous 3 days. The electrocardiograms of 3 days were analyzed. The presence of typical AF electrocardiogram indicated the successful establishment of AF model induced by CaCl_2 -Ach mixture.

Rat grouping and medication

The rats were divided into a control group, a model group, a 100 mg/kg PNS group, and a 150 mg/kg PNS group, with 15 rats in each group. In later two groups, from the 4th day (the 1st day of medication), the rats were given with 100 mg/kg and 150 mg/kg PNS by intraperitoneal injection, respectively, followed by injection of CaCl_2 -Ach mixture after 1 hour. The rats in model group were given with equal volume of normal saline, followed by injection of CaCl_2 -Ach mixture after 1 hour. The treatment was performed once per day, for continuous 7 days. The rats in control group were given with equal

volume of normal saline during the whole experiment.

Measurement of duration of AF

On the 3rd, 4th, 6th, 8th and 10th day of the whole experiment, the duration of AF was determined from the standard lead II electrocardiogram. The disappearance of P wave with appearance of typical AF wave (f wave) was used as the marker of AF onset, and the restoration of sinus rhythm (f wave disappearance and P wave occurrence) was used as the marker of AF termination. The duration from AF onset to AF termination was recorded as the duration of AF.

Measurement of atrial effective refractory period

On the 10th day of experiment, the rats were anesthetized with chloral hydrate, and then the heart was taken, and washed immediately in the oxygen saturated Krebs-Henseleit solution [12], and then the auricle was carefully separated. Each end of the auricle was threaded and placed in a bath containing Krebs-Henseleit solution. One end of the thread was fixed, and the other end was connected with a tension transducer. The preload was adjusted at 1 g. After stabilization for 40 minutes, the threshold of evoked action potential was measured by programmed stimulation. The atrial effective refractory period (ERP) was measured by continuous double stimulation method. The stimulation parameters were as follows frequency, 10 Hz; wave width, 3 ms; 1.5 times threshold voltage. The double-pulse interval increased gradually from 50 ms. The increase amplitude was 1 ms. When the second stimulation caused the visible contraction, the double-pulse interval was recorded as the ERP.

Detection of blood biochemical indexes

On the 10 day of experiment, the rats were anesthetized, and blood was taken by extracting the eyeball blood. After centrifuging at 3000 r/min, the supernatant was obtained. The glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD) levels in serum were detected using colorimetry assays. The serum tumor necrosis factor- α (TNF- α), intercellular cell adhesion molecule-1 (ICAM-1), hypersensitive C-reactive protein (hs-CRP), matrix

Panax notoginseng saponins against atrial fibrillation

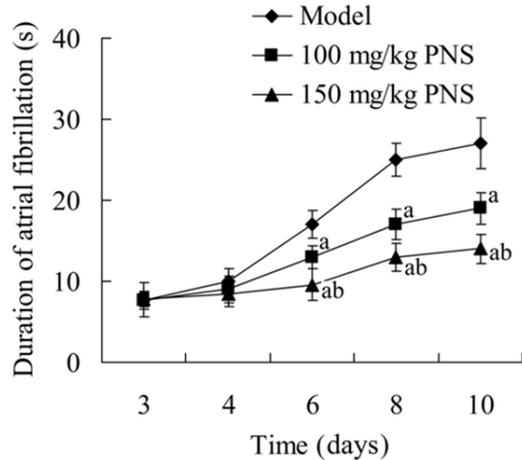


Figure 1. Duration of atrial fibrillation in rats in four groups. ^a $p < 0.05$ compared with the model group; ^b $p < 0.05$ compared with the 100 mg/kg PNS group. PNS, *Panax notoginseng* saponins.

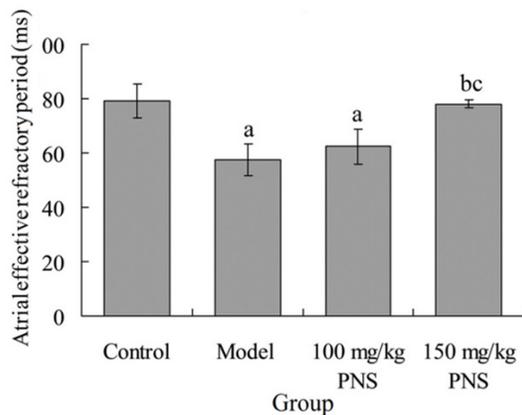


Figure 2. Atrial effective refractory period of rats in four groups. ^a $p < 0.05$ compared with the control group; ^b $p < 0.05$ compared with the model group; ^c $p < 0.05$ compared with the 100 mg/kg PNS group. PNS, *Panax notoginseng* saponins.

metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2) levels were determined using enzyme linked immunosorbent assay. The detection operations were strictly according to the instruction of kits.

Statistical analysis

SPSS 18.0 software (SPSS, US) was adopted for One-Way ANOVA and post-hoc test. Measurement data are presented as mean \pm standard deviation. SNK test was employed for group comparison. The significance level $p < 0.05$ was supposed to be significant.

Results

PNS shortening duration of AF in rats

Figure 1 demonstrates the changes of duration of AF in rats in model, 100 mg/kg PNS and 150 mg/kg PNS groups during the experimental period. On the 3rd day (successful establishment of AF model), the duration of AF had no remarkable difference among 3 groups. From the 4th day (the 1st day of medication), the duration of AF in each group was gradually prolonged with the increase of experimental days. Compared with the model group, the duration of AF in 100 mg/kg PNS group and 150 mg/kg PNS groups was remarkably shortened on the 6th, 8th, and 10th day, respectively ($p < 0.05$).

PNS extending atrial ERP of AF rats

At the end of treatment, the atrial ERP in model group was 57.56 ± 5.88 ms, which was remarkably shorter than 79.07 ± 6.34 ms in control group ($p < 0.05$). The atrial ERP in 100 mg/kg PNS group and 150 mg/kg PNS group was 62.32 ± 6.35 ms and 78.02 ± 1.44 ms, respectively. The atrial ERP in 150 mg/kg PNS group was remarkably longer than that in model group ($p < 0.05$) (**Figure 2**).

PNS increasing serum GSH and SOD levels and decreasing MDA level of AF rats

After treatment, compared with control group, the serum GSH and SOD levels in model group were remarkably decreased, respectively ($p < 0.05$), and the serum MDA level in model group was remarkably increased ($p < 0.05$). Compared with model group, the serum GSH level in 150 mg/kg PNS group and SOD level in 100 mg/kg PNS group and 150 mg/kg PNS group were remarkably increased, respectively ($p < 0.05$), and the serum MDA level in 100 mg/kg PNS group and 150 mg/kg PNS group was remarkably decreased, respectively ($p < 0.05$) (**Table 1**).

PNS decreasing serum TNF- α , ICAM-1 and hs-CRP levels of AF rats

As shown in **Table 2**, after treatment, compared with control group, the serum TNF- α , ICAM-1 and hs-CRP levels in model group were increased, respectively ($p < 0.05$). Compared with the model group, the serum TNF- α and

Panax notoginseng saponins against atrial fibrillation

Table 1. Serum GSH, MDA, and SOD levels of rats in four groups

Group	GSH (g/L)	MDA (mmol/L)	SOD (U/ml)
Control	10.11±1.23	3.16±0.56	124.35±20.21
Model	7.24±1.02 ^a	5.34±0.78 ^a	76.73±11.29 ^a
100 mg/kg PNS	7.67±1.17 ^a	4.87±0.67 ^{a,b}	91.46±9.22 ^{a,b}
150 mg/kg PNS	9.02±1.44 ^{a,b,c}	3.99±0.48 ^{a,b,c}	103.45±13.61 ^{a,b,c}

^a*p* < 0.05 compared with the control group; ^b*p* < 0.05 compared with the model group; ^c*p* < 0.05 compared with the 100 mg/kg PNS group. PNS, *Panax notoginseng* saponins; GSH, glutathione; MDA, malondialdehyde; SOD, superoxide dismutase.

Table 2. Serum TNF- α , ICAM-1, and hs-CRP levels of rats in four groups

Group	TNF- α (μ g/L)	ICAM-1 (μ g/L)	hs-CRP (μ g/L)
Control	9.56±1.62	77.67±10.42	148.74±16.34
Model	13.32±1.67 ^a	138.78±15.92 ^a	198.36±21.36 ^a
100 mg/kg PNS	12.89±1.43 ^a	129.41±14.33 ^a	173.43±19.73 ^{a,b}
150 mg/kg PNS	10.65±1.39 ^{a,b,c}	83.38±9.51 ^{a,b,c}	165.83±18.16 ^{a,b,c}

^a*p* < 0.05 compared with the control group; ^b*p* < 0.05 compared with the model group; ^c*p* < 0.05 compared with the 100 mg/kg PNS group. PNS, *Panax notoginseng* saponins; TNF- α , serum tumor necrosis factor- α ; ICAM-1, intercellular cell adhesion molecule-1; hs-CRP, hypersensitive C-reactive protein.

Table 3. Serum MMP-2 and TIMP-2 levels of rats in four groups

Group	MMP-2 (pg/ml)	TIMP-2 (ng/ml)
Control	241.52±23.62	4.47±0.48
Model	406.71±38.78 ^a	3.76±0.56 ^a
100 mg/kg PNS	353.26±40.31 ^{a,b}	3.99±0.43 ^a
150 mg/kg PNS	302.36±33.45 ^{a,b,c}	4.37±0.59 ^{b,c}

^a*p* < 0.05 compared with the control group; ^b*p* < 0.05 compared with the model group; ^c*p* < 0.05 compared with the 100 mg/kg PNS group. PNS, *Panax notoginseng* saponins; MMP-2, matrix metalloproteinase-2; TIMP-2, tissue inhibitor of metalloproteinase-2.

ICAM-1 levels in 150 mg/kg PNS group and the serum hs-CRP levels in 100 mg/kg PNS group 150 mg/kg PNS group were decreased, respectively (*p* < 0.05).

PNS decreasing serum MMP-2 level and increasing TIMP-2 level of AF rats

After treatment, compared with control group, in model group the serum MMP-2 level was increased (*p* < 0.05), and the serum TIMP-2 level was decreased (*p* < 0.05). Compared with the model group, the serum MMP-2 level in

100 mg/kg PNS group 150 mg/kg PNS group were decreased, respectively (*p* < 0.05), and the serum TIMP-2 level in 150 mg/kg PNS group was increased (*p* < 0.05) (Table 3).

Discussion

In the present study, the rat AF model was established, and the therapeutic effects of PNS against AF in rats were investigated. The results show that, compared with the model group, the duration of AF in 100 mg/kg PNS group and 150 mg/kg PNS group was significantly shortened on the 6th, 8th, and 10th day, respectively. In addition, on the 10th day, the atrial ERP in 150 mg/kg PNS group was significantly longer than that in model group. This suggests that, PNS can mitigate the AF in rats.

There are many viewpoints on the pathogenesis of AF. Studies [13, 14] have shown that, the oxidative

stress plays an important role in the occurrence of AF. The increased peroxidation may cause the change in the direction of calcium current in the heart, promote the myocardial fibrosis, or cause the gap junction damage and other pathological changes, thus leading to the occurrence of AF. The levels of MDA, SOD and GSH in plasma can be used as independent predictors of AF [15-17]. GSH can reduce the hydrogen peroxide and protect the structure and function of cell membrane. It is a low-molecular weight scavenger, which can scavenge $\cdot\text{O}_2^-$, H_2O_2 , LOOH and so on. The lack or depletion of GSH aggravates the toxicity of many chemicals or environmental factors, which may be related to increased oxidative damage. Therefore, the content of GSH is an important factor to measure the body's antioxidant capacity [18]. MDA can be produced naturally in body and is a marker of oxidative stress. SOD is an important antioxidant enzyme *in vivo*. Both of them can reflect the degree of oxidative stress in body [19]. The results of this study show that, compared with control group, the serum GSH and SOD levels in model group were decreased, and the serum MDA level in

model group was increased. Compared with model group, in 150 mg/kg PNS group the serum GSH and SOD levels were increased, and the serum MDA level was decreased. This suggests that the mechanism of PNS in mitigating AF may be related to its inhibition of oxidative stress in body.

TNF- α is mainly produced by mononuclear macrophages. Neutrophils, stellate cells, endothelial cells and smooth muscle cells can also produce TNF- α . TNF- α can damage the endothelial cells, promote the coagulation, inhibit the fibrinolysis, promote the monocyte adhesion to endothelial cells, and promote the migration and proliferation of vascular smooth muscle cells. It not only acts as a mediator of host's defense, immunity and homeostasis, but also is a pathological factor of infection, inflammation and injury [20]. It is reported that, TNF- α is involved the occurrence of AF [21]. ICAM-1 belongs to the immunoglobulin super-family. When the endothelial dysfunction occurs, VCAM-1 activates the monocytes and neutrophils and promote them to adhere to the surface of endothelial cells. The activated monocytes and neutrophils can release some toxic products, causing the local inflammation [22]. VCAM-1 plays a role in the occurrence and development of AF [23]. Hs-CRP is a common inflammatory factor in body. The change of its level is related to the occurrence of AF [24]. In the present study, compared with the control group, the serum TNF- α , ICAM-1 and hs-CRP levels in the model group were increased. Compared with the model group, the serum TNF- α and ICAM-1 levels in 150 mg/kg PNS group were decreased. This indicates that, PNS can inhibit the inflammatory response, thus exerting the therapeutic effect on AF.

MMPs and TIMPs play an important role in atrial fibrosis of AF. MMPs are the most important proteolytic system for degradation of extracellular matrix components. Under pathological conditions, MMPs with increased expression or activity can degrade normal extracellular matrix components and synthesize collagens those have no structure and function, leading to the structural remodeling [25]. As the endogenous inhibitors of MMPs, TIMPs can alleviate the degradation of extracellular matrix components by MMPs. The balance between MMPs and TIMPs determines the balance of cardiac

matrix synthesis and degradation, which plays an important role in maintaining the integrity of cardiac structure and function [26]. MMP-2 and TIMP-2 are the representational types of MMPs and TIMPs, respectively, and closely related to many cardiovascular diseases. In the present study, compared with model group, the serum MMP-2 level in 100 mg/kg PNS group 150 mg/kg PNS group were decreased, and the serum TIMP-2 level in 150 mg/kg PNS group was increased. This suggests that, PNS can regulate the MMP-2 and TIMP-2 expressions, thus lighten the AF in rats.

In conclusion, PNS can shorten the duration of AF and atrial ERP of rats with AF. The mechanism may be related to its inhibition of oxidative stress and inflammatory response and regulation of MMP-2/TIMP-2 expression. However, the exact molecular mechanisms deserve further investigation.

Disclosure of conflict of interest

None.

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Panax notoginseng saponins against atrial fibrillation

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