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Original Article

Effect of berberine on acetylcholine-induced atrial fibrillation in rabbit

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Abstract: The purpose of this study was to test the efficacy of Berberine (Ber) on atrial fibrillation (AF) induced by acetylcholine (ACh) and explore its underlying mechanisms of action. In vivo electrophysiology experiments were performed in adult anesthetized rabbits. Single atrial myocytes were isolated from rabbit hearts and action potentials recorded using patch clamp techniques. AF was induced by rapid atrial burst pacing during intravenous (IV) ACh infusion alone or with IV Ber. Compared to the Baseline, IV Ber (2 mg/kg) prolonged the RR interval and effective refractory period (195 ± 10 vs. 215 ± 11 msec; 80 ± 4 vs. 85 ± 5 msec, respectively; both $P<0.05$). The induced rate of sustained 1 min AF was lower during ACh infusion with Ber than during ACh infusion alone (4/10 vs. 30/35, $P<0.01$). The termination rate of ACh-induced AF was higher with IV Ber (1 mg/kg) than with IV saline (sustained 1 min AF: 6/8 vs. 6/20, sustained 10 min AF: 8/10 vs. 1/6, both $P<0.05$). ACh perfusion significantly shortened the action potential duration (APD) of isolated atrial myocytes (APD_{50}: 152 ± 13 vs. 81 ± 10 msec; APD_{90}: 256 ± 19 vs. 132 ± 13 msec, both $P<0.01$). Application of Ber reversed the APD shortening induced by ACh (APD_{50}: 81 ± 10 vs. 134 ± 15 msec; APD_{90}: 132 ± 13 vs: 213 ± 17 msec, both $P<0.01$). We conclude that Ber suppresses ACh-induced AF in the rabbit by increasing atrial effective refractory period and prolonging the APD of atrial myocytes.

Keywords: Berberine, atrial fibrillation, acetylcholine, effective refractory period, action potential duration

Introduction

Atrial fibrillation (AF) is an arrhythmia that has gained growing clinical concern because of its increasing prevalence, and significant morbidity and mortality [1, 2]. Given the growing number of affected individuals, development of safe and effective treatment options for AF has become a worldwide priority [1, 2]. Although substantial progress has been made in AF treatment, such as catheter ablation, antiarrhythmic drugs (AADs) remain the first and most appropriate therapy because of contraindications or patient unwillingness to undergo invasive treatment. The currently available AADs for restoration and maintenance of sinus rhythm have limitations because of their low or modest efficacy, and adverse effects such as proarrhythmia and extracardiac toxicity [3]. The potential advantages of sinus rhythm maintenance encourage continued efforts to identify novel pharmacological means to restore and maintain sinus rhythm. Therefore, finding more effective and safer AADs for chronic AF treatment remains imperative [1, 2].

Berberine (Ber), a natural compound from Barberry, exhibits numerous beneficial cardiovascular pharmacological effects in conditions such as myocardial ischemia, hyperlipidemia, hypertension, and thrombosis [4]. Ber has long been considered as a promising AAD and has been used for arrhythmia treatment for years [5]. However, most clinical studies have focused on ventricular arrhythmia and studies of isolated ventricle myocytes [5, 6]. Studies regarding the action of Ber on AF and its potential mechanisms are limited. Therefore, the present study was undertaken to assess the efficacy of Ber against AF and explore the potential electrophysiologic mechanisms of its actions.

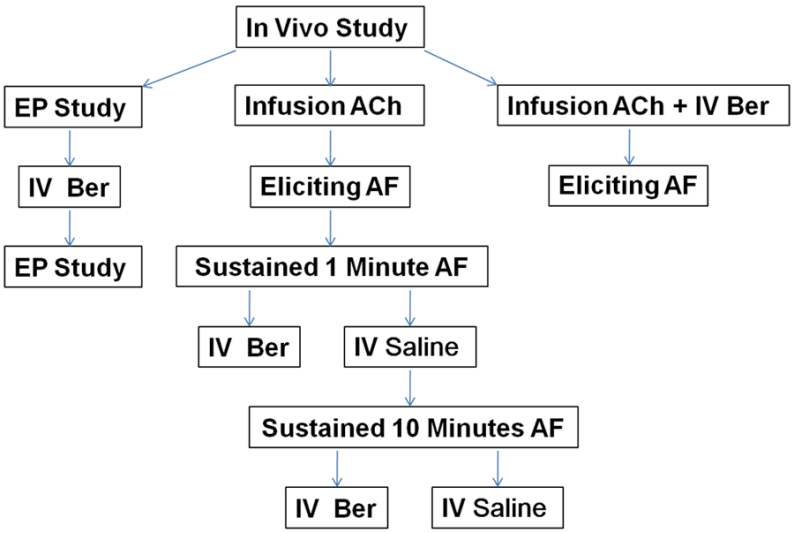

Figure 1. Electrophysiologic study protocols for *in vivo* studies showing the three sets of experiments (left, middle and right columns).

Methods

Ethics statement

Animal care protocols were reviewed and approved by the Animal Experimentation Ethics Committee of Xuhui Center Hospital. All animals used in this study received humane care in compliance with the Chinese Association for Accreditation of Laboratory Animal Care. All surgery was performed under general anesthesia, and every effort was made to minimize pain and suffering.

Animal preparation

Adult New Zealand white rabbits (32 males and 28 females) weighing 2.0-2.5 kg were used for these experiment. The study was performed in a cardiac catheterization laboratory equipped with fluoroscopy.

After 12 hours of fasting, a safety closed IV catheter system (trocar cannula) was placed in an ear edge vein for administration of fluids including anesthesia and investigational drugs. Animals were anesthetized with 30 mg/kg sodium pentobarbital for induction supplemented by 2-5 mg/kg/h in intermittent bolus injections as needed. A limb lead electrocardiogram (ECG) (Cardiofax S, ECG-1250, NIHON KOHDEN, China) was monitored from subcutaneous needle electrodes. A multipole right ventricular electrode was advanced into the right atrium through the left external jugular vein under fluoroscopic guidance and was connected to the ECG precardial V_1 lead and a cardiac electrophysiological stimulator system (SuZhou Dong-Fang Electronics Co. Ltd., China). The ECG signal was viewed on a personal computer with ECG Viewer II software. The atrial and ventricular endocardial waves were easily identified on the computer for analysis. After the stimulation voltage threshold (2-3 mV) for atrium pacing was determined, the multipole right ventricular electrode was fixed in place.

Electrophysiologic study protocol

One group of animals (**Figure 1**, left column) was subjected to regular stimuli with an added stimulus (S1S2) to determine the atrial effective refractory period (ERP) before and after 2 mg/kg intravenous Ber (Northeast Pharmaceutical Group Co., Ltd, China). ERP was determined as the longest interval of S1S2 that did not allow the capture of the atrial response [7]. The RR interval was also measured and analyzed.

In another group of animals (**Figure 1**, center and right column) AF was induced by high-frequency (800-1000 beats/min) burst atrial pacing during intravenous infusion (10 ml/h) of 10 mM ACh (SIGMA, St. Louis, Mo, USA) with or without IV Ber (1 mg/kg). The end point of the high-frequency burst pacing was the elicitation of sustained AF for 1 min, or 1 hour of the total stimulation time. AF was defined as a totally irregular atrial rhythm with an average cycle length <150 ms, and totally irregular ventricular rhythm [7].

Animals with sustained 1 min AF induced by ACh infusion alone (**Figure 1**, center column) were then divided into two groups receiving either IV Ber or IV saline. The IV Ber group received 1 or 2 mg/kg Ber to terminate AF. The IV saline groups received an injection of same volume of saline as a control. We regarded the treatment as successful if the sustained AF

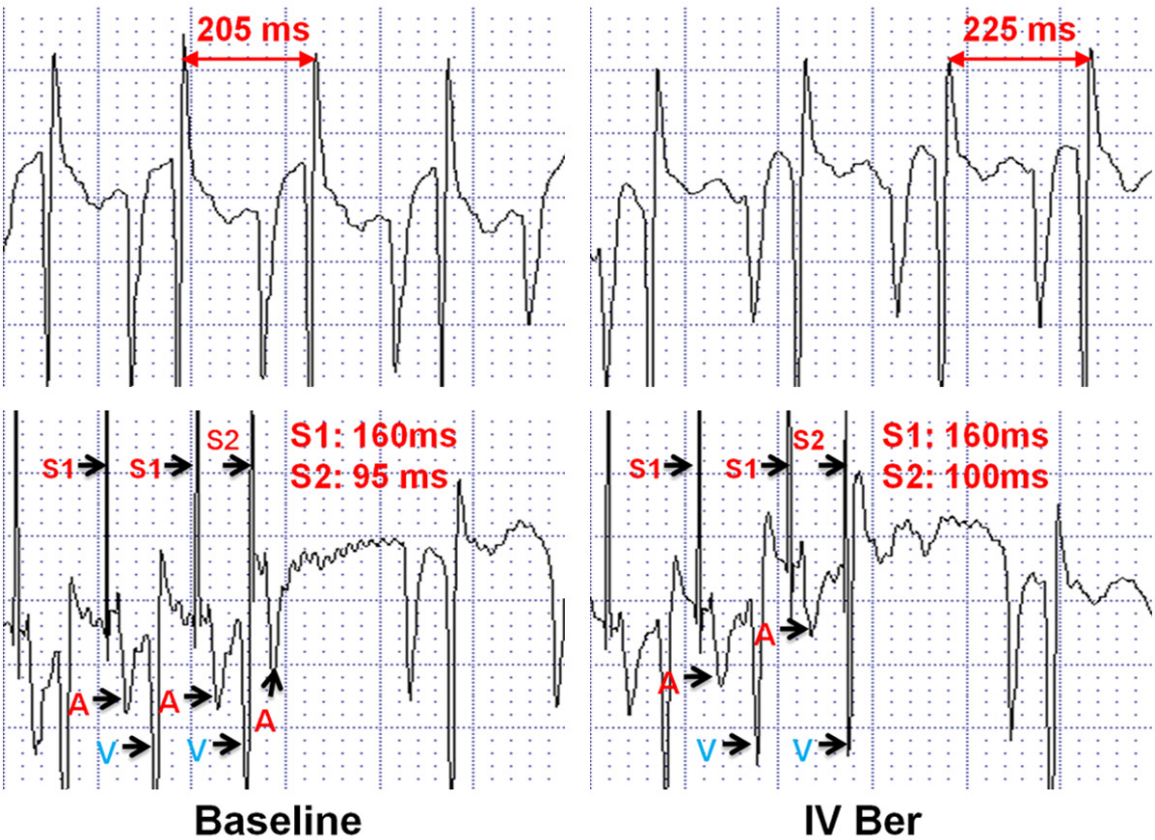

Figure 2. Effects of IV Ber on the RR interval (upper) and the effective refractory period (lower) (ERP). Compared with baseline (Left), Ber prolongs RR interval and ERP (Right). A: atrial endocardial wave; V: ventricular endocardial wave; S1: the regular stimuli; S2: added extra stimulus; Ber: Berberine.

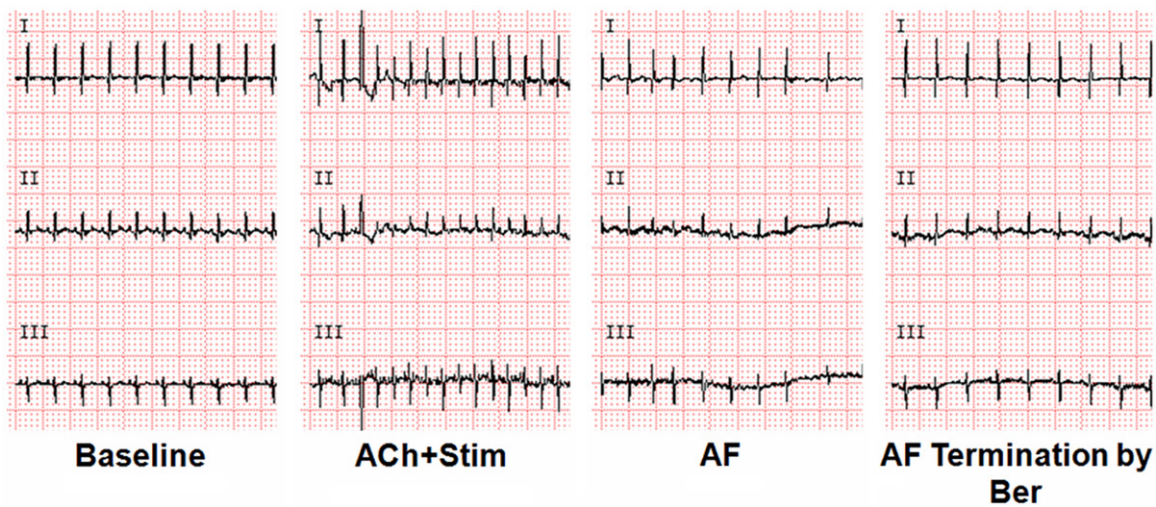

Figure 3. Examples of ECGs showing that AF was induced by high-frequency burst pacing during ACh intravenous infusion and AF was terminated by IV Ber. ACh: acetylcholine; AF: atrial fibrillation; Stim: stimulation; Ber: Berberine.

was terminated within 5 min from the beginning of treatment.

If AF was terminated spontaneously within 10 min for the IV saline group, high-frequency

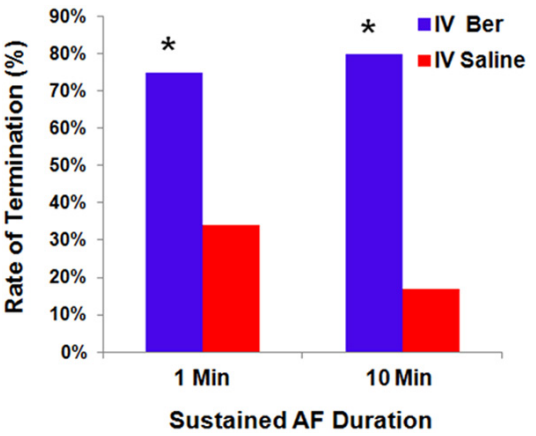

Figure 4. Summary of the rate of termination of induced sustained 1 min and 10 min AF by IV Ber (blue) compared to saline control (red). *, $P<0.05$ Ber vs Saline. AF: atrial fibrillation; Ber: Berberine.

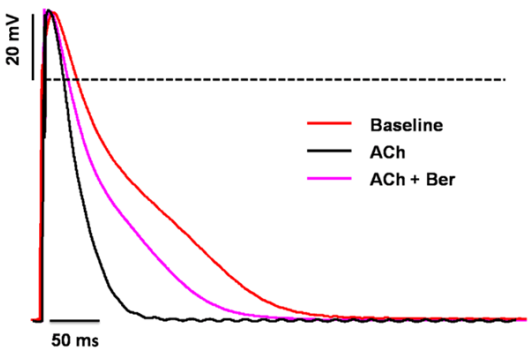

Figure 5. Action potentials recorded from isolated atrial myocytes. ACh shortens the atrial AP duration while Ber reverses this effect. ACh: acetylcholine; Ber: Berberine. Horizontal and vertical calibration bars show 50 ms and 20 mV, respectively. The dashed line represents 0 mV.

burst atrial pacing with ACh intravenous infusion was continued until the AF duration reached 10 min. Then we repeated the previous procedure used for the sustained 1 min AF protocol with IV Ber or IV saline.

After these studies were completed, 10 randomly selected surviving rabbits were injected intramuscularly with 200,000 unit penicillium sodium daily for 3 d to prevent infections. These rabbits were used for the isolation of atrial myocytes at least 20 d after the *in vivo* study to allow washout of drugs. The other rabbits were humanely sacrificed under deep anesthesia.

Action potential of atrial myocytes

Atrial myocytes were enzymatically isolated from the hearts of surviving rabbits described above using previous methods [8]. Only Ca^{2+}-tolerant, rod-shaped cells with clear cross-striation and without spontaneous contractions were selected for *in vitro* patch clamp studies.

Myocytes were patch-clamped in the whole-cell configuration as previously described [11]. Voltage or current signals were obtained with a MultiClamp 700B patch-clamp amplifier using a Digidata 1440 acquisition board driven by pCLAMP 10 software (Molecular Devices, Sunnyvale, CA) and stored on a computer for analysis.

For action potential (AP) recordings, pipettes (resistance 3 to 5 MΩ) were filled with an internal solution of the following composition (in mmol/L): 110 K-aspartate, 30 KCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 5 MgATP, 5 Na_2-phosphocreatine, 0.05 cAMP, and pH 7.2 adjusted with KOH. The cells were superfused with Tyrode's solution of the following composition (in mmol/L): 136 NaCl, 4.0 KCl, 0.33 Na_2PO_4, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES, and pH 7.4 adjusted with NaOH. APs were elicited with 2 ms duration, 2 to 4 nA amplitude square pulses at a pacing rate of 1 Hz. Following baseline recordings, cells were superfused with Tyrode's solution containing 3 μM ACh for at least 30 min, and AP recordings repeated. Next, 10 μM Ber was added to the Tyrode's solution with 3 μM ACh and cells superfused for at least 30 min and APs were recorded again.

At least 50 AP waveforms were recorded for each condition and averaged. Measured parameters included AP amplitude (APA, mV), maximum upstroke velocity of the AP (V_{max}, V/s), and the AP duration (APD, msec) from the onset of the action potential spike to 50% and 90% repolarization (APD_{50} and APD_{90}).

Statistical analysis

Values of RR, ERP and APD were expressed as mean ± 1 standard deviation. The significance of differences among the above parameters was determined using Student's t-test for comparisons of two groups. The Chi-Square Test was used to compare the induction and termination of AF. A P value of <0.05 was considered to be statistically significant, and all analyses

Table 1. Effects of Acetyhlcholine and Berberine on Atrial Action Potential Duration (ms)

	APD_{50}	APD_{90}
Baseline	152 ± 13	256 ± 19
ACh	81 ± 11*	132 ± 13*
Ach+Ber	134 ± 15#	213 ± 17#

*P<0.05 vs. Baseline, #P<0.05 vs. ACh+Ber. Units of APD are msec. ACh: acetylcholine, Ber: berberine, APD_{50}: duration of the action potential to 50% of repolarization; APD_{90}: duration of the action potential to 90% of repolarization.

were performed using SPSS version 18.0 (SPSS, Chicago, Illinois).

Results

A total of 60 animals were used in this study, three of which died from anesthesia accident, and two others died during high-frequency burst atrial pacing with IV ACh infusion. Two additional animals died after the termination of the induced sustained 1 min AF with IV Ber 2 mg/kg due to extreme bradycardia and cardiac arrest.

One group of 10 animals (**Figure 1**, left column) was used to determine the effects of Ber on RR interval and ERP. Compared to baseline, 2 mg/kg Ber IV significantly prolonged both the RR interval (195 ± 10 vs. 215 ± 11 msec; P<0.05) and the ERP (80 ± 4 vs. 85 ± 5 msec; P<0.05) (**Figure 2**).

A second group of 10 animals (**Figure 1**, right column) was used to elicit AF with IV ACh infusion plus IV Ber, while 35 animals (**Figure 1**, center column) were used to elicit AF during ACh infusion alone. The rate of sustained 1 min AF was significantly lower in the group receiving ACh infusion plus IV Ber than with ACh infusion alone (4/10 vs. 30/35, P<0.05).

For sustained AF induced with ACh infusion alone, the termination rate of the sustained AF was higher in the Ber group (1 mg/kg) than saline group (Sustained 1 min AF: 6/8 vs. 6/20, Sustained 10 min AF: 8/10 vs. 1/6, both P<0.05) (**Figures 3**, **4**).

APs were recorded in atrial myocytes isolated from 10 animals following their recovery from the *in vivo* experiments. As shown in **Figure 5** and **Table 1**, 3 μM ACh perfusion significantly shortened the APD_{50} and APD_{90} of atrial myocytes (APD_{50}: 152 ± 13 vs. 81 ± 10 msec; APD_{90}: 256 ± 19 vs. 132 ± 13 msec, both P<0.01). Application of Ber (10 μM) markedly attenuated the APD shortening induced by 3 μM ACh (APD_{50}: 81 ± 10 vs. 134 ± 15 msec; APD_{90}: 132 ± 13 vs: 213 ± 17 msec, both P<0.01). No significant differences in values of APA and V_{max} were observed among the various groups.

Discussion

The present study investigated the effects of Ber on ACh-induced AF in rabbits and the mechanisms of its action. High rate burst pacing elicited sustained AF in the majority of the rabbits with ACh infusion alone while the rate of elicited sustained AF was significantly lower for infusion of ACh with Ber. Ber also terminated the majority of the ACh-induced AF. Importantly, Ber prolonged the RR interval and ERP, and reduced the shortening of APD induced by ACh in isolated atrial myocytes. The effect of ACh to abbreviate atrial repolarization and refractoriness underlies its ability to facilitate the induction of AF [7, 9, 10]. By prolonging atrial repolarization and ERP, Ber treatment results in the termination of the ACh-induced AF.

Cellular effects of Ber

Several laboratories have shown that Ber possesses anti-arrhythmic actions through the prolongation of APD [11] as a result of blocking several ion channels, including K^+ channels. Sanchez-Chapula [12] showed that Ber could prolong APD by blocking I_{Kr} at concentrations of 0.3-30 μM. He also found that at concentrations higher than 10 μM Ber inhibited the transient outward current (I_{to}). Wang et al [13] demonstrated a beneficial effect of Ber on ischemia-induced arrhythmias which they attributed to inhibition of K_{ATP} channel activation. Rodriguez-Menchaca et al [14] showed that Ber blocked hERG (I_{Kr}) and KCNQ1/KCNE1 (I_{Ks}) channels expressed in HEK-293 cells and *Xenopus* oocytes. A study in a rat type 2 diabetic myocardial infarction model suggested that the effects of Ber on I_{K1}/Kir2.1 may be an important mechanism for producing its anti-arrhythmic effects [6]. Therefore, combined with its derivative CPU86017 already considered a class III anti-arrhythmic agent, Ber may also be classified a class III anti-arrhythmic agent and a broad-spectrum AAD [15].

ACh abbreviates atrial repolarization and refractoriness through activation of the atrial muscarinic activated potassium current and facilitates AF induction [9]. Although Ber is not an ACh antagonist, it exerts electrophysiologic effects that abrogate those of ACh in atrial myocardium. This action of Ber may be due, at least in part, to blockade of the components of the delayed rectifying potassium current I_{Kr} and/or I_{Ks} [6, 13, 14]. Thus, Ber prolongs atrial repolarization by virtue of its inhibition of several ion channels thereby terminating the ACh-induced AF, just as our study has shown.

Clinical evidence for an anti-arrhythmic effect of Ber

Ber had been tested as an anti-arrhythmic drug in clinical trials for many years. A randomized clinical trial tested the effects of Ber in patients with chronic congestive heart failure [5]. In this study, 79 of 156 patients were treated with Ber while all patients received a conventional therapeutic regimen consisting of angiotensin-converting enzyme inhibitors, digoxin, diuretics, and nitrates. The Ber-treated group showed significantly lower rates of ventricular premature complexes, hospital admission for congestive heart failure, and long-term mortality [5]. Zeng et. al. showed that the decrease in frequency and complexity of ventricular premature beats in patients with plasma Ber concentrations higher than 0.11 mg/L was significantly greater than at concentrations lower than 0.11 mg/L [16].

Another study showed that treatment of 100 arrhythmic patients with Ber (300 mg, qid) for 1 to 4 weeks resulted in a greater than 89% reduction in premature beats in 62 patients and a 50% reduction in the other 38 patients, indicating that Ber significantly reduced premature beats. Other clinical trials yielded similar results with Ber [4, 17].

Effect of Ber on AF and its potential advantage

AF is more common in older people and often complicated by myocardial ischemia, hypertension, and/or diabetes. “Upstream’ therapy” is the term used to describe treatments that could reduce the substrate for AF by reversing or preventing atrial remodeling [1, 2]. These treatments are aimed primarily at the cardiovascular conditions that underlie AF such as hypertension, ischemic heart disease, or heart failure. AF patients with other diseases should be treated with AADs as well as other drugs such anti-coagulant, anti-atherosclerotic, and anti-hypertensive agents [1, 2, 18].

As mentioned previously, evidence shows that Ber has a broad array of cardiovascular pharmacological effects. Ber has anti-arrhythmic actions as well as anti-oxidant, anti-inflammatory, anti-atherosclerosis, anti-myocardial ischemia, and even anti-thrombosis effects [19-23]. Therefore, Ber is potentially more suitable for treating chronic AF than other similar AADs.

Adverse effects are a frequent concern for all AADs. As a traditional medicine Ber has definite potential for use in a wide spectrum of clinical applications. Ber has been used for a very long time in developing countries of Africa and Asia especially China to treat gastroenteritis, abdominal pain and diarrhea owing to its antimicrobial, anti-diabetic and anti-inflammatory actions [24]. Clinical studies have shown that Ber can be used for long term treatment without severe adverse effects [22, 24]. Therefore, Ber is a candidate drug for the treatment of chronic AF.

Study limitation

Several limitations in this study should be mentioned. Similar to past studies, AF was induced with high-frequency burst atrial pacing during ACh infusion which may not be the same as spontaneous AF [7, 10]. Second, the dose of Ber used to terminate AF was only 50% of the dose used to prolong RR interval and ERP. The lower dose of Ber that terminated ACh-induced AF was inadequate to significantly prolong RR interval and ERP. Furthermore, *in vitro* the dose of Ber used to prolong RR interval and ERP can led to extreme bradycardia and cardiac arrest during ACh infusion. Although we do not completely understand the reasons for these differences, the higher concentration of ACh and Ber together may adversely affect the cardiac conduction system and heart function [25]. Additional work must be performed to understand the mechanism of the differences mentioned above.

Conclusion

Berberine has the ability to suppress the induction of ACh-mediated AF. The mechanism may

be related to prolonging ERP and APD. Therefore, Ber may be a promising drug for AF management because of its numerous cellular pharmacological effects and its proven safety in humans.

Acknowledgements

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Disclosure of conflict of interest

None.

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