Novel beta-blocker pretreatment prevents alcohol-induced atrial fibrillation in a rat model

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BACKGROUND A case report published in 2019 described a patient who presented with difficult-to-manage atrial fibrillation (AF) that consistently was associated with alcohol consumption. After the patient did not respond to drug therapy, a novel beta-blocker (BB) pretreatment regimen initiated immediately before alcohol consumption successfully prevented AF occurrence.

OBJECTIVE The purpose of this study was to test the hypothesis that a novel prophylactic BB therapy given before alcohol consumption could prevent AF in a rat model.

METHODS An alcohol-induced AF model was developed in adult Sprague-Dawley rats of both sexes by administering alcohol (2 g/kg intraperitoneal [IP]) once every other day for a total of 4 times. Three groups were enrolled: alcohol (EtOH; n = 10); alcohol plus BB (metoprolol 50 mg/kg IP) pretreatment (EtOH+BB; n = 10); and control (n = 9). Cardiac function (assessed by echocardiography and left ventricular hemodynamics) and *in vivo* atrial electrophysiology and AF inducibility tests were performed 24 hours after the last injection.

RESULTS All but 1 rat completed the study. Alcohol exposure did not significantly impact cardiac function and the atrial effective refractory period. However, alcohol exposure significantly increased AF inducibility [median (first and third quartile [Q1–Q3]) 0% (0%–0%) in control vs 60% (25%–100%) in the EtOH group; $P < .05$] and AF duration [0 second (0–0 second) in control vs 0.81 second (0.24–3.67 seconds) in the EtOH group; $P < .05$]. Compared to the EtOH group, the EtOH+BB group had significantly reduced AF inducibility [0% (0%–22.5%); $P < .05$] and duration [0 second (0–0.2 second); $P < .05$].

CONCLUSION Metoprolol pretreatment before alcohol administration significantly decreased AF induction in rats. These findings suggest that BB pretreatment is a promising prophylaxis regimen for alcohol-induced AF.

KEYWORDS Alcohol; Atrial fibrillation; Atrial fibrillation inducibility; Beta-blocker; Effective refractory period

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Introduction

Atrial fibrillation (AF) is recognized as the most common clinically significant arrhythmia. Many risk factors are known to be associated with increased AF prevalence in patients, such as aging, hypertension, and structural heart diseases.[1,2] It is well recognized that excessive alcohol consumption is associated with increased AF incidence. Alcohol-induced AF is sometimes known as the "holiday heart syndrome."[3] In patients with alcohol-induced AF, alcohol cessation probably is the best management strategy. This is supported by a recent clinical study that reported abstinence from alcohol reduced arrhythmia recurrences in regular drinkers with AF.[4] However, alcohol cessation often is difficult in habitual drinkers. Currently no known medications can be used to prevent alcohol-induced AF.

We recently reported the case of a patient who developed a novel "as needed" medical option in order for him to tolerate mild-to-moderate alcohol consumption that was associated with AF.[5] The patient previously had attempted antiarrhythmic drug therapy and exhibited proarrhythmia (more regularized and symptomatic atrial flutter). He also had difficulty tolerating maintenance-dose beta-blocker (BB) therapy due to symptomatic hypotension. He devised a novel alternative administration modality, taking a BB just before alcohol consumption, which proved universally effective and was well tolerated. Forty-four episodes of AF were prevented with this novel BB pretreatment.[5]

If this novel, "as needed" BB pretreatment modality could prevent alcohol-induced AF, it would greatly help those people who cannot abstain from consuming alcohol. This "as

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KEY FINDINGS

- The results of this controlled, blinded rat study support those of a recent published human case, in which a novel beta-blocker pretreatment prior to alcohol consumption prevented alcohol-induced atrial fibrillation (AF).
- Alcohol administration in rats resulted in a significant increase in AF inducibility.
- Beta-blocker pretreatment with metoprolol, in alcohol-exposed rats, resulted in significantly decreased AF inducibility.
- Cardiac function and atrial effective refractory periods were not significantly affected by alcohol exposure.

needed" novel approach also has the obvious benefit that the patient does not need to take the medication regularly. In order to test the hypothesis, a binge drinking animal model was developed to examine whether BB pretreatment can prevent or decrease alcohol-associated AF inducibility in rats.

Materials and methods

Animal model and study design

Adult Sprague-Dawley rats (3–4 months old; both sexes) were used in this study. A rat binge drinking model was developed according to a method described in a previous report.[6] In brief, alcohol group rats received intraperitoneal (IP) administration of ethanol (2 g/kg, diluted in saline) once every other day for a total of 4 times (ie, on days 1, 3, 5, and 7). Nonalcohol control animals received equivalent amounts of saline injection IP once every other day for 4 times. The animals in this study were randomized, and the person performing the AF inducibility test was blinded to the treatment.

The study consisted of 3 groups of animals: an alcohol-injected group (EtOH; n = 10); an alcohol plus BB pretreatment group (EtOH+BB; n = 10); and a control group (n = 9). In the EtOH+BB group, metoprolol (50 mg/kg IP; Sigma-Aldrich, St. Louis, MO) was administered just before each alcohol injection. The metoprolol dosage used in this study was in accordance with previous reports that 20–100 mg/kg/day were typically used in rats.[7,8]

All animals underwent echocardiography, left ventricular (LV) hemodynamics, atrial electrophysiology, and AF inducibility tests 24 hours after the last alcohol or saline injection (on day 8), as previously reported.[6] The use of animals was approved by the Institutional Animal Care and Use Committee at New York Institute of Technology College of Osteopathic Medicine and was in accordance with the Guide for the Care and Use of Laboratory Animals. All rats were housed in our institutional animal care facility and kept on a 12-hour light/dark cycle with food and water available *ad libitum*.

Echocardiographic measurement

Echocardiography was performed in all rats just before the terminal experiment (on day 8) using a GE Vivid 7 Dimension System (GE Vingmed Ultrasound A/S, Horten, Norway) coupled with a GE M12L linear (matrix) array ultrasound transducer probe (5–13 MHz), as previously reported.[9,10] Animals were anesthetized and maintained with isoflurane (1.5%). Two-dimensional echocardiograms were obtained from the short-axis (at the papillary muscle level) and long-axis views of the LV. A 2-dimensionally targeted M-mode echocardiogram was used to determine LV wall thickness and chamber dimensions in systole and diastole from the short-axis view. Left atrial (LA) diameter was determined at the aortic valve level from the long-axis view. The following parameters were measured: anterior wall thickness in end-diastole and end-systole; LV diastolic and systolic internal diameters; posterior wall thickness in end-diastole and end-systole; LV fractional shortening; and LA diameter.

LV hemodynamic measurements

After echocardiographic measurements, a 1.9F Scisense pressure catheter (Transonic Scisense, London, Ontario, Canada) was inserted through the right carotid artery and advanced into the LV, as previously described.[9,10] After about 20 minutes of stabilization, the following LV hemodynamic data were collected: LV systolic pressure, LV end-diastolic pressure, positive change in LV pressure over time, and negative change in LV pressure over time.

In vivo atrial electrophysiology and AF inducibility test

In vivo atrial electrophysiology and AF inducibility tests were performed as previously described.[9,10] In brief, a 1.6F octapolar Millar electrophysiology catheter (EPR-802; Millar Instruments, Inc, Houston, TX) was advanced into the right atrium (RA) through the right jugular vein. The catheter has 8 poles, with 3 pairs of electrodes for recording RA electrograms and 1 pair for pacing. Standard surface electrocardiogram lead II and 3 RA intracardiac electrograms were recorded using a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO).

Standard S1-S2 pacing protocol was used to determine the atrial effective refractory period (ERP). The atria were paced at 3× threshold at a cycle length of 150 ms. Atrial ERP was defined as the longest coupling intervals that did not capture the atria. ERP was first narrowed down by shortening the S2 interval in 5-ms decrements and then determined by 1-ms steps.

Burst pacing consisting of 200 impulses at 100 Hz was used to induce AF. Each rat received burst pacing 10 times, and the duration of subsequent AF after each burst pacing was documented. AF was defined as rapid irregular atrial activations with varying electrographic morphology lasting $\geq$0.5 second, as reported previously.[9] For each animal, AF inducibility was calculated as a percentage of the times AF was induced out of the 10 times burst pacing was delivered.

Table 1 Echocardiographic parameters in the 3 studied groups

	Control (n = 9)	EtOH (n = 9)	EtOH+BB (n = 10)
AWTd (mm)	1.18 ± 0.08	1.21 ± 0.04	1.19 ± 0.05
AWTs (mm)	2.14 ± 0.08	2.16 ± 0.05	2.15 ± 0.10
LVDd (mm)	7.15 ± 0.73	7.00 ± 0.54	7.02 ± 0.40
LVDs (mm)	3.78 ± 0.54	3.89 ± 0.50	4.01 ± 0.34
PWTd (mm)	1.19 ± 0.07	1.21 ± 0.04	1.19 ± 0.05
PWTs (mm)	2.19 ± 0.09	2.20 ± 0.05	2.15 ± 0.10
FS (%)	46.1 ± 3.6	44.4 ± 4.6	43.2 ± 2.4
LADd (mm)	3.48 ± 0.51	3.55 ± 0.34	3.45 ± 0.39

AWTd = left ventricular anterior wall thickness in diastole; AWTs = left ventricular anterior wall thickness in systole; EtOH = alcohol injected group; EtOH+BB = alcohol-injected rats pretreated with beta-blocker; FS = left ventricular fractional shortening; LADd = left atrial diameter in diastole; LVDd = left ventricular diameter in diastole; LVDs = left ventricular diameter in systole; PWTd = left ventricular posterior wall thickness in diastole; PWTs = left ventricular posterior wall thickness in systole.

AF duration for each animal was calculated based on the average AF duration induced by the 10 burst pacing trials.

Statistical analysis

Data are expressed as mean ± SD where appropriate. Body weights and cardiac functional parameters were normally distributed, and comparisons among the 3 groups were first evaluated using 1-way analysis of variance, followed by *post hoc* analysis with Bonferroni correction for multiple comparisons. Nonnormally distributed AF inducibility and duration data are expressed as median with first and third quartile (Q1–Q3) values. A nonparametric Kruskal-Wallis test followed by Mann-Whitney U tests were used to compare AF inducibility and AF duration data. All tests were 2-tailed. $P < .05$ was considered significant.

Results

General condition

Only 1 rat in the alcohol group died suddenly (presumably due to ventricular arrhythmia); all other animals completed the study. Animals in both alcohol groups lost weight. Compared with weights before alcohol injection, body weights decreased by 11.8% ± 5.9% in the EtOH group and by 12.4% ± 5.5% in the EtOH+BB group (both $P < .01$), whereas a slight increase in body weight was observed in the saline control group (2.0% ± 2.9%; $P > .05$).

Echocardiographic parameters

Alcohol injection did not significantly affect LV wall thickness or chamber dimension in rats. No significant differences in all echocardiographic parameters among the 3 studied groups were observed (Table 1).

LV hemodynamic parameters

Alcohol injection did not significantly affect LV hemodynamic in rats. No significant differences in LV hemodynamic parameters among the 3 studied groups were observed (Figure 1).

Atrial electrophysiology, AF inducibility, and AF duration

Heart rates were lower in both alcohol groups compared with the control group (267 ± 27 bpm EtOH; 259 ± 35 bpm EtOH+BB; 281 ± 23 bpm control), but the difference was not statistically significant ($P > .05$).

The EtOH group had the shortest atrial ERP and the BB group had the longest ERP (36.8 ± 9.9 ms EtOH; 42.9 ± 8.2 ms EtOH+BB; 38.1 ± 4.3 ms control) (Figure 2). However, the difference among the 3 groups did not reach statistical significance ($P > .05$).

Significant differences were observed in AF inducibility and duration among the 3 studied groups (Figure 3). In the control group, AF was induced in 1 of 9 rats, with calculated AF inducibility of 0% (0%–0%) and duration of 0 second (0–0 second). In the EtOH group, AF was induced in 8 of 9 rats, with calculated AF inducibility of 60% (25%–100%) and duration of 0.81 second (0.24–3.67 seconds). In the EtOH+BB group, AF was induced in 4 of 10 rats, with AF inducibility of 0% (0%–22.5%) and duration of 0 second (0–0.2 second). Thus, compared to the control group, the EtOH group had significantly increased AF inducibility ($P < .05$) as well as AF duration ($P < .05$). Compared with the EtOH group, BB pretreatment decreased AF inducibility and AF duration (both $P < .05$).

Discussion

Major findings

This study demonstrated that alcohol injection in rats significantly increased AF inducibility and AF duration. Our results are consistent with a previous study that used the same alcohol injection protocol but AF inducibility was tested *in vitro* in isolated mouse and rabbit hearts.[6] This study confirmed our hypothesis that BB (metoprolol) pretreatment can significantly reduce alcohol-enhanced AF inducibility in rats, which is consistent with a previous case report that BB pretreatment prevented alcohol consumption–induced AF in a patient.[5]

Alcohol consumption and AF

Excessive alcohol consumption or binge drinking is associated with increased AF risk, even in young, apparently healthy adults.[2,11] The arrhythmias induced by binge drinking are also known as the "holiday heart syndrome," and AF is the most frequently diagnosed arrhythmia in holiday heart syndrome.[3] Alcohol consumption can promote AF, but the exact mechanism(s) underlying alcohol-enhanced AF has not been fully elucidated.

More than half a century ago, Gimeno et al[12] reported that ethanol at concentrations between 24 and 192 mM (110–880 mg/100 mL) depressed the contractility of rat atria and shortened action potential duration *in vitro*. They suggested that the decreased action potential duration may predispose the atria to arrhythmias, such as ectopic beats and AF. A recent report showed that ethanol at much lower concentrations (1–3 mM) could enhance AF vulnerability via shortening of atrial ERP

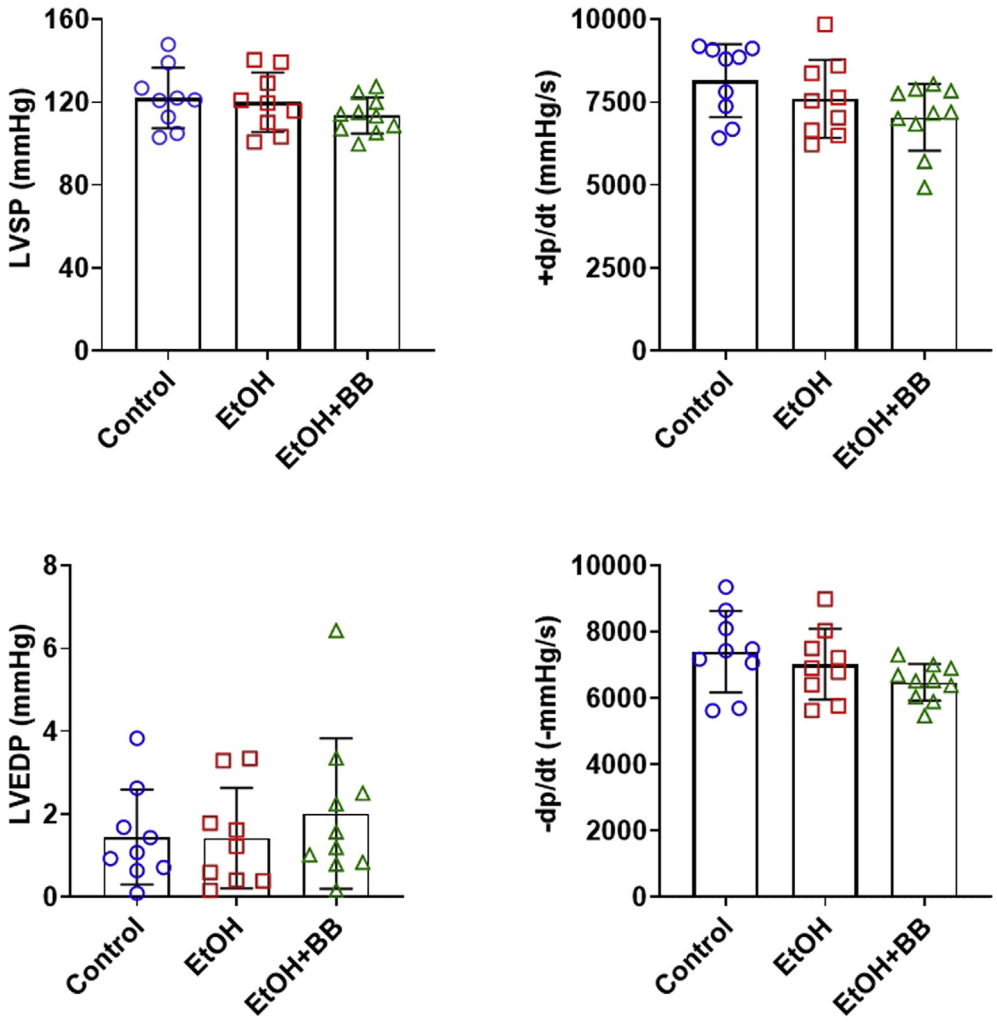

Figure 1 Left ventricular hemodynamics. Values are presented as mean ± SD. +dP/dt = positive change in pressure over time; –dP/dt = negative change in pressure over time; EtOH = alcohol-injected group; EtOH+BB = alcohol-injected rats pretreated with beta-blocker; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure.

and decreasing conduction velocity in isolated rat hearts.[13] The ERP-abbreviating effect of alcohol has also been reported in patients.[14] In this study, although atrial ERP was shorter in the alcohol group, the difference was not statistically significant. This most likely is due to the timing when the measurement was taken. In this study, ERP was measured 24 hours after alcohol consumption, at which time the alcohol had already been eliminated from the body, as previously reported.[6]

Alcohol-induced cardiac ryanodine receptor dysfunction is another potential mechanism mediating alcohol-enhanced AF. A recent study showed that alcohol could result in cardiac ryanodine receptor dysfunction and calcium leak, enhancing AF arrhythmogenesis, which reportedly was mediated by the stress kinase JNK and calmodulin kinase II pathway.[6] The role of ryanodine receptor dysfunction in AF arrhythmogenesis is becoming a topic of interest,[15] and its role in alcohol-enhanced AF deserves further investigation.

Of note, in this study the rats in both alcohol groups lost body weight, whereas the control animals gained some weight by the end of the 8-day experiment. This finding is different from a previous report that alcohol injection did not affect body weight.[6] The reason for this discrepancy is unknown. Cardiac function was not significantly affected by alcohol injection, as evaluated by both echocardiography and LV hemodynamic measurements in this study. These findings are consistent with a previous report in mice and rabbits[6] but are in contrast to the cardiac depressive effect observed during alcohol perfusion in isolated hearts.[12] Again this probably is due to the fact that in our study, cardiac function was determined 24 hours after alcohol injection, at which time the alcohol had already been eliminated from the body.[6]

BB therapy in AF

The utility of BB therapy in the management of AF has been firmly established.[16–18] In particular, by inhibiting atrioventricular conduction, BBs are useful in achieving ventricular rate control, one of the pillars of therapy along with anticoagulation that help to control the symptoms of AF and provide stroke prophylaxis. BBs have also been

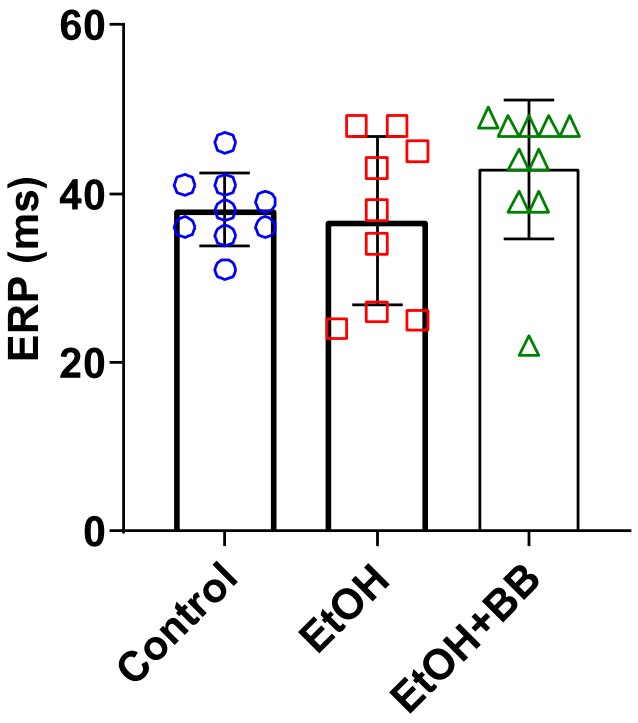

Figure 2 Atrial effective refractory period (ERP). Values are presented as mean ± SD. EtOH = alcohol-injected group; EtOH+BB = alcohol-injected rats pretreated with beta-blocker.

demonstrated to reduce AF risk, especially in patients with coronary heart disease and heart failure.[19] Although BBs can be used for AF prevention, they may not be completely effective in every scenario,[20,21] and the mechanisms underlying the preventive benefits of BBs have not been completely elucidated.

In the case report discussed previously, a novel BB pretreatment regimen given 30 minutes before alcohol consumption could prevent alcohol-induced AF.[5] To provide further evidence to confirm this important observation, the current study was designed to investigate whether BB pretreatment can prevent/reduce AF in an animal model. Our results clearly confirmed our hypothesis that BB pretreatment can significantly decrease alcohol-enhanced AF inducibility in rats.

Rats pretreated with BB had the longest atrial ERP among the 3 studied groups, but the difference was not statistically significant. This probably is due to the fact that alcohol had already been eliminated from the blood when atrial ERP was measured 24 hours after alcohol consumption. Based on previous reports that alcohol shortens atrial ERP,[12,13] it could be speculated that if atrial ERP had been measured sooner (ie, within a few hours after alcohol injection), it could have been significantly shorter. Nevertheless, it is interesting and important to further investigate the potential mechanisms through which BB pretreatment exerts benefits in preventing alcohol-enhanced AF.

Study limitations

This proof-of-concept study was designed to confirm whether BB pretreatment can prevent alcohol-enhanced AF. It was not designed to explore in detail the underlying mechanism(s). Thus, whether BB pretreatment can prevent

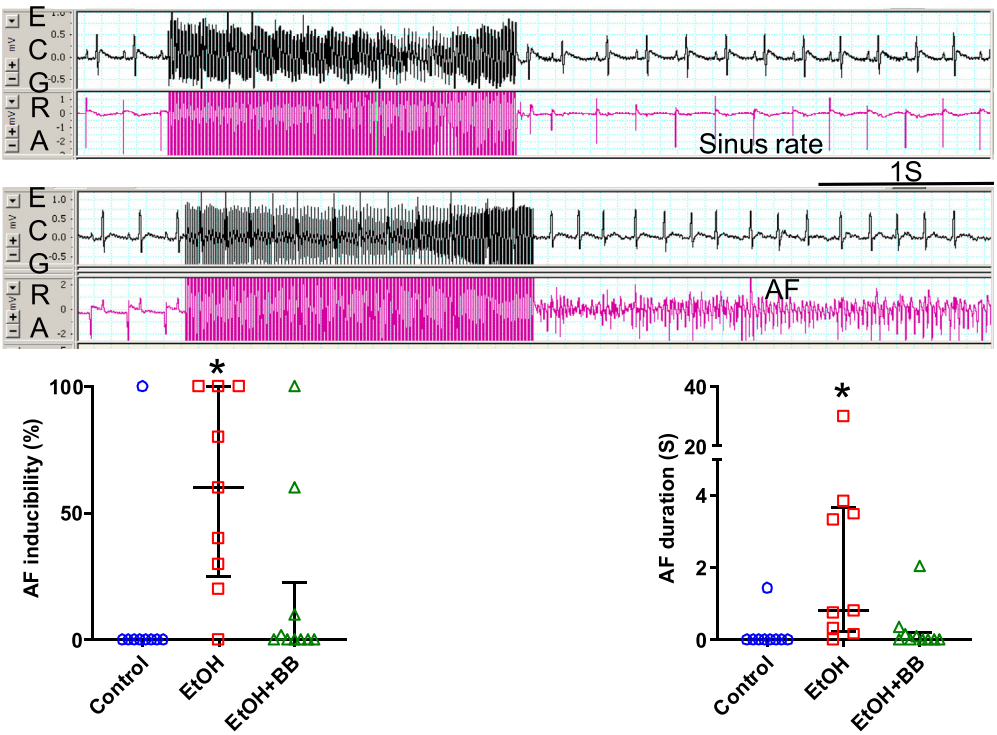

Figure 3 Atrial fibrillation (AF) inducibility and duration. **Top, middle:** Original electrocardiogram (ECG) (lead II) and right atrial electrocardiogram (RA) traces. **Top:** Burst pacing did not induce AF. **Middle:** AF was induced immediately after burst pacing. **Bottom:** AF inducibility **(left)** and duration **(right)** data were not normally distributed. Values are presented as median (first and third quartile), with original numbers shown. *P <.05 vs control and EtOH+BB groups. EtOH = alcohol-injected group; EtOH+BB = alcohol-injected rats pretreated with beta-blocker.

alcohol-induced atrial ERP shortening and/or stabilize the cardiac ryanodine receptor remain to be studied.

Although the current study confirmed the potential benefits of BB pretreatment in preventing/reducing alcohol-enhanced AF in rats, only 1 case report has suggested it is an effective treatment in patients.[5] More studies in humans and animals are warranted to further confirm the clinical efficacy and delineate potential mechanisms underlying this promising strategy.

Conclusion

This study evaluated the effects of a novel BB pretreatment regimen given immediately before alcohol exposure in a rat model as an alternative method of preventing alcohol-induced AF. Alcohol significantly increased AF inducibility in rats, and pretreatment with metoprolol significantly reduced AF inducibility. The results correlate well with a previously published clinical case report. The effectiveness of this treatment modality should be explored further in a more extensive human trial.

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