

Research Article

# Effects of Artemisinin on Peak Sodium Current in Ventricular Myocytes

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## Abstract

**Background:** Previous studies have confirmed that artemisinin can prevent arrhythmia by inhibiting K<sup>+</sup> currents. Recent findings have shown that artemisinin attenuates sodium current in nodose ganglion and endocrine cells of rats. This study investigated the effects of artemisinin on peak sodium current in ventricular myocytes.

**Methods:** Rat ventricular myocytes were isolated by Langendorff reverse aortic perfusion method. Peak

sodium current was recorded using the whole-cell patch clamp technique.

**Results:** The  $I_{Na}$  was reduced by 50  $\mu$ M artemisinin, and the steady-state activation and inactivation curves were shifted toward the left. The time constant  $\tau$  of the steady-state recovery curve increased from 2.89 ms to 7.13 ms.

**Conclusions:** Artemisinin attenuates  $I_{Na}$  by modulating the voltage dependence of the Na<sup>+</sup> channel similar to the class I anti-arrhythmia agents.

**Keywords:** Artemisinin; Peak Sodium Current; Anti-arrhythmias

## Introduction

Artemisinin (Art), as an effective antimalarial agent [1], has been widely used for decades. Several other pharmacological effects of artemisinin have been demonstrated, such as cytotoxicity against tumors and cancers [2-3] and antiarrhythmic effects [4]. Previous studies have shown that inhibition of multiple potassium channels, including inwardly rectifying potassium current ( $I_{K1}$ ), transient outward potassium current ( $I_{to}$ ), and delayed outward rectifier potassium current ( $I_K$ ) with similar potency, as well as prolongation of action potential duration (APD), are the major mechanisms of the antiarrhythmic effects of artemisinin [5]. A previous study reported that artemisinin affected the amplitude of ionic current in intact nodose ganglion neurons of adult rats by blocking the voltage-gated  $Na^+$ ,  $K^+$ , and N-type  $Ca^{2+}$  channels, suggesting the probable mechanism of anti-arrhythmia [6]. Recent research has shown the ability of artemisinin to attenuate the voltage-gated  $Na^+$  ( $I_{Na}$ ) and delayed-rectifier  $K^+$  current ( $I_{K(DR)}$ ) in endocrine or neuroendocrine cells [7]. All these data show the inhibitory effects of artemisinin on voltage-gated  $Na^+$  channels in various cells, but little is known about its effects on ventricular myocytes.

Our previous research confirmed that artemisinin has an antagonistic effect on ventricular arrhythmias induced by increased left ventricular after load [8]. Hence, the present study was undertaken to investigate the effects of artemisinin on peak sodium currents of ventricular myocytes to further understand the mechanism of antiarrhythmic actions of artemisinin.

## Abbreviations

APD: Action potential duration

Art: Artemisinin

$I_K$ : Delayed outward rectifier potassium current

$I_{K1}$ : Inwardly rectifying potassium current

$I_{Na}$ : Peak sodium current

$I_{to}$ : Transient outward potassium current

## Materials and methods

**Solution** The standard Tyrode's solution (in mM): NaCl 135, KCl 5.4, HEPES 10,  $NaH_2PO_4$  0.33,  $MgCl_2$  1,  $CaCl_2$  1.8, Glucose 10, pH adjusted to 7.4 with NaOH. KB solution(in mM): KCl 40, HEPES 10, EGTA 0.5,  $MgCl_2$  3, Glucose 10, taurine 20, L-Glutamic acid 50,  $KH_2PO_4$  20, pH adjusted to 7.4 with KOH.  $I_{Na}$  Bath solution(in mM): NaCl 20, Choline Chloride 115,  $MgCl_2$  1,  $CaCl_2$  1.8,  $BaCl_2$  0.3, CsCl 5.4, Glucose 10, HEPES 10,  $CdCl_2$  0.1, pH adjusted to 7.4 with NaOH.  $I_{Na}$  Pipet solution(in mM): CsCl 120, HEPES 10, EGTA 10,  $Na_2ATP$  5,  $MgCl_2$  5,  $CaCl_2$  1, Glucose 10, pH adjusted to 7.2 with CsOH.

Artemisinin was dissolved with DMSO and diluted with deionized water to prepare concentration of 50  $\mu M$ . Nifedipine was prepared at a concentration of 10 mM to inhibit the calcium current. All reagents were purchased from Sigma-Aldrich (Beijing, China).

## Isolation of the ventricular myocytes

The animal experiments complied with the Guide for Care and Use of Laboratory Animals drafted by the Institutional Medical Ethics Review Board of Peking University People's Hospital.

The animals used in this study were Eleven to twelve-weeks old male Wistar rats, purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.. The method of isolation of the ventricular myocytes was described previously [9]. Briefly, heart was quickly removed and cannulated on a Langendorff device and

perfused with Tyrode's solution (36.5°C) for 5 min. Then changing to Ca<sup>2+</sup> free Tyrode's solution (standard Tyrode's solution without CaCl<sub>2</sub>) for 10 minutes. Next the heart was enzymatically digested for a period of 15-20 min with a solution containing collagenase II (200-230 U · L<sup>-1</sup>). Left ventricular tissue was then excised from the softened hearts and minced gently and filtered with mesh filter. The myocytes were incubated in the KB storage medium at 4°C temperature.

### Whole-cell patch clamp recordings

$I_{Na}$  were recorded in a single ventricular myocyte by whole-cell patch clamp in a voltage clamp mode. The resistance of micropipettes with pipette solution were 2-4 MΩ. An HEKA EPC10 USB single amplifier was used to record current with low pass filtered rate at 3kHz and digital rate at 10kHz. The junction potentials and series resistance were electrically compensated and no leakage correction was applied.

### Statistical analyses

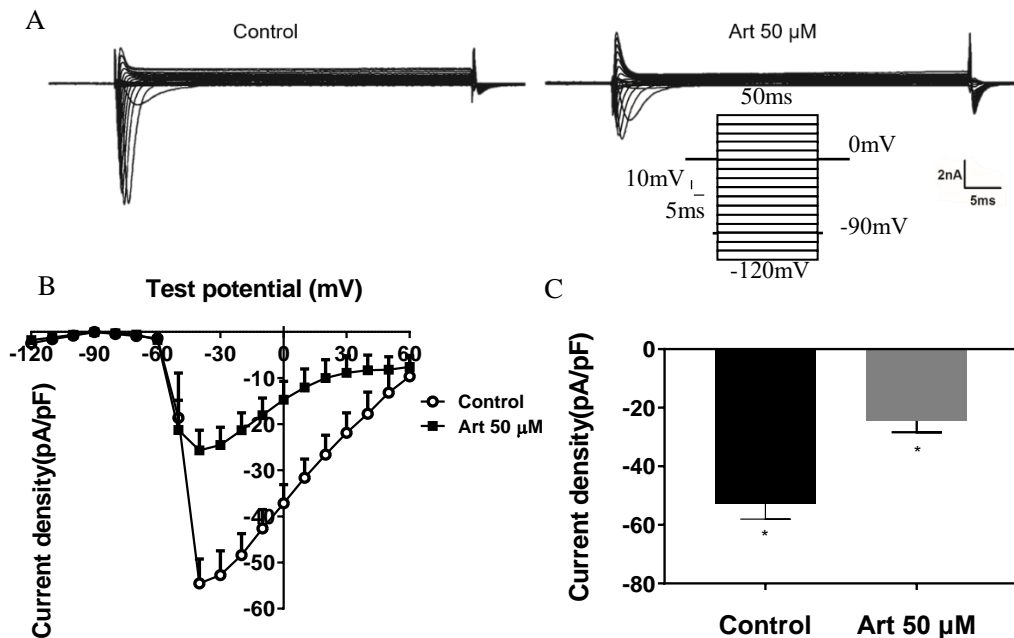
In order to correct the current error caused by a discrepancy in the cardiomyocyte size, the current was shown as the current density alternatively. All continuous variables were represented by the mean ± SEM. The differences between the two groups were determined by student's t-test. A p value of <0.05 was considered statistically significant. All analyses were performed by SPSS version 20.0, and curve fitting was performed by GraphPad Prism version 7.0.

## Results

### Artemisinin decreased $I_{Na}$ in ventricular myocytes

$I_{Na}$  was record by a 50 ms depolarization pulse increasing from -120 mV to 50 mV with holding potential at -90 mV. As shown from current traces in (Figure 1A),  $I_{Na}$  was inhibited significantly with 50 μM artemisinin in the ventricular myocytes. The current-

voltage relationship illustrated the effect of artemisinin on  $I_{Na}$ , especial from -40 mV to 40 mV testing potential (Figure 1B). At -30 mV testing potential, the maximum current density of  $I_{Na}$  was downregulated from  $-52.7 \pm 5.28$  to  $24.51 \pm 3.85$  (n = 5, p < 0.05, Figure 1C).

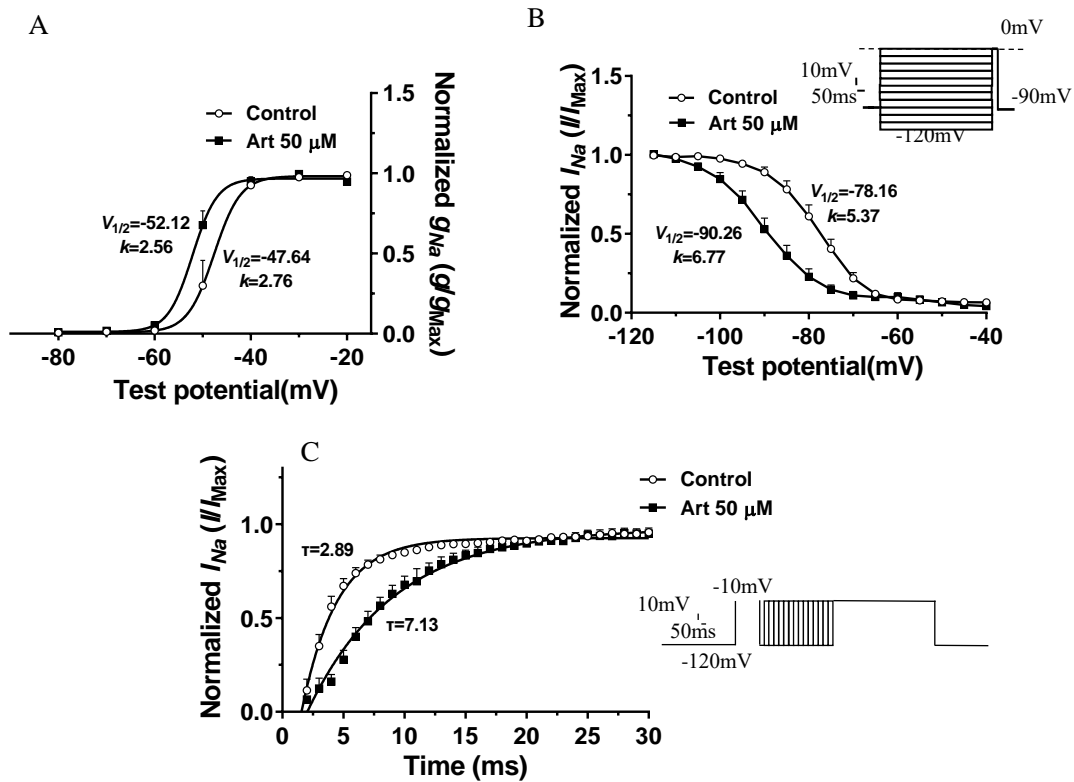


**Figure 1:** Effects of artemisinin on  $I_{Na}$  in ventricular myocytes. (A) current traces of  $I_{Na}$  in the absence or presence 50  $\mu$ M artemisinin. (B) current-voltage (I-V) relationship of  $I_{Na}$  before and after application of 50  $\mu$ M artemisinin. (C) Summarized data of maximum current density of  $I_{Na}$  ( $p < 0.05$  vs. control).

### The voltage dependence of sodium channel was shifted more negatively by artemisinin

In order to illustrate the inhibitory effects of artemisinin on  $I_{Na}$ , voltage dependence tests of the  $Na^+$  channel were conducted. We analyzed the steady-state activation curve with the same stimulus protocol as mentioned above in current-voltage recording. As shown in Figure 2A, exposure to 50  $\mu$ M artemisinin, the steady-state activation shifted towards the left.  $V_{1/2}$  was decreased from -47.64 mV to -52.12 mV and the slope factor  $k$  changed from 2.76 to 2.56. This suggested that 50  $\mu$ M artemisinin altered the voltage-dependence of the steady-state activation of  $I_{Na}$  negatively.

We also examined the effect of artemisinin on inactivation kinetics for  $I_{Na}$ . The steady-state inactivation curve was conducted by a stimulus, which a condition pulse increasing from -120 mV to 0 mV was used to inactivate  $Na^+$  channel adequately prior to the testing pulse to 0 mV. In presence of 50  $\mu$ M artemisinin, the steady-state inactivation curve illustrated in Figure 2B was also shifted negatively.  $V_{1/2}$  was decreased from -78.16 mV to -90.26 mV and the slope factor  $k$  changed from 5.37 to 6.77. These results demonstrated that a further negative potential was required for the activation and inactivation of the  $Na^+$  channel, resulting in facilitating activation and inactivation at rest potential.



**Figure 2:** Effects of artemisinin on steady-state activation(A), inactivation(B), and recovery curves(C) of Na<sup>+</sup> channel.  $V_{1/2}$  was reduced from -47.64 mV and -78.16 mV to -52.12 mV and -90.26 mV, respectively, and the slope factor  $k$  changed from 2.76 and 5.37 to 2.56 and 6.77, respectively. Time constant  $\tau$  was increased from 2.89 ms to 7.13 ms.

### Artemisinin delayed recovery of sodium channel

The steady-state recovery curve was conducted by a condition pulse to -10 mV with holding potential at -120 mV, following a testing pulse to -10 mV with a time interval increasing by 1 ms. As illustrated in Figure 2C, the steady-state recovery curve became less sharp with 50  $\mu$ M artemisinin and the time constant  $\tau$  was increased from 2.89 ms to 7.13 ms which indicated that Na<sup>+</sup> channel recovered slower from inactivation by 50  $\mu$ M artemisinin.

### Discussion

In the present study, we found that artemisinin inhibited  $I_{Na}$  in ventricular myocytes and changed the voltage dependence and recovery of sodium channel.

Artemisinin attenuating  $I_{Na}$  shows similar action to class I antiarrhythmic agents, which decreasing the automaticity and conduction [10]. Three states of Na<sup>+</sup> channel (rest, activated, and inactivated) vary in their affinity to the class I antiarrhythmic agents, and activation and inactivation states are higher than rest. Further, different agents show a discrepancy in affinity with these three states of Na<sup>+</sup> channels. For example,

propafenone has the strongest affinity with open channels, while lidocaine and mexiletine mainly bind to inactivated channels. Furthermore,  $I_{Na}$  blockers have an unlikely dissociation rate which determines use dependence. Lidocaine and mexiletine dissociate quickly from the  $Na^+$  channel and shows no blocking effect on  $I_{Na}$  at a normal heart rate. However, other  $I_{Na}$  blockers with longer dissociation duration block  $I_{Na}$  at a non-tachycardic heart rate [11]. It's exactly the diversity of dynamic characteristics interacting with  $Na^+$  channel determines the different functions of  $I_{Na}$  blockers.

The present study showed that artemisinin promoted a leftwards shift of the steady-state activation and inactivation curves with more negative  $V_{1/2}$ . The results indicated that the threshold potential of  $Na^+$  channel was close to the resting potential, facilitating opening of  $Na^+$  channels, but inactivation of  $Na^+$  channel was also promoted with more negative voltage dependence. As a consequence,  $I_{Na}$  was downregulated by less  $Na^+$  channel available at the resting potential. In addition, the recovery time constant  $\tau$  was increased accompanied by a less sharp slope of the recovery curve indicating that the  $Na^+$  channels recovered gently which enhanced their blocking actions in presence of a slower heart rate. By and large, artemisinin may work on all three state channels of  $Na^+$  channel, which contributes to the opening of the  $Na^+$  channel, but more negative inactivation voltage and slower recovery reduce the number of available channels leading to  $I_{Na}$  diminution. But, which state of  $Na^+$  channel showing stronger affinity with artemisinin hasn't make out in our study.

## Conclusion

Previous research demonstrated the nonselective inhibition on potassium currents might be the antiarrhythmic mechanism of artemisinin. In present study we showed artemisinin inhibited the peak sodium

current but the antiarrhythmic effect of decreasing automaticity and conduction we haven't demonstrated. These results show that artemisinin may play both class I and class III antiarrhythmic functions. There we didn't include the concentration dependence of artemisinin on peak sodium current, so the applicability of artemisinin needs further study to illustrate.

## Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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## Author contributions

Participated in research design: Huanqiu Song, Yuansheng Liu and Dong Han

Conducted experiments: Huanqiu Song, Lihua Liu and Jiatong Wu.

Contributed new reagents or analytic tools: Huanqiu Song and Yuansheng Liu

Performed data analysis: All authors

Wrote or contributed to the writing of the manuscript: Huanqiu Song, Yuansheng Liu and Dong Han

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