L-type Calcium Current ($I_{Ca,L}$) and Inward Rectifier Potassium Current (I_{K1}) are Involved in QT Prolongation Induced by Arsenic Trioxide in Rat

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Key Words

As₂O₃ • QT prolongation • Rat ventricular cardiomyocytes • Action potential • I_{K1} • $I_{Ca,L}$ • Intracellular calcium

Abstract

The present study was designed to study the effects of As₂O₃ on QT interval prolongation and to explore the potential ionic mechanisms in isolated rat ventricular cardiomyocytes. The rats of As₂O₃ group were treated with 0.8 mg·kg⁻¹·d⁻¹ As₂O₃ intravenously for 7 days consecutively and the control group with saline. The ECG was recorded to calculate heart rate-corrected QT interval (QTc). Single cardiomyocytes were isolated by using collagenase II, and the action potential duration (APD) and ion currents were recorded by whole-cell patch clamp. [Ca²⁺]_i was examined by confocal laser scanning microscopy. Our data showed that both QTc and APD were prolonged significantly after As₂O₃ treatment. Meanwhile, As₂O₃ suppressed I_{K1} and shifted the reversal potential to more positive direction. Moreover, the density of $I_{Ca,L}$ was augmented significantly, and the steady-state activation curve became more negative, whereas, the inactivation and reactivation of $I_{Ca,L}$ were not changed notably after As₂O₃ administration. Furthermore, the

maximal [Ca²⁺]_i was enhanced obviously by either KCl or caffeine stimulation in As₂O₃-treated cardiomyocytes. Our results show that the potential mechanism of As₂O₃-induced QT interval prolongation in rat might be relative to disturbing the fine balance of transmembrane currents (increasing $I_{Ca,L}$ and decreasing I_{K1}) and causing APD prolongation.

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Introduction

Acute promyelocytic leukaemia (APL) is known as a fatal disease in hematological system that strikes especially young people, and the use of As₂O₃ to treat APL was began in the 1970s at Harbin Medical University [1]. Thereafter, in the mid-1990s, As₂O₃ was reported as an effective remedy for successfully curing as many as 90% of patients with APL [2]. Since then, As₂O₃ has widely been used for the therapy of patients with relapsed or refractory acute promyelocytic leukaemia [3-5]. However, the clinical use of As₂O₃ has been restricted by its dose-dependent toxicity including cardiac toxicity, such as QT interval prolongation. In the previous clinical report, the occurrence of QT interval prolongation and

tachycardia (torsades de pointes) observed in the APL patients after As₂O₃ showed a great impact on ventricular fibrillation and sudden cardiac death [6-9]. Extensive attention to the adverse effect of As₂O₃, especially its cardiac toxicity, has been drawn by both clinical and basic researchers.

It has been well documented that the membrane repolarization of cardiomyocytes, especially action potential (AP) plateau phase, is maintained by an exquisite equilibrium between inward and outward currents. The prolongation of action potential duration (APD) in isolated papillary muscle of guinea pig has been reported by the treatment with clinically equivalent concentrations of As₂O₃ [10].

It is well known that inward Na⁺ currents generate the depolarization, whereas, outward K⁺ currents produce the repolarization of the myocytes. Calcium current (I_{Ca}) [11-13], the primary inward current of the plateau phase, is also a major determinant of the rate of the cellular membrane repolarization. It has been demonstrated that As₂O₃ could induce QT interval prolongation and the increase in I_{Ca} as well in isolated ventricular cardiomyocytes of guinea pig [8]. The L-type calcium channels functionally expressed in myocardium play a key role in managing the intracellular Ca²⁺ ([Ca²⁺]_i) and also the APD of the rat cardiomyocytes since apparent I_{Kr} and I_{Ks} are lacking in this species [14, 15]. An increase in [Ca²⁺]_i has been considered as one of the crucial signals of inducing cell apoptosis [16]. [Ca²⁺]_i increase in the cardiac tissue has been related to a variety of abnormalities, including ventricular arrhythmias and contractile dysfunction [17].

In addition, the K⁺ currents (I_K) [18-20] are also substantial in controlling the speed of membrane repolarization. Function and density alterations of the cardiac K⁺ channel might be responsible for some pathophysiological results in various cardiac diseases, such as heart failure, myocardial ischemia, and also malignant arrhythmias [21, 22]. A study on the molecular mechanisms for the abnormalities of cardiac electrophysiology induced by As₂O₃ indicated decreased trafficking of hERG channels to cell surface as an underlying ionic mechanism for the QT interval prolongation and torsades de pointes in patients receiving As₂O₃ administration [23].

Nevertheless, to the best of our knowledge, the exact mechanisms of QT interval abnormality by As₂O₃ have not been well elucidated, thus, in the present study, we used male Wistar rats that were treated with As₂O₃ for 7 consecutive days with a relevant clinical concentration

to explore the potential mechanisms of As₂O₃-induced QT interval prolongation.

Materials and Methods

Animals

Male Wistar rats (230~300 g) selected in the present study were provided by the Experimental Animal Center of Harbin Medical University (Grade II), China. The animals were conditioned at room temperature (23 ± 1°C), with a constant humidity (55 ± 5%) for a week, and had free access for food and tap water. The protocols for animal handling were pre-approved by the Institutional Animal Care and Use Committee of Harbin Medical University.

Measurement of QT Interval

The three-lead surface electrocardiogram (ECG) were collected on anaesthetized rats treated with sodium pentobarbitone (40 mg·kg⁻¹, intraperitoneally). The experimental animals with normal ECG were randomly divided into two groups. The As₂O₃ group: 0.8 mg·kg⁻¹·d⁻¹ As₂O₃ was administered intravenously for 7 consecutive days; and the control group: the same volume of saline was given under the same experimental condition. The ECG was recorded from both groups for two hours before and after 7 days, and the heart rate-corrected QT interval (QTc) was calculated with the Bazett formula: QTc = QT/(RR)^{1/2}, an accepted method for correcting QT interval in rats [24]. The measurement of QT interval was started from the onset of QRS complex to the end of the T wave, whereas RR interval was considered as the distance between the apexes of consecutive R waves.

Isolation of Adult Rat Ventricular Cardiomyocytes

The procedure for cardiomyocytes dissociation was described previously [25, 26]. Briefly, after pentobarbital sodium anesthetization, the heart of rat was rapidly excised, clipped, and perfused in a retrograde fashion through the aorta with Ca²⁺-containing Tyrode's solution (in mM: NaCl 136, KCl 5.4, MgCl₂ 1, CaCl₂ 1.8, Hepes 10, and Glucose 10, pH 7.4 with NaOH) at 37 °C until the effluent was clear of blood. Then the heart was switched to Ca²⁺-free Tyrode's solution for about 10 min at a constant rate of 10 ml·min⁻¹ until the heart stopped beating, and then followed by enzyme perfusion with the same solution containing collagenase (type II, 7.8 mg : 50 ml) and bovine serum albumin (8 mg : 50 ml). The ventricular tissue was removed after it softened, minced, and preserved in KB solution (in mM: Glutamic acid 70, Taurine 15, MgCl₂ 0.5, KCl 30, KH₂PO₄ 10, Hepes 10, EGTA 0.5, and Glucose 10, pH 7.4 with KOH). All solutions were oxygenated at 37° C with 95% O₂ and 5% CO₂. Single cell was obtained by gentle pipetting and stored at 4°C for 1 ~ 2 h before patch-clamp recording. Only single rod-shaped quiescent cells with clear cross-striation were selected for electrophysiological measurements.

Patch-clamp Recording

The cardiomyocytes were laid in a recording chamber placed on an inverted microscope (Nikon Diaphot Eclipse TE

Fig. 1. Effects of As_2O_3 on QT interval recorded from surface ECG in rat *in vivo* and APD in rat ventricular cardiomyocytes. (A) Representative traces of ECG. QT intervals from control (upper panel) and As_2O_3 -treated rats (lower panel). (B) Relative levels of corrected QT interval ($\text{QTc} = \text{QT}/(\text{RR})^{1/2}$). (C) Representative action potential traces from control (solid line) and As_2O_3 -treated group (dotted line). APD was prolonged significantly in As_2O_3 -treated group. (D) APD_{50} and APD_{90} were prolonged significantly in the As_2O_3 -treated group compared with control. Average data were mean $\pm$ SEM. $n = 10$ rats for Figure A, B and $n = 11, 14$ ventricular cardiomyocytes for Figure C, D, $**P < 0.01$ vs. control.

2000, Japan) and bathed in an extracellular solution for about 10 min at room temperature ($23 \pm 1^\circ\text{C}$) before superfusion at a rate of $0.5 \sim 1 \text{ ml} \cdot \text{min}^{-1}$.

Borosilicate glass electrodes prepared by a two-stage pull on a vertical puller (PP-830, Narishige, Japan) were filled with pipette solution to obtain the tip resistance of $2 \sim 4 \text{ M}\Omega$.

Membrane currents and AP were recorded in the voltage-clamp and current-clamp mode, respectively, using an Axopatch-200B amplifier (Axon Instruments).

Command pulses were generated by a D/A converter (Digidata 1322, Axon Instruments) driven by pCLAMP 9.2 software. Data were low-pass filtered at 1 kHz. Junction potentials were offset before formation of the giga-seal in extracellular solution. After giga-seal, the membrane was ruptured by gentle suction to establish the whole-cell configuration, as described previously [25, 26]. The whole-cell capacitance and series resistance were compensated (typically $75\% \sim 85\%$) and the leak currents were subtracted. The measured currents were normalized by whole-cell capacitance and presented as the current density (pA/pF).

The solution composition for Ca^{2+} currents recording: extracellular solution (in mM: Tris-Cl 136, CsCl 5.4, CaCl_2 2, MgCl_2 1, HEPES 10, and Glucose 5, pH 7.4 with Tris-OH); pipette solution (in mM: CsCl 20, MgCl_2 1, Mg-ATP 5, EGTA 10, and CsOH 110, Aspartate 110, pH 7.2 with CsOH). For AP and K^+ currents recording: extracellular solution, the same composition as Ca^{2+} -containing Tyrode's solution; pipette solution (in mM: KCl 20, KOH 110, Aspartate 110, MgCl_2 1, Hepes 5, EGTA 10, $\text{Na}_2\text{-ATP}$ 5, pH 7.2 with KOH).

Measurement of Intracellular Calcium Concentration ($[\text{Ca}^{2+}]_i$)

Intracellular Ca^{2+} imaging of ventricular cardiomyocytes loaded Fluo-3/AM ($5 \mu\text{M} \cdot \text{L}^{-1}$) [27] was performed by a Confocal Laser Scanning Microscope (Olympus FV-300) with 488 nm beam for excitation from an argon ion laser and 530 nm

beam for emission. Acquisition rate of $[\text{Ca}^{2+}]_i$ was one frame per 10 s for about 300 s in 30 mM KCl-induced group bathed in normal Ca^{2+} -containing Tyrode's solution, while one frame per 0.5 s for 120 s in 10 mM caffeine-stimulated group bathed in Ca^{2+} -free Tyrode's solution. KCl was added between 1st and 2nd scan, while caffeine between 9th and 10th scan. The magnitude of fluorescent signals was quantified in terms of FI/F_0 , where F_0 is the fluorescence of basal level and FI is the value after drug administration [28].

Drugs and Materials

As_2O_3 was the gift from Harbin YI-DA Pharmaceutical Limited Company. Fluo-3-acetoxymethyl (Fluo-3/AM) (Molecular Probes, Eugene, Oregon, USA) was dissolved in DMSO ($< 0.2\%$) and stored at -20°C in the dark. Caffeine (Sigma, USA) was stored at room temperature. Collagenase (type II, Sigma, USA) was stored at 4°C away from light.

Data Analysis

All results were expressed as mean $\pm$ SEM. Statistic analysis was performed by using paired and unpaired Student's *t*-test. Significant differences between the two groups were considered when $P < 0.05$ (two-tailed). Boltzmann function was used to fit voltage-dependent activation and inactivation curves and single exponential function was used to fit the curve of recovery from inactivation (reactivation). All graphs were drawn by GraphPad Prism 5.0.

Results

Effects of As_2O_3 on QT interval prolongation in rat hearts *in vivo*

We studied the effects of As_2O_3 ($0.8 \text{ mg} \cdot \text{kg}^{-1}$) on QTc , and the representative traces of ECG of the two

Groups	n	APD (ms)	RMP (mV)	OS (mV)	APD ₅₀ (ms)	APD ₉₀ (ms)
Control	14	134.3 ± 4.1	-70.4 ± 0.5	38.9 ± 0.9	74.7 ± 4.4	111.2 ± 4.4
As ₂ O ₃	11	234.2 ± 5.5**	-68.2 ± 1.0*	36.7 ± 1.3	105.9 ± 4.2**	194.4 ± 5.0**

Table 1. Effects of As₂O₃ on APD prolongation in rat ventricular cardiomyocytes. RMP, resting membrane potential; OS, overshoot; APD₅₀ and APD₉₀, action potential duration at 50% and 90% of repolarization, respectively. Average values were described as mean ± SEM, *n* = 11, 14 indicates the number of rat ventricular cardiomyocytes. ***P* < 0.01, **P* < 0.05 vs. control.

Fig. 2. Effects of As₂O₃ on I_{K1} in rat ventricular cardiomyocytes. (A) Voltage protocol (top panel). Representative current traces recorded from cardiomyocytes isolated from control (middle panel) and As₂O₃-treated rats (bottom panel). (B) I-V curves of I_{K1}: amplitudes measured at 300 ms for each voltage were normalized to whole-cell capacitance to obtain the current densities (pA/pF). (C) Inward current conductance (mS/cm²) of I_{K1} was calculated by the slope between -120 to -80 mV. Average values were described as mean ± SEM, *n* = 11, 16 ventricular cardiomyocytes, ***P* < 0.01 and **P* < 0.05 vs. control.

groups were presented in Fig. 1A. As described in Fig. 1B, As₂O₃ prolonged QTc significantly compared with that of control (*P* < 0.01, *n* = 10).

Effects of As₂O₃ on APD prolongation of rat ventricular cardiomyocytes

APs from isolated ventricular cardiomyocytes were recorded at 1.5 times threshold stimulus and 3 ms duration, and cells were paced at 0.1 Hz [29, 30]. Fig. 1C illustrated the representative traces showing the effects of As₂O₃ on APD prolongation in rat ventricular cardiomyocytes. Resting membrane potential (RMP), overshoot (OS), 50% and 90% repolarization of AP duration (APD₅₀ and APD₉₀, respectively) were used to describe the changes in AP waveform. The results indicated that As₂O₃ significantly prolonged APD, APD₅₀ and APD₉₀ in rat ventricular cardiomyocytes compared with that of control (*n* = 11, 14, *P* < 0.01, Table 1; Fig. 1D). Moreover, there was a decrease in the mean value of RMP or OS in the As₂O₃ group, and only the former had the significant difference compared with control (-70.4 ± 0.5 mV and -68.2 ± 1.0 mV for the control and As₂O₃ group, respectively, *P* < 0.05, Table 1).

Effects of As₂O₃ on I_{K1} in rat ventricular cardiomyocytes

I_{K1} plays an important role in the maintenance of RMP. Except for APD prolongation, As₂O₃ also depolarized APD, suggesting that the current density of I_{K1} might be down-regulated in the presence of As₂O₃. To this regard, I_{K1}'s whole-cell currents were investigated. In this experiment, the ventricular cardiomyocytes were superfused with Ca²⁺-containing Tyrode's solution. Calcium current and transient outward potassium current (I_{to}) were removed by bath application of 0.3 mM Cd²⁺ and 2.0 mM 4-AP, respectively. Sodium current was inactivated by adding 30 μmol · L⁻¹ tetrodotoxin (TTX). I_{K1} was elicited by a 300 ms depolarizing current pulse stepped from -120 mV to +50 mV with an increment of 10 mV from a holding potential of -40 mV, and the interval was 3 s (Fig. 2A). The measured current values at 300 ms were considered as the magnitudes of I_{K1}, and the normalized current values were used to describe I-V curve. As shown in Fig. 2B, compared with control group, As₂O₃ significantly decreased the current density of I_{K1} (*n* = 11, 16, ***P* < 0.01, **P* < 0.05), and the reversal potential in

Groups	Steady-state activation		Steady-state inactivation		Reactivation time constant τ (ms)
	$V_{1/2}$ (mV)	$S_{1/2}$	$V_{1/2}$ (mV)	$S_{1/2}$	
Control	-3.54 ± 1.58	6.75 ± 0.22	-20.1 ± 1.73	-6.27 ± 0.52	114.3 ± 11.9
As ₂ O ₃	$-9.56 \pm 0.82^{**}$	6.33 ± 0.12	-20.7 ± 1.83	-6.21 ± 0.48	133.3 ± 15.9

Table 2. Effects of As₂O₃ on kinetic properties of $I_{Ca,L}$ in rat ventricular cardiomyocytes. $V_{1/2}$ represents the voltage at 50% activation or inactivation. $S_{1/2}$ represents the slope factor of Boltzmann function. τ indicates the time constant of reactivation. Values were described as mean $\pm$ SEM, $n = 8$, 12 rat ventricular cardiomyocytes from each group. $^{**}P < 0.01$ vs. control.

Fig. 3. Effects of As₂O₃ on $I_{Ca,L}$ and its voltage-dependent properties in rat ventricular cardiomyocytes. (A) Voltage protocol (top panel). Representative current traces recorded from cardiomyocytes isolated from control (middle panel) and As₂O₃-treated rats (bottom panel). (B) I-V curves of $I_{Ca,L}$. The curve of As₂O₃-treated group was shifted to more negative direction vs. control. (C) The steady-state activation curve in As₂O₃-treated group was shifted to more negative and the half activation voltage ($V_{1/2}$) was changed obviously vs. control (refer to Table 2). (D) The steady-state inactivation curves and the kinetic parameters ($V_{1/2}$, $S_{1/2}$) of the two groups were not statistically different; the inset represents the recording protocol. (E) The reactivation curves and the time constant (τ) from each group were not changed significantly. The inset indicates the recording protocol. The values of $V_{1/2}$, $S_{1/2}$, and τ were given in the text. Average values were described as mean $\pm$ SEM, $n = 8$, 12 ventricular cardiomyocytes from each group. $^{**}P < 0.05$ and $^{*}P < 0.01$ vs. control.

As₂O₃ group was shifted to more positive potential (-55.1 ± 2.35 mV vs. -61.9 ± 1.81 mV, $P < 0.05$). I_{K1} inward current conductance (mS/cm²) was measured by obtaining the slope of the linear section of the I-V relationship from -120 to -80 mV [31], and the conductance of As₂O₃ group was decreased by $\sim 24.6\%$ compared with that of control, which was significantly different ($n = 11, 16$, $P < 0.01$, Fig. 2C).

Effects of As₂O₃ on $I_{Ca,L}$ and its voltage-dependent properties in rat ventricular cardiomyocytes

As described in Fig. 3A, $I_{Ca,L}$ was elicited by a 300 ms depolarizing step from -30mV to +50 mV with an increment of 10 mV from a holding potential of -40 mV, and the current was suppressed by 0.3 mM Cd²⁺. In the present experiment, the peak inward currents were measured as the magnitude of $I_{Ca,L}$. Under our experimental condition, the run-down of $I_{Ca,L}$ was less than 5% during the observation period in control (data not shown).

Our results showed that the peak current density of $I_{Ca,L}$ was significantly increased by $\sim 75.8\%$ in ventricular cardiomyocytes of As₂O₃-treated rats ($n = 16$) compared with control group ($n = 11$, $P < 0.01$, Fig. 3B). Moreover, the shape of I-V curve was changed and the voltage at the peak value of the current was shifted negatively from +10 mV to 0 mV after As₂O₃ administration (Fig. 3B).

In order to understand whether the voltage-dependent property of L-type Ca²⁺ channel was modulated by application of As₂O₃, we further examined the kinetics of $I_{Ca,L}$ and the kinetic parameters of $I_{Ca,L}$ of ventricular cardiomyocytes from both groups which were summarized in Table 2. To further demonstrate whether the increase in the peak current was related to a faster voltage-dependent activation, the voltage-dependent activation of $I_{Ca,L}$ was fitted by Boltzmann equation and showed that the activation curve of As₂O₃-treated group was shifted to more hyperpolarized direction (Fig. 3C), and the half activation voltage of As₂O₃-treated group

Fig. 4. Effects of As_2O_3 on $[\text{Ca}^{2+}]_i$ in rat ventricular cardiomyocytes. (A) Representative images of $[\text{Ca}^{2+}]_i$ changes before (a) and after (b, at peak) 30 mM KCl exposure for control, and before (c) and after (d, at peak) for As_2O_3 group. (B) The curves illustrated the effects of 30 mM KCl on $[\text{Ca}^{2+}]_i$ in both groups, and the As_2O_3 -treated group increased much more than the control group. (C) Representative images of $[\text{Ca}^{2+}]_i$ changes before (a) and after (b, at peak) 10 mM caffeine exposure for control, and before (c) and after (d, at peak) for As_2O_3 group. (D) Effects of caffeine (10 mM) on $[\text{Ca}^{2+}]_i$ in cardiomyocytes in both groups, and the As_2O_3 -treated group increased much more than the control group. (E) The average ratio of peak value ($F_{\text{Imax}} : F_{I_0}$) in each group after 30 mM KCl or 10 mM caffeine stimulation. Average data were described as mean $\pm$ SEM, $n = 16, 26$ ventricular cardiomyocytes from each group. $**P < 0.01$ and $*P < 0.05$ vs. control.

($V_{1/2} = -9.56 \pm 0.82$ mV, $n = 11$) was dramatically different from that in control ($V_{1/2} = -3.54 \pm 1.58$ mV, $n = 11$, $P < 0.01$). However, the slope factor ($S_{1/2}$) of the activation for each group had no significance (6.33 ± 0.12 vs. 6.75 ± 0.22 , $P > 0.05$).

We then recorded the voltage-dependent inactivation of $I_{\text{Ca,L}}$ by clamping to different prepulses from -120 mV to $+10$ mV for 300 ms and then applying a test voltage to $+10$ mV for the same time. The holding potential was -80 mV and the interval was 5 s. Our analyzed data showed that although the steady-state inactivation curve of $I_{\text{Ca,L}}$

was switched to a more negative voltage by As_2O_3 , it was not significantly changed compared with that from control ($P > 0.05$, $n = 8$ for control and $n = 9$ for the As_2O_3 group, Fig. 3D), and the kinetic parameters ($V_{1/2}$, $S_{1/2}$) did not differ notably from those vs. control (Table 2).

Recovery from inactivation (reactivation) was also examined by a double pulses' protocol stepped from -80 mV to $+10$ mV and the two 300 ms identical pulses were separated by an increasing interpulse interval, which was ranged from 5 to 385 ms with an increment of 20 ms [32]. The holding potential was -80 mV and the interval was 5 s. Data was described and fitted by single exponential function. The results showed that the reactivation curves of $I_{\text{Ca,L}}$ from both groups were nearly coincidence (Fig. 3E) and the time constants for recovery (τ) were not statistically different between the two groups (Table 2).

Effects of As_2O_3 on $[\text{Ca}^{2+}]_i$ in rat ventricular cardiomyocytes

We first determined whether As_2O_3 was able to alter $[\text{Ca}^{2+}]_i$ at resting conditions in normal Tyrode's solution or Ca^{2+} -free solution, and it appeared that As_2O_3 did not induce significant changes in $[\text{Ca}^{2+}]_i$ under resting conditions vs. control ($P > 0.05$, data not shown). We then observed whether As_2O_3 affected KCl-stimulated Ca^{2+} mobilization in standard Tyrode's solution, and the representative fluorescent images of $[\text{Ca}^{2+}]_i$ changes in ventricular cardiomyocytes of two groups were shown in Fig. 4A. The average data were summarized and described in Fig. 4B. Our data indicated that, after the application of 30 mM KCl, the average ratio of peak value ($F_{\text{Imax}} : F_{I_0}$) was significantly increased to 6.25 ± 0.44 times in the As_2O_3 group ($n = 16$) compared with 3.98 ± 0.13 times in the control group ($n = 26$, $P < 0.01$, Fig. 4E).

It has been well documented that caffeine is usually considered as a useful index of intercellular Ca^{2+} release from sarcoplasmic reticulum (SR); therefore, we applied 10 mM caffeine to detect the response of cardiomyocytes in either group with 2.0 mM EGTA in Ca^{2+} -free Tyrode's solution in order to completely remove the extracellular calcium. The representative images of $[\text{Ca}^{2+}]_i$ changes in ventricular cardiomyocytes of either group were shown in Fig. 4C, and the trace of caffeine-induced Ca^{2+} release was quite different vs. control in Fig. 4D. As a result, we found that the peak value of $[\text{Ca}^{2+}]_i$ was increased by 3.12 ± 0.88 times in the As_2O_3 group ($n = 19$) compared with that from control (1.92 ± 0.61 times, $n = 18$, $P < 0.01$, Fig. 4E).

Discussion

The results of our study demonstrated that As_2O_3 could prolong QTc intervals in rats by $\sim 36.4\%$ compared with control (Fig. 1B), and the multiple relevant ion channel mechanisms underlying QTc prolongation in the presence of As_2O_3 were also confirmed and the As_2O_3 -induced down-regulation of I_{K1} and up-regulation of $I_{\text{Ca,L}}$ might both be involved in the QTc prolongation in As_2O_3 -treated ventricular cardiomyocytes of rats.

It has been reported that if a lengthened QT interval is observed on the surface of ECG, a prolongation of APD could also be recorded in ventricular cardiomyocytes [33, 34]. The trajectory of cardiac AP recorded from isolated ventricular cardiomyocytes differs significantly in different sections of the heart, especially atrium and ventricle [35]. In the present study, we only selected the ventricular cardiomyocytes for whole-cell patch clamp examination. The APD in As_2O_3 -treated group was increased nearly 74.4% compared with control (Fig. 1C). Moreover, As_2O_3 also notably lengthened APD_{50} and APD_{90} by $\sim 41.8\%$ and 74.8% vs. control, respectively (Table 1). In addition, the mean value of RMPs in cardiomyocytes treated by As_2O_3 was smaller than the control group ($P < 0.05$), suggesting that it is easier to trigger AP depolarization. Once the trajectory of AP changes, the currents of some ion channels might be altered.

I_{K1} is a key regulator for the repolarization of phase 3 and also the maintenance of RMP. It has been reported that down-regulation of I_{K1} provokes membrane depolarization, APD prolongation, and also both early and delayed after-depolarizations [36, 37]. Our study in rat ventricular cardiomyocytes detected that I_{K1} was markedly suppressed by $\sim 19.7\%$ at -120 mV after 7 days treatment of As_2O_3 (Fig. 2B), which was partly consistent with the study by Sun et al. [38]: I_{K1} decreased significantly after acute application of 50 or 100 μM As_2O_3 in guinea pig ventricular myocytes, while the change of reversal potential was not indicated. We also detected that the reversal potential of I_{K1} was shifted to more positive direction in the I-V curve (Fig. 2B). The study by Ficker et al. [23] demonstrated inversely I_{K1} did not change upon overnight exposure to 3 μM As_2O_3 in the cultured guinea pig

myocytes. This discrepancy might be related to the differences of dosage, the ways of given [39], and the experimental condition. Our study suggested that As_2O_3 -induced I_{K1} down-regulation might be one of the important reasons causing increase in APD and consequently QTc prolongation.

Calcium current (I_{Ca}) [12, 13] is the primary inward current of the AP plateau and plays a key role in maintaining the rate of the membrane repolarization in ventricular cardiomyocytes. Our data indicated that the peak amplitude of $I_{\text{Ca,L}}$ in cardiomyocytes treated with As_2O_3 was $\sim 75.8\%$ larger than that in control group (Fig. 3B). Moreover, we examined the voltage-dependent properties of $I_{\text{Ca,L}}$ and confirmed that the steady-state activation curve of $I_{\text{Ca,L}}$ was shifted to more negative potential, while the inactivation and the reactivation curves of $I_{\text{Ca,L}}$ were not significantly altered in As_2O_3 -treated cardiomyocytes, suggesting that As_2O_3 could produce the APD prolongation and results in malignant arrhythmias through the acceleration of activation of process of $I_{\text{Ca,L}}$.

In addition, the L-type Ca^{2+} channels expressed in ventricular cardiomyocytes play a key role in handling the $[\text{Ca}^{2+}]_i$. In our present study, the $[\text{Ca}^{2+}]_i$ was largely increased in the As_2O_3 -treated group (Fig. 4A, 4B) induced by 30 mM KCl. At the same time, it was observed that 10 mM caffeine still augmented $[\text{Ca}^{2+}]_i$ in the As_2O_3 -treated group (Fig. 4C, 4D), suggesting that the ryanodine receptor (RyR) might be involved in this cardiac abnormalities.

Taken all data together, I_{K1} down-regulation and $I_{\text{Ca,L}}$ up-regulation might be the underlying ion channel mechanisms involved in APD prolongation of ventricular cardiomyocytes in As_2O_3 -treated rats, which may help us to comprehend why QT prolongation and torsades de pointes is so pervasive among patients with APL treated with As_2O_3 .

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