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# Arrhythmogenic Effects of Arsenic Trioxide in Patients With Acute Promyelocytic Leukemia and an Electrophysiological Study in Isolated Guinea Pig Papillary Muscles

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**Background** Arsenic trioxide ($As_2O_3$) is a new promising regimen for patients with a relapse of acute promyelocytic leukemia (APL), but causes life-threatening arrhythmias. This study aimed to investigate the incidence and mechanism of arrythmogenesis caused by $As_2O_3$.

**Methods and Results** Standard 12-lead ECGs were monitored throughout $As_2O_3$ therapy in 20 APL patients. $As_2O_3$ (0.15 mg/kg) significantly prolonged the corrected QT interval (QTc: 445±7 to 517±17 ms, means±SE, $p<0.01$), and also increased the QTc dispersion and transmural dispersion of repolarization. Non-sustained ventricular tachycardias and torsades de pointes occurred in 4 and 1 patients, respectively. The action potentials and isometric contraction were measured in guinea pig papillary muscles during $As_2O_3$ perfusion (350 μmol/L). The action potential duration was prolonged ($APD_{90}$: 150±11 to 195±12 ms at 60 min, $p<0.01$, n=5) and perfusion of $As_2O_3$ in a low $K^+$ solution with a low stimulation rate augmented the prolongation of APD, and provoked early after-depolarizations and triggered activities. The prolonged exposure to $As_2O_3$ induced muscle contracture, after-contractions, triggered activities and electromechanical alternans. Tetrodotoxin or butylated hydroxytoluene partially prevented the $As_2O_3$-induced prolongation of APD.

**Conclusions** The prolonged QTc and spatial heterogeneity are responsible for the $As_2O_3$-induced ventricular tachyarrhythmias. In addition to prolongation of the APD, cellular $Ca^{2+}$ overload and lipid peroxidation might contribute to the electrophysiological abnormalities caused by $As_2O_3$. (*Circ J* 2006; **70:** 1407 – 1414)

**Key Words:** Action potential; Arsenic; QT interval; Triggered activity; Ventricular arrhythmias

Recently, arsenic trioxide ($As_2O_3$) has been shown to induce complete remission of acute promyelocytic leukemia (APL)[1] and both the USA Food and Drug Administration (FDA) and the Ministry of Health, Labor and Welfare of Japan have approved $As_2O_3$ for the treatment of APL. However, $As_2O_3$ is also a poison that causes multiple organ failure. Toxic effects on the cardiac system include: torsades de pointes (TdP), T-U wave alternans, ST-T change and QT interval prolongation[2–4] The FDA reported that the incidence of QT interval prolongation was 40%, and recommended particular attention be paid to QT interval prolongation. We previously reported that QT interval prolongation occurred in all of 8 APL patients treated with $As_2O_3$, and recent studies in isolated guinea pig papillary muscles have also shown that $As_2O_3$ prolongs the action potential duration (APD) in a reverse frequency-dependent way, as well as blocking both $I_{kr}$ and $I_{ks}$ in HERG- or KCNQ1 + KCNE1-transfected CHO cells[5,6] However, the toxic effects of $As_2O_3$ are complex and the involvement of other undetermined mechanisms other than prolongation of APD is implied. Previous studies have shown cellular $Ca^{2+}$ overload, generation of reactive oxygen species (ROS), and decreased intracellular ATP concentration ($[ATP]_i$);[7–9] which may provoke triggered activities because of early- and delayed afterdepolarization (EAD and DAD). In fact, we have observed not only TdP but also non-sustained monomorphic ventricular tachycardias in patients treated with $As_2O_3$.[2]

In order to investigate the arrhythmogenesis of $As_2O_3$, we evaluated the changes of in ECG parameters in patients with APL during $As_2O_3$ therapy, and also examined the electromechanical effects of $As_2O_3$ in isolated guinea pig papillary muscles.

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## Methods

*ECG Changes*

This investigation conforms to the principles outlined in the Declaration of Helsinki (Cardiovascular Research 1997; 35: 2–4). Twenty patients with APL who had relapsed after previous extensive therapies with all-trans retinoic acid and other chemotherapies were treated with $As_2O_3$ ($0.15\ mg \cdot kg^{-1} \cdot day^{-1}$). The 12-lead ECG and chest X-ray were recorded once a week and telemetry ECG was monitored throughout the admission period. The following parameters

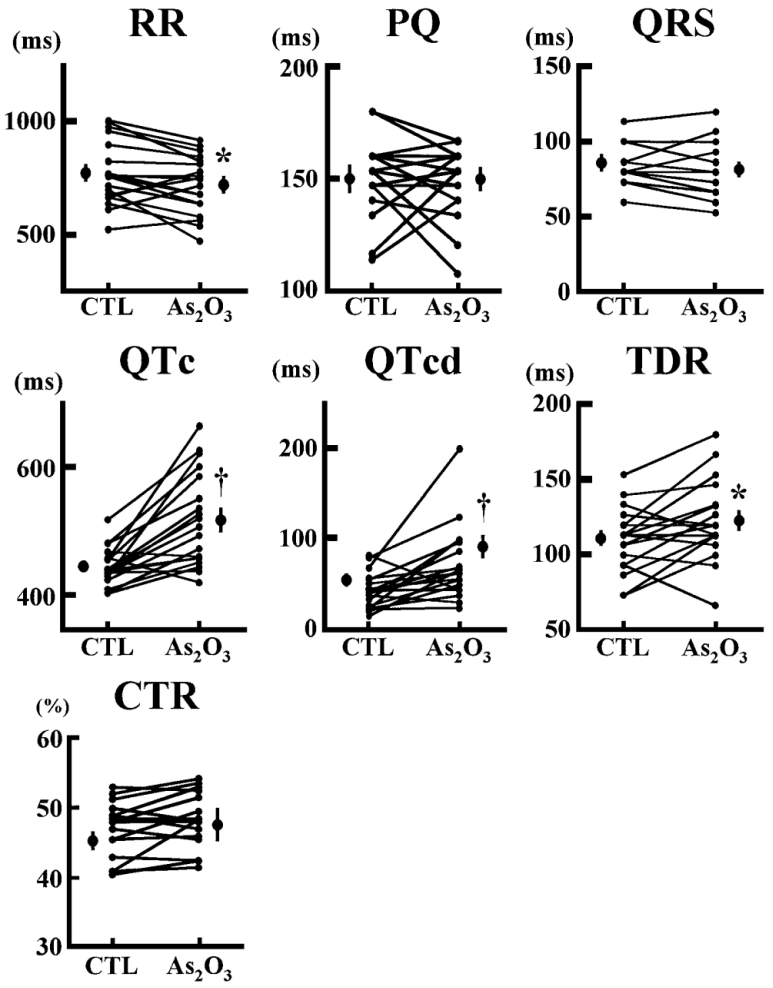


Fig 1. Changes in the clinical parameters of 20 patients with relapsed acute promyelocytic leukemia during treatment with arsenic trioxide ($As_2O_3$). RR, RR interval; PQ, PQ interval; QRS, QRS duration; QTc, corrected QT interval; QTcd, dispersion of QTc; TDR, transmural dispersion of repolarization; CTR, cardio-thoracic ratio; CTL, before the administration of $As_2O_3$; $As_2O_3$, after the administration of $As_2O_3$ (0.15 mg/kg daily). Values are mean ± SE in 20 patients. *$p<0.05$ and †$p<0.01$ vs CTL by using a paired t-test.

were evaluated: PQ, RR, QT interval, corrected QT interval (QTc) and QRS duration. The QT dispersion and QTc dispersion (QTcd) were calculated by subtracting the shortest QT or QTc interval in any lead for each beat from the longest one. The interval between the peak and the end of a T wave was measured as an index of transmural dispersion of repolarization (TDR). The cardiothoracic ratio (CTR) was measured on the chest X-ray.

*Measurement of Action Potentials and Contraction in Guinea Pig Papillary Muscles*

This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No.85-23, revised 1996). Papillary muscles were obtained from the right ventricles of guinea pig (300–400 g) hearts. In brief, the heart was quickly removed after cervical dislocation, and thin papillary muscles were dissected in an oxygenated physiological salt solution (pH 7.4) that had the following composition (in mmol/L): NaCl, 113.1; KCl, 4.6; $CaCl_2$, 2.45; $MgCl_2$, 1.2; $NaHCO_3$, 21.9; $NaH_2PO_4$, 3.5 and glucose, 5.

The muscle was mounted in a Perspex bath perfused with oxygenated solution at 37±0.2°C. The mural end of the muscles was pinned to the bottom of the bath, and the tendinous end was tied to a rod of an isometric transducer (model TB-651T; Nihon Kohden, Tokyo, Japan) by a short length of silk thread. The length of the muscle was adjusted until the resting tension was 50–100 mg. Stimulation was applied to the basal part of the preparation through a bipolar Ag/AgCl electrode. The action potentials were recorded with a 3 mol/L KCl-filled microelectrode (8–10 MΩ), which was connected to a high-impedance amplifier (model MEZ-8201; Nihon Kohden, Tokyo, Japan) by an Ag/AgCl pellet. Action potentials and tension were displayed on a storage oscilloscope (model 5113; Tektronix, Tokyo, Japan) and recorded on both a pen recorder (model WS-641G; Nihon Kohden) and a digital audiotape recorder (model RD-120T; TEAC, Tokyo, Japan) for later analysis. All experiments were preceded by an equilibration period of 60 min in the control solution, after which the action potentials and tension were continuously monitored during perfusion of 35 μmol/L and 350 μmol/L $As_2O_3$[5,10,11]

*Reagents*

Both $As_2O_3$ and tetrodotoxin (TTX) were purchased from WAKO (Tokyo, Japan). Butylated hydroxytoluene (BHT) was obtained from Sigma Chemical (St Louis, MO, USA).

*Statistical Analyses*

Statistical analyses of the data were performed using the Student's t-test for paired data or repeated measure ANOVA with Fisher's test among 3 groups. In this study, data are presented as mean ± SE. A p-value $<0.05$ was considered to be statistically significant.

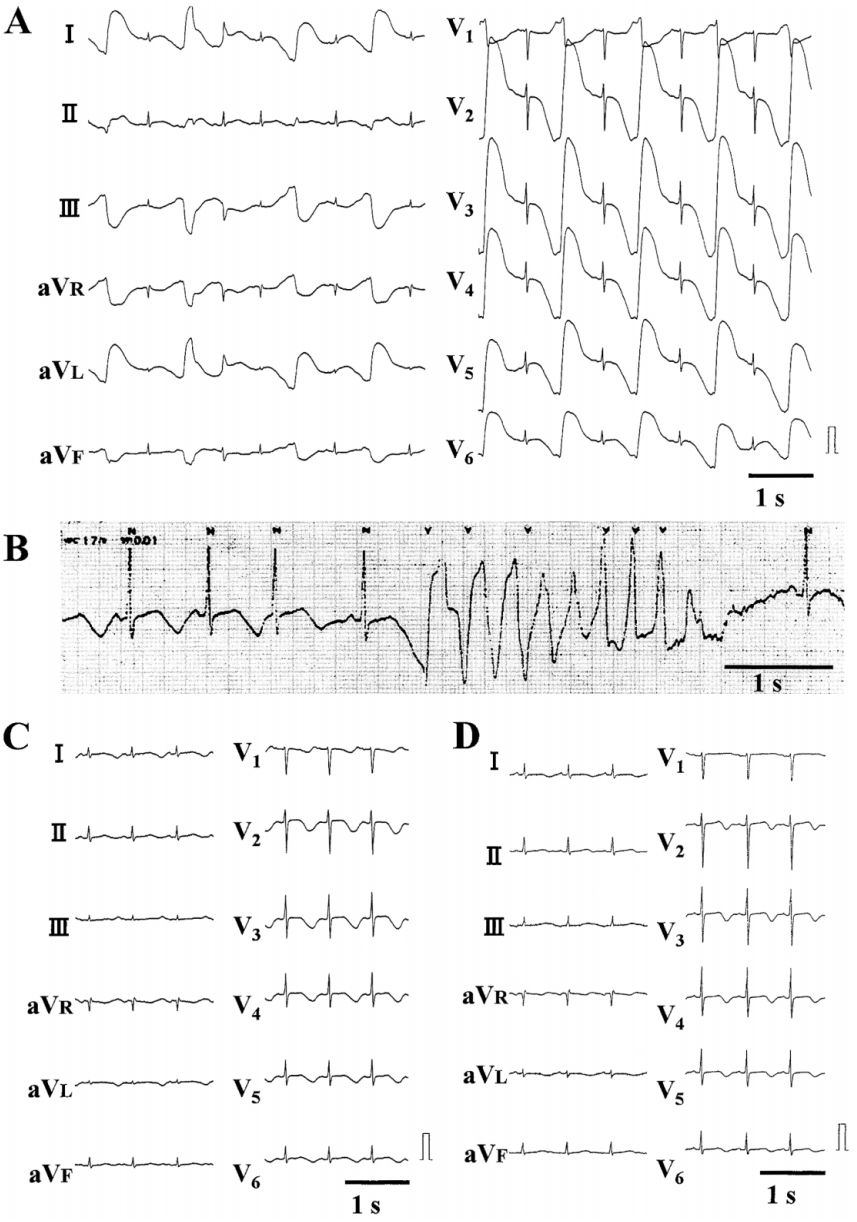


Fig 2. QT prolongation and ventricular arrhythmias during treatment with arsenic trioxide ($As_2O_3$) in a woman aged 20 years. (A) 12-lead ECG on day 38 of $As_2O_3$ therapy shows marked QT prolongation and ventricular bigeminy. (B) The telemetry ECG recording demonstrates torsades de pointes. (C) The 12-lead ECG immediately after lidocaine infusion. (D) 12-lead ECG 9 days after cessation of $As_2O_3$ therapy. The premature ventricular contractions have disappeared and the QT interval has shortened to the pretreatment level.

## Results

*Effect of $As_2O_3$ on Clinical Parameters*

Fig 1 shows the changes in the ECG parameters and CTR in the 20 APL patients during treatment with $As_2O_3$. The QTc was prolonged from 445±7 ms to 517±17 ms ($p<0.01$ determined by paired t-test). Both the QTcd and TDR increased and the RR interval shortened, but there were no significant changes in the PQ interval or in QRS duration. No patient developed heart failure and there was no increase in CTR.

Monomorphic non-sustained ventricular tachycardias appeared in 4 of the 20 patients. In 1 case (female, 20 years old), both a remarkable QT prolongation and TdP occurred during treatment with $As_2O_3$ (Fig 2). On day 38 of the therapy, she complained of chest discomfort, and the 12-lead ECG showed ventricular bigeminy and marked prolongation of the QT interval (Fig 2A). The QTc, QTcd and TDR were 663, 201 and 120 ms, respectively. Thereafter, the telemetry ECG showed TdP, which returned to sinus rhythm spontaneously (Fig 2B). At that time, she was treated with fluconazole and her serum $K^+$ concentration was within the normal range. The premature ventricular contractions disappeared and the QT interval shortened immediately after infusion of lidocaine (Fig 2C). At the 10th day after cessation of $As_2O_3$, the QT interval returned to the pretreatment level (Fig 2D).

*Effect of $As_2O_3$ in Guinea Pig Papillary Muscles*

To investigate the mechanisms of $As_2O_3$-induced ventricular arrhythmias, both the action potentials and isometric contraction in papillary muscle were monitored during perfusion of a solution containing either 35 μmol/L or 350 μmol/L $As_2O_3$. The muscles were stimulated at 1 Hz throughout the experiment. Fig 3A shows typical examples of the changes in the action potentials and isometric contraction. In this muscle, the developed tension increased after 60 min perfusion of $As_2O_3$ (350 μmol/L), and at that time, the APD was obviously prolonged. The resting tension began to increase after 90 min, which resulted in muscle

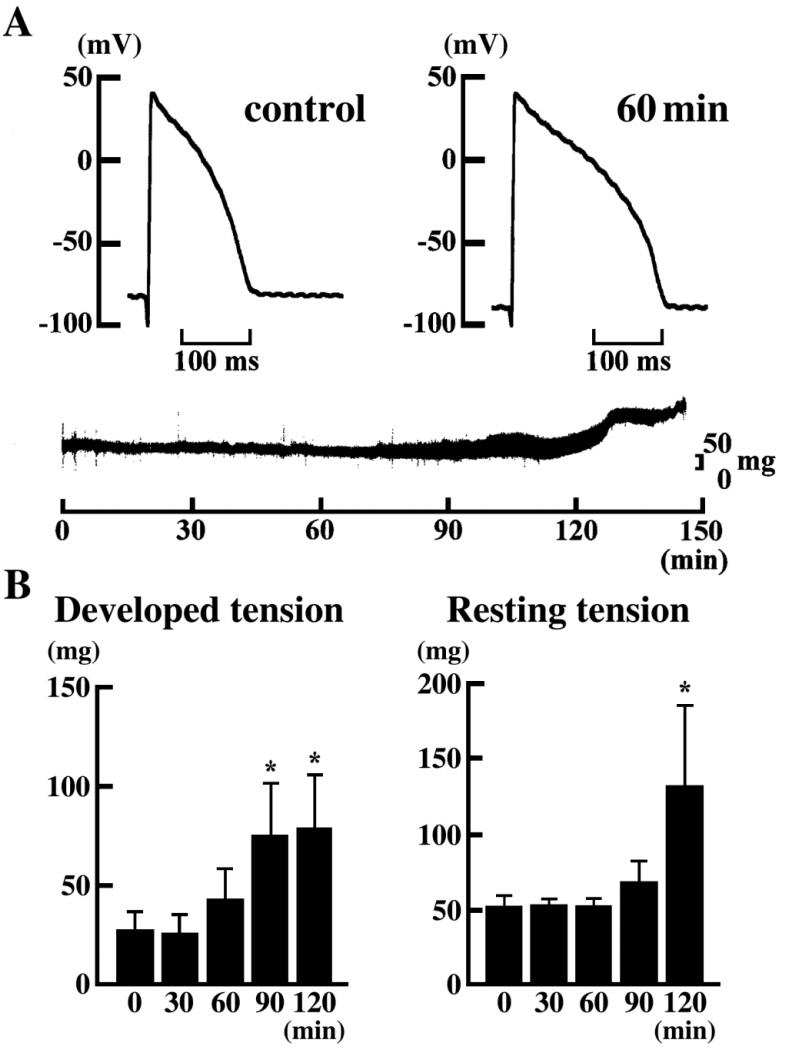


Fig 3. Effects of arsenic trioxide ($As_2O_3$) on action potentials and isometric contraction. (A) Typical example of $As_2O_3$-induced changes of the action potentials and isometric contraction in a guinea pig papillary muscle. (Upper) Action potentials recorded before and 60 min after exposure to $As_2O_3$ (350 μmol/L). (Lower) Continuous recording of isometric contraction stimulated at 1 Hz. Perfusion of $As_2O_3$ prolongs the action potential duration and there is a transient increase in developed tension, followed by muscle contracture. (B) Summary of the changes in developed tension (Left panel) and resting tension (Right panel). Developed tension increases significantly at 90 min after exposure to $As_2O_3$ (350 μmol/L). Resting tension increases significantly after 120 min. Values are mean ± SE from 5 papillary muscles. *$p<0.05$ vs 0 min by 1-way repeated measure ANOVA with Fisher's test.

**Table 1 Effects of $As_2O_3$ on Electrophysiological Parameters**

| | *$As_2O_3$ (35 μmol/L)* | | | *$As_2O_3$ (350 μmol/L)* | | |
|---|---|---|---|---|---|---|
| | *Control* | *30 min* | *60 min* | *Control* | *30 min* | *60 min* |
| *RMP (mV)* | *–82±2* | *–78±5* | *–78±3* | *–78±6* | *–75±5* | *–77±5* |
| *APA (mV)* | *32±3* | *33±2* | *27±4* | *33±4* | *35±6* | *35±6* |
| *$APD_{90}$ (ms)* | *151±3* | *165±10†* | *162±16†* | *150±11* | *184±13†* | *195±12†* |

*RMP, resting membrane potential; APA, action potential amplitude; $APD_{90}$, action potential duration measured at 90% repolarization. Values are means ± SE in 5 papillary muscles.*
*†$p<0.01$ vs control by 1-way repeated measure ANOVA with Fisher's test.*

contracture. As summarized in Table 1, the APD at 90% repolarization ($APD_{90}$) was significantly prolonged in a dose- and time-dependent manner during the perfusion of $As_2O_3$. Neither the resting membrane potential nor the action potential amplitude altered significantly. Fig 3B shows the significant increase in developed tension that was observed 90 min after exposure to $As_2O_3$, which was followed by a significant increase in resting tension at 120 min.

*Triggered Activity Due to EAD Caused by $As_2O_3$*

In the next series of experiments, the action potentials were measured at various stimulation rates (0.1, 0.5 and 1 Hz) and at different extracellular $K^+$ concentrations ($[K^+]_o$) during perfusion of $As_2O_3$ (350 μmol/L). As shown in Fig 4A, the prolongation of the APD by $As_2O_3$ was more prominent at lower stimulation frequencies, indicating reverse frequency dependency. In addition, the prolongation of APD by $As_2O_3$ was dependent on $[K^+]_o$ (Fig 4B). When $As_2O_3$ (350 μmol/L) was administered in a low $[K^+]_o$ (3.0 mmol/L) solution, the increase in $APD_{90}$ was augmented compared with that when the normal solution was used (4.6 mmol/L).

Prolonged perfusion with $As_2O_3$ (350 μmol/L) at the low stimulation rate (0.1 Hz) in the low $[K^+]_o$ (3.0 mmol/L) solution caused a marked prolongation of APD and provoked an EAD at 120 min (Fig 4C). Thereafter, triggered activities caused by EADs appeared at 180 min. The EADs and triggered activities appeared in 3 and 1 of 5 preparations,

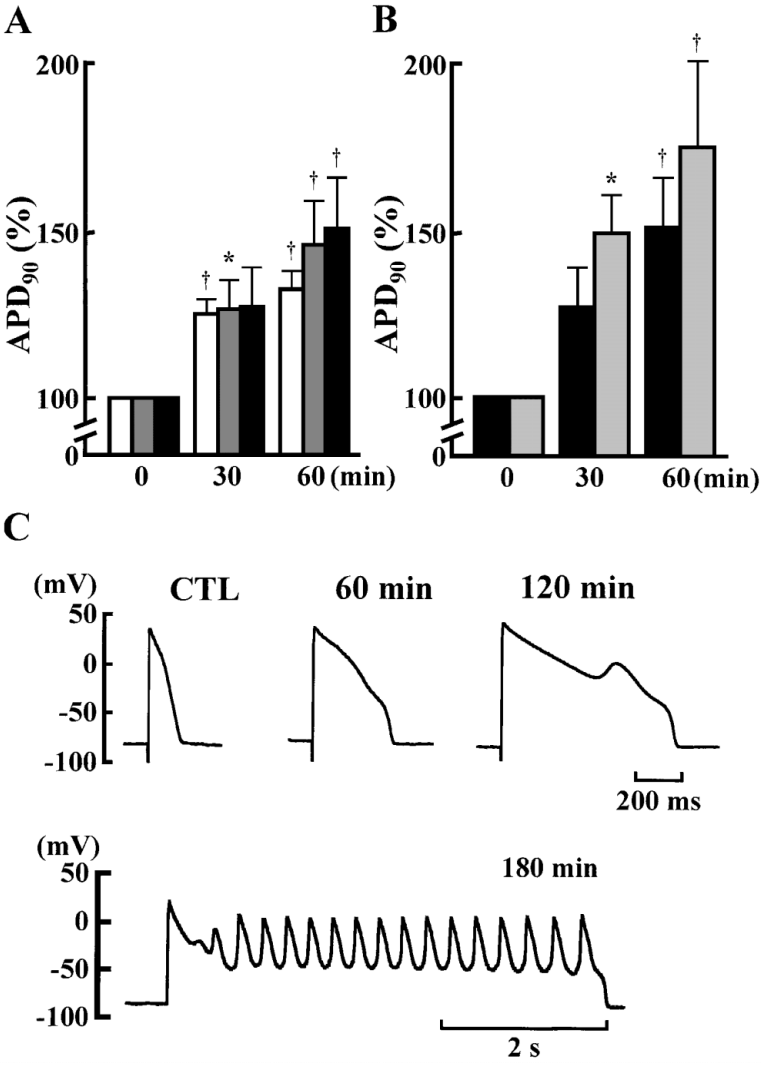


Fig 4. Effects of alterations in stimulation rate and extracellular $K^+$ concentration ($[K^+]_o$) on the arsenic trioxide ($As_2O_3$)-induced prolongation of action potential duration (APD). (A) The relative changes in APD at 90% repolarization ($APD_{90}$) at 30 and 60 min after the exposure to $As_2O_3$ (350 μmol/L) at different stimulation rates. The columns indicate $APD_{90}$ (%) at 1 Hz (open bars), 0.5 Hz (hatched bars), and 0.1 Hz stimulation (filled bars), respectively. (B) The relative changes in $APD_{90}$ at 30 and 60 min after the exposure to $As_2O_3$ (350 μmol/L) at different $[K^+]_o$. The columns show $APD_{90}$ (%) in the normal $[K^+]_o$ (4.6 mmol/L, filled bars), and low $[K^+]_o$ (3.0 mmol/L, dotted bars). Value are mean ± SE from 5 experiments, *$p<0.05$ and †$p<0.01$ vs 0 min by repeated measure ANOVA with Fisher's test. (C) The action potentials recorded before (CTL), and at 60, 120 and 180 min after the exposure to $As_2O_3$ (350 μmol/L) at 0.1 Hz stimulation in a low $[K^+]_o$ solution (3.0 mmol/L). The record at 120 min shows marked prolongation of the APD and an early-after-depolarization (EAD). Thereafter, triggered activities caused by EADs appeared at 180 min.

respectively. Without $As_2O_3$, neither EAD nor triggered activity occurred under these conditions (n=5).

*Triggered Activity and Electromechanical Alternans During Rapid Stimulation*

The finding that $As_2O_3$ caused muscle contracture following a transient increase in developed tension suggests that $As_2O_3$ may induce intracellular $Ca^{2+}$ overload. Thus, rapid stimulation at a cycle length of 200 ms in trains of 20 stimuli was applied every 10 s. With perfusion of the control solution, there was neither aftercontraction nor triggered activity after the rapid stimulation for 240 min (Fig 5A). Perfusion with $As_2O_3$ (350 μmol/L) for 80–120 min induced aftercontractions in all of the 5 muscles (Fig 5A) and in 2 of them triggered activities appeared at 180–210 min after exposure to $As_2O_3$ (Fig 5A).

Exposure to $As_2O_3$ for more than 240 min induced electromechanical alternans in all 5 muscles (Fig 5B,C). It is evident that mechanical alternans was associated with the APD alternans. Thus, longer action potentials related to larger contractions, whereas the subsequent shorter action potentials coincided with smaller contractions.

*Effects of TTX on the $As_2O_3$-Induced Changes in Action Potentials in Guinea Pig Papillary Muscles*

Because $As_2O_3$ induced prolongation of the APD and muscle contracture, we examined whether inhibition of the $Na^+$ window current would prevent the prolongation of the APD. After pretreatment with TTX (3 μmol/L) for 15 min, the papillary muscles were exposed to $As_2O_3$ (350 μmol/L) in the continuous presence of TTX. The shortening of the $APD_{90}$ by TTX was not statistically significant (94±5% of control, p=NS by a paired t-test). In the presence of TTX, $As_2O_3$ did not prolong the $APD_{90}$ significantly until 60 min, whereas the $APD_{90}$ was prolonged at both 30 and 60 min in the absence of TTX (Fig 6A).

*Effects of BHT on $As_2O_3$-Induced Changes in Action Potentials in Guinea Pig Papillary Muscles*

Finally, because arsenic is reported to cause lipid peroxidation,[12] we examined whether BHT, an inhibitor of lipid peroxidation, would prevent the $As_2O_3$-induced prolongation of APD. When the papillary muscles were pretreated with BHT (50 μmol/L) for 30 min, the subsequent addition of $As_2O_3$ (350 μmol/L) did not prolong the $APD_{90}$ during the 60 min perfusion (Fig 6B). However, BHT could not inhibit the prolongation of the APD when the muscles were exposed to $As_2O_3$ for longer than 60 min (data not shown).

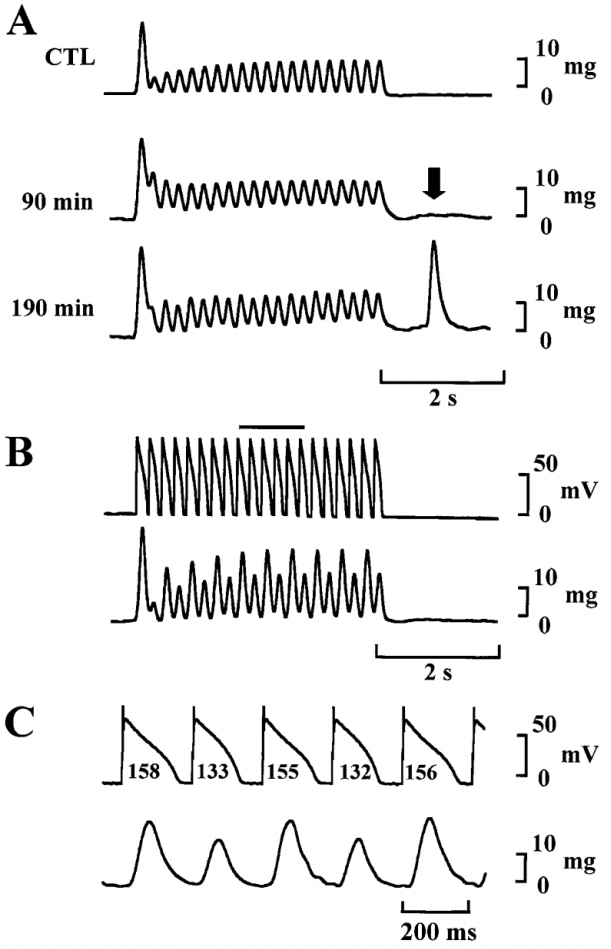


Fig 5. Aftercontractions, triggered activities and electromechanical alternans by arsenic trioxide ($As_2O_3$). (A) The records of isometric contraction before (Top), 90 min (Middle), and 190 min (Bottom) after the exposure to $As_2O_3$ (350 μmol/L). Rapid stimulation at a cycle length of 200 ms in trains of 20 stimuli was applied every 10 s An aftercontraction (arrowhead) and triggered activity occurred at 90 and 190 min, respectively. (B) Action potentials (Upper) and tension (Lower) recorded at 240 min after exposure to $As_2O_3$ (350 μmol/L). Rapid stimulation induced an alternans in isometric contraction. (C) More detailed records for a shorter time interval (indicated by the bar in Fig 5B). The numbers in action potentials indicate action potential duration (APD) at 90% repolarization ($APD_{90}$) (ms). It is evident that the mechanical alternans was associated with the APD alternans.

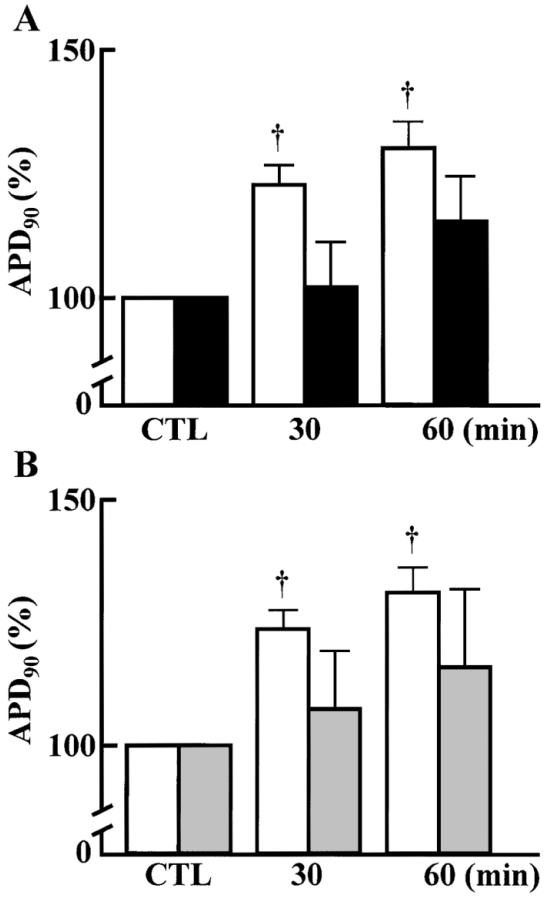


Fig 6. Effects of tetrodotoxin (TTX) (A) and butylated hydroxytoruene (BHT) (B) on the arsenic trioxide ($As_2O_3$)-induced prolongation of action potential duration (APD). The relative changes in APD at 90% repolarization ($APD_{90}$) are shown at 30 and 60 min after the exposure to $As_2O_3$ (350 μmol/L) in the absence (open bars) and presence of TTX (3 μmol/L, filled bars) or BHT (50 μmol/L, dotted bars). The papillary muscles were stimulated at 1.0 Hz in the normal extracellular $K^+$ concentrations ($[K^+]_o$) solution (4.6 mmol/L). Values are mean ± SE from 5 experiments. †$p<0.01$ vs before the administration of $As_2O_3$ (CTL) by 1-way repeated measure ANOVA with Fisher's test. Both TTX and BHT prevented prolongation of the APD by $As_2O_3$.

## Discussion

This study investigated the arrhythmogenic effects of $As_2O_3$ using clinical and basic electromechanical examinations, and demonstrated that: (1) in patients with relapsed APL, treatment with $As_2O_3$ prolongs the QTc, QTcd and TDR, which are associated with the occurrence of ventricular tachyarrhythmias including TdP; (2) in guinea pig papillary muscles, $As_2O_3$ prolonged the APD and provoked EAD; (3) $As_2O_3$ also induced muscle contracture, aftercontraction, triggered activities, and electromechanical alternance; and (4) TTX and BHT partially prevented the $As_2O_3$-induced prolongation of the APD. Thus, it is suggested that in addition to prolongation of the APD, cellular $Ca^{2+}$ overload and lipid peroxidation caused by ROS generation may contribute to the electrophysiological abnormalities associated with $As_2O_3$ therapy.

*Effects of $As_2O_3$ on ECG Parameters in Patients With APL*

$As_2O_3$ has been reported to induce various effects on ECG. Little et al reported a case of arsenic poisoning in which QTc prolongation, prominent U wave, T-U wave alternans and TdP were recorded.[4] In contrast, as a therapeutic agent for APL, Unnikrishnan et al depicted 3 cases of APL with QTc prolongation and TdP after treatment with $As_2O_3$.[3]

In the present study, we showed changes in the ECG parameters of APL patients treated with $As_2O_3$, and reported a case in which $As_2O_3$ induced marked QTc prolongation and TdP. Although fluconazole is known to prolong the QT interval and to induce TdP,[13] it did not cause ECG abnormalities in that particular case during a previous administration or before treatment with $As_2O_3$. Although an interaction between fluconazole and $As_2O_3$ cannot be ruled out, the prolongation of the QT interval could be one of the mechanisms responsible for the arrhythmogenic effects of $As_2O_3$.

In addition to QTc prolongation, $As_2O_3$ increased the QTcd and TDR. The TDR has been reported as a marker of

electrical dispersion[14] and the development of a large TDR would provide the substrate for intra-mural re-entry[15] In fact, 4 of the 20 patients showed monomorphic, non-sustained ventricular tachycardias. In the clinical situation, therefore, the precise mechanisms for the arrhythmogenic effects of $As_2O_3$ remain undetermined but may be heterogeneous.

*Effects of $As_2O_3$ on Action Potentials and Contraction in Guinea Pig Papillary Muscles*

Several studies have shown effects of $As_2O_3$ on the action potential and ion channels. Chiang et al suggested that $As_2O_3$-induced prolongation of the APD could be caused by blockade of $I_{kr}$, because $As_2O_3$ prolonged the APD in a reverse frequency dependent way[5] Drolet et al reported that $As_2O_3$ blocked both $I_{kr}$ and $I_{ks}$ in HERG- or KCNQ1 + KCNE1-transfected CHO cells, and that the sensitivity to $As_2O_3$ was higher in $I_{kr}$ than in $I_{ks}$[6] In the present study of guinea pig papillary muscles, $As_2O_3$ (35–350 μmol/L) prolonged the APD without changing other action potential parameters (Table 1). A previous clinical study indicated that the mean peak plasma $As_2O_3$ level was 6.9 μmol/L (range 5.54–7.3 μmol/L) in patients with APL receiving $As_2O_3$ (10 mg/day) intravenously[10] Chiang et al reported that the perfusion of $As_2O_3$ (10 and 25 μmol/L) prolonged the APD significantly in isolated guinea pig papillary muscles only when they were stimulated at a low rate (0.1 Hz)[5] In Langendorff-perfused rabbit hearts, $As_2O_3$ at 300 μmol/L prolonged the QT interval, whereas lower concentrations (30–100 μmol/L) did not[11] Thus, relatively high concentrations of $As_2O_3$ were necessary to induce prolongation of the APD in both the present and previous studies compared with the plasma $As_2O_3$ level that was reported in the clinical study. There could be several reasons for the discrepancy. As the effects of $As_2O_3$ on the APD are dependent on the heart rate (Fig 4A), a simple extrapolation from the experimental study to the clinical situation is difficult. In addition, most patients treated with $As_2O_3$ had been receiving therapy with anthracyclin, which could modify the sensitivity to $As_2O_3$. Finally, the toxicity of $As_2O_3$ is reported to be different in various animal species[16]

There are several explanations for the prolongation of the APD by $As_2O_3$. We showed that it was dependent on both the pacing rate and $[K^+]_o$. The prolongation of the APD by $As_2O_3$ was prominent at 0.1 Hz stimulation, but less at 1 Hz. In addition, when $As_2O_3$ (350 μmol/L) was administered in a low $[K^+]_o$ (3.0 mmol/L) solution, the increase in the $APD_{90}$ was augmented compared with that in the normal solution (4.6 mmol/L). The reverse rate-dependency and the $[K^+]_o$-dependency of the prolongation of the APD may predict blockade of $I_{kr}$ and are clinically important phenomena, because bradycardia and hypokalemia are the risk factors for acquired long QT syndrome and TdP[15,17] In fact, we showed that $As_2O_3$ induced marked prolongation of the APD and triggered activities caused by EAD in muscle stimulated at a low rate in a low $[K^+]_o$ solution (Fig 4C). Second, $As_2O_3$ did not prolong the APD in the presence of TTX (Fig 6A). An increase in the $Na^+$ window current might contribute to the $As_2O_3$-induced prolongation of the APD, although we did not examine whether TTX could inhibit the EAD induced by $As_2O_3$. The recovery of the QT interval by lidocaine in the patient with TdP supports this idea (Fig 2C). We have also reported the prophylactic antiarrhythmic effect of oral mexiletine HCl in APL patients treated with $As_2O_3$[2] Finally, pretreatment with BHT prevented the $As_2O_3$-induced prolongation of the APD, at least during the initial 60 min. Thus, generation of ROS and the resulting lipid peroxidation might be responsible for the modification of these ion channels by $As_2O_3$. In fact, it has been reported that $As_2O_3$ increases the $H_2O_2$ level in leukemia cells, and that ROS cause prolongation of the APD by a marked decrease in the time-dependent $K^+$ current in patch-clamped guinea pig ventricular myocytes[18,19] Furthermore, 4-hydroxynonenal, the aldehydic product of lipid peroxidation, has been shown to increase the $Na^+$ window current[20] However, BHT failed to inhibit the prolongation of APD when the muscles were exposed to $As_2O_3$ for longer than 60 min, presumably because lipid peroxidation is a chain reaction, and ROS reacted not only with fatty acids but also with crucial enzymatic proteins. Nakaya et al also reported that in isolated papillary muscles, BHT inhibited cumene hydroperoxide-induced electrical changes, but could not prevent the unexcitability of muscles induced by prolonged exposure to hydroperoxide[21]

*Triggered Activities and Electromechanical Alternans During Rapid Stimulation*

The present study demonstrated that not only EAD but also DAD contributed to the triggered activities induced by $As_2O_3$ (Fig 5). Both DADs and aftercontraction are related to the oscillatory $Ca^{2+}$ release from the sarcoplasmic reticulum (SR), which usually results from cellular $Ca^{2+}$ overload[7] In fact, $As_2O_3$ has been shown to increase intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) in esophageal carcinoma cells[22] In the present study, $As_2O_3$ caused muscle contracture following a transient increase in developed tension, and induced aftercontractions and triggered activities only in muscles stimulated by a rapid train of stimuli. These observations lend support to the idea of $As_2O_3$-induced $Ca^{2+}$ overload.

Although the precise mechanisms for the $Ca^{2+}$ overload are unclear, $As_2O_3$ has been reported to inhibit pyruvate dehydrogenase and to decrease $[ATP]_i$[8,9] The decrease in $[ATP]_i$ could inhibit $Na^+/K^+$ ATPase and result in an increase in $[Na^+]_i$, leading to $Ca^{2+}$ overload via the $Na^+/Ca^{2+}$ exchange. The decrease in $[ATP]_i$ may also depress SR $Ca^{2+}$-ATPase activity, which causes $Ca^{2+}$ overload. Additionally, ROS generated by $As_2O_3$, per se, could directly induce $Ca^{2+}$ overload[23] In fact, we have already shown $H_2O_2$-induced triggered activities caused by DADs, contracture following a transient increase in developed tension, and an increase in $[Ca^{2+}]_i$ in fura-2-loaded guinea pig ventricular myocytes[24] Beresewicz and Horackova have also shown that ROS can induce both EADs and DADs in rat and guinea pig ventricular myocytes[25] Finally, the prolongation of the APD by $As_2O_3$ might cause cellular $Ca^{2+}$ overload by increasing the $Ca^{2+}$ influx via L-type $Ca^{2+}$ channels[26]

The present study showed that $As_2O_3$ induced electromechanical alternans, which is intriguing because T-U wave alternans has been reported in patients with $As_2O_3$ poisoning[4] As the $As_2O_3$-induced electromechanical alternans coincided with aftercontractions and triggered activities, electromechanical alternans might also be related to the abnormal cellular $Ca^{2+}$ handling. Supporting this hypothesis, Shimizu and Antzelevitch have shown in left ventricular preparations that electromechanical alternans was produced by a sea anemone toxin at rapid stimulation, and blocked by either ryanodine or low $[Ca^{2+}]_o$[27]

In conclusion, the treatment of relapsed APL with $As_2O_3$

prolongs the QTc and increases the spacial heterogeneity of repolarization. These effects are associated with the occurrence of ventricular tachyarrhythmias, including TdP. The arrhythmogenic effects of $As_2O_3$ seem to be heterogeneous, and might involve triggered activities caused by EADs and DADs. These electrophysiological abnormalities are caused at least, in part, by lipid peroxidation caused by $As_2O_3$-induced ROS generation. ECG monitoring during the $As_2O_3$ therapy is recommended, and special attention to the maintenance of normal $K^+$ concentration and avoidance of bradycardia are also crucial to prevent fatal ventricular tachyarrhythmias. Immediate treatment with class Ib $Na^+$ channel blockers may be effective if these do occur.

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