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 (As2O3) 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 arrhythmogenesis caused by As2O3.

Methods and Results  Standard 12-lead ECGs were monitored throughout As2O3 therapy in 20 APL patients. As2O3 (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 As2O3 perfusion (350 μmol/L). The action potential duration was prolonged (APD90: 150±11 to 195±12 ms at 60 min, p<0.01, n=5) and perfusion of As2O3 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 As2O3 induced muscle contracture, aftercontractions, triggered activities and electromechanical alternans. Tetrodotoxin or butylated hydroxytoluene partially prevented the As2O3-induced prolongation of APD.

Conclusions  The prolonged QTc and spatial heterogeneity are responsible for the As2O3-induced ventricular tachyarrhythmias. In addition to prolongation of the APD, cellular Ca2+ overload and lipid peroxidation might contribute to the electrophysiological abnormalities caused by As2O3. (Circ J 2006; 70: 1407–1414)

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

Recently, arsenic trioxide (As2O3) has been shown to induce complete remission of acute promyelocytic leukemia (APL), but causes life-threatening arrhythmias. This study aimed to investigate the incidence and mechanism of arrhythmogenesis caused by As2O3.

The toxic effects of As2O3 are complex and the involvement of other undetermined mechanisms other than prolongation of APD is implied. Previous studies have shown cellular Ca2+ overload, generation of reactive oxygen species (ROS), and decreased intracellular ATP concentration ([ATP]i),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 As2O3.

In order to investigate the arrhythmogenesis of As2O3, we evaluated the changes of in ECG parameters in patients with APL during As2O3 therapy, and also examined the electromechanical effects of As2O3 in isolated guinea pig papillary muscles.

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 As2O3 (0.15 mg·kg⁻¹·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...
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; CaCl₂, 2.45; MgCl₂, 1.2; NaHCO₃, 21.9; NaH₂PO₄, 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 As₂O₃.

Reagents

Both As₂O₃ 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.
Arrhythmogenic Effects of Arsenic Trioxide

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Results

Effect of As2O3 on Clinical Parameters

Fig 1 shows the changes in the ECG parameters and CTR in the 20 APL patients during treatment with As2O3. 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 As2O3 (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 As2O3, the QT interval returned to the pretreatment level (Fig 2D).

Effect of As2O3 in Guinea Pig Papillary Muscles

To investigate the mechanisms of As2O3-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 As2O3. 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 As2O3 (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 contraction.
contracture. As summarized in Table 1, the APD at 90% repolarization (APD90) was significantly prolonged in a dose- and time-dependent manner during the perfusion of As2O3. 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 As2O3, which was followed by a significant increase in resting tension at 120 min.

**Triggered Activity Due to EAD Caused by As2O3**

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 As2O3 (350 μmol/L). As shown in Fig 4A, the prolongation of the APD by As2O3 was more prominent at lower stimulation frequencies, indicating reverse frequency dependency. In addition, the prolongation of APD by As2O3 was dependent on [K+]o (Fig 4B). When As2O3 (350 μmol/L) was administered in a low [K+]o (3.0 mmol/L) solution, the increase in APD90 was augmented compared with that when the normal solution was used (4.6 mmol/L).

Prolonged perfusion with As2O3 (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,

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**Table 1 Effects of As2O3 on Electrophysiological Parameters**

<table>
<thead>
<tr>
<th></th>
<th>As2O3 (35 μmol/L)</th>
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<td></td>
<td>Control 30 min 60 min</td>
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<td>Control 30 min 60 min</td>
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<td>RMP (mV)</td>
<td>–82±2 –78±5 –78±3</td>
<td>–78±6 –75±5 –77±5</td>
<td>–33±4 35±5 35±6</td>
<td>–150±11 184±13 195±12</td>
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<tr>
<td>APA (mV)</td>
<td>32±3 32±2 27±4</td>
<td>165±10 162±16</td>
<td>165±10 162±16</td>
<td>165±10 162±16</td>
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<tr>
<td>APD90 (ms)</td>
<td>151±3 165±10† 162±16†</td>
<td></td>
<td>150±11 184±13† 195±12†</td>
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RMP, resting membrane potential; APA, action potential amplitude; APD90, 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.
respectively. Without As2O3, neither EAD nor triggered activity occurred under these conditions (n=5).

**Triggered Activity and Electromechanical Alternans During Rapid Stimulation**

The finding that As2O3 caused muscle contracture following a transient increase in developed tension suggests that As2O3 may induce intracellular Ca2+ 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 As2O3 (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 As2O3 (Fig 5A).

Exposure to As2O3 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 As2O3-Induced Changes in Action Potentials in Guinea Pig Papillary Muscles**

Because As2O3 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 As2O3 (350 μmol/L) in the continuous presence of TTX. The shortening of the APD90 by TTX was not statistically significant (94±5% of control, p= NS by a paired t-test). In the presence of TTX, As2O3 did not prolong the APD90 significantly until 60 min, whereas the APD90 was prolonged at both 30 and 60 min in the absence of TTX (Fig 6A).

**Effects of BHT on the As2O3-Induced Changes in Action Potentials in Guinea Pig Papillary Muscles**

Finally, because arsenic is reported to cause lipid peroxidation, we examined whether BHT, an inhibitor of lipid peroxidation, would prevent the As2O3-induced prolongation of APD. When the papillary muscles were pretreated with BHT (50 μmol/L) for 30 min, the subsequent addition of As2O3 (350 μmol/L) did not prolong the APD90 during the 60 min perfusion (Fig 6B). However, BHT could not inhibit the prolongation of the APD when the muscles were exposed to As2O3 for longer than 60 min (data not shown).
This study investigated the arrhythmogenic effects of As$_2$O$_3$ using clinical and basic electromechanical examinations, and demonstrated that: (1) in patients with relapsed APL, treatment with As$_2$O$_3$ prolongs the QTc, QTcd and TDR, which are associated with the occurrence of ventricular tachyarrhythmias including TdP; (2) in guinea pig papillary muscles, As$_2$O$_3$ prolonged the APD and provoked EAD; (3) As$_2$O$_3$ also induced muscle contracture, aftercontraction, triggered activities, and electromechanical alternance; and (4) TTX and BHT partially prevented the As$_2$O$_3$-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$_2$O$_3$ therapy.

**Discussion**

This study investigated the arrhythmogenic effects of As$_2$O$_3$ using clinical and basic electromechanical examinations, and demonstrated that: (1) in patients with relapsed APL, treatment with As$_2$O$_3$ prolongs the QTc, QTcd and TDR, which are associated with the occurrence of ventricular tachyarrhythmias including TdP; (2) in guinea pig papillary muscles, As$_2$O$_3$ prolonged the APD and provoked EAD; (3) As$_2$O$_3$ also induced muscle contracture, aftercontraction, triggered activities, and electromechanical alternance; and (4) TTX and BHT partially prevented the As$_2$O$_3$-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$_2$O$_3$ therapy.

**Effects of As$_2$O$_3$ on ECG Parameters in Patients With APL**

As$_2$O$_3$ 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. 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$_2$O$_3$. Although fluconazole is known to prolong the QT interval and to induce TdP, it did not cause ECG abnormalities in that particular case during a previous administration or before treatment with As$_2$O$_3$.

In the present study, we showed changes in the ECG parameters of APL patients treated with As$_2$O$_3$, and reported a case in which As$_2$O$_3$ induced marked QTc prolongation and TdP. Although fluconazole is known to prolong the QT interval and to induce TdP, it did not cause ECG abnormalities in that particular case during a previous administration or before treatment with As$_2$O$_3$.

In addition to QTc prolongation, As$_2$O$_3$ increased the QTcd and TDR. The TDR has been reported as a marker of...
electrical dispersion and the development of a large TDR would provide the substrate for intra-mural re-entry. 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 As₂O₃ remain undetermined but may be heterogeneous.

**Effects of As₂O₃ on Action Potentials and Contraction in Guinea Pig Papillary Muscles**

Several studies have shown effects of As₂O₃ on the action potential and ion channels. Chiang et al suggested that As₂O₃-induced prolongation of the APD could be caused by blockade of Ikr, because As₂O₃ prolonged the APD in a reverse frequency dependent way. Drolet et al reported that As₂O₃ blocked both Ik and Ikᵣ in HERG- or KCNQ1 + KCNE1-transfected CHO cells, and that the sensitivity to As₂O₃ was higher in Ik than in Ikᵣ. In the present study of guinea pig papillary muscles, As₂O₃ (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 As₂O₃ level was 6.9 μmol/L (range 5.54–7.3 μmol/L) in patients with APL receiving As₂O₃ (10 mg/day) intravenously.

Chiang et al reported that the perfusion of As₂O₃ (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). In Langendorff-perfused rabbit hearts, As₂O₃ at 0.1 Hz stimulation, but less at 1 Hz. As₂O₃ was prominent at 0.1 Hz stimulation, but less at 1 Hz. As₂O₃ was higher in Ikᵣ than in Ikᵣ. In the present study of guinea pig papillary muscles, As₂O₃ (35–350 μmol/L) prolonged the APD by a marked decrease in the time-dependent K⁺ current following a transient increase in developed tension, and an increase in [Ca²⁺]ᵢ in fura-2-loaded guinea pig ventricular myocytes. Beresewicz and Horackova have shown that ROS generated by As₂O₃, per se, could directly induce Ca²⁺ overload. In fact, As₂O₃ has been shown to increase intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) in esophageal carcinoma cells. In the present study, As₂O₃ caused muscle contraction 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 As₂O₃-induced Ca²⁺ overload.

Although the precise mechanisms for the Ca²⁺ overload are unclear, As₂O₃ has been reported to inhibit pyruvate dehydrogenase and to decrease [ATP]. The decrease in [ATP] could inhibit Na⁺/K⁺ ATPase and result in an increase in [Na⁺], leading to Ca²⁺ overload via the Na⁺/Ca²⁺ exchange. The decrease in [ATP] may also depress SR Ca²⁺-ATPase activity, which causes Ca²⁺ overload. Additionally, ROS generated by As₂O₃, per se, could directly induce Ca²⁺ overload. In fact, we have already shown H₂O₂-induced triggered activities caused by DADS, contracture following a transient increase in developed tension, and an increase in [Ca²⁺]ᵢ in fura-2-loaded guinea pig ventricular myocytes. Beresewicz and Horackova have also shown that ROS can induce both EADs and DADs in rat and guinea pig ventricular myocytes. Finally, the prolongation of the APD by As₂O₃ might cause cellular Ca²⁺ overload by increasing the Ca²⁺ influx via L-type Ca²⁺ channels.

The present study showed that As₂O₃-induced electromechanical alternans, which is intriguing because T-U wave alternans has been reported in patients with As₂O₃ poisoning. As the As₂O₃-induced electromechanical alternans coincided with aftercontractions and triggered activities, electromechanical alternans might also be related to the abnormal cellular Ca²⁺ 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 [Ca²⁺]ᵢ.

In conclusion, the treatment of relapsed APL with As₂O₃...
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 As2O3 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 As2O3-induced ROS generation. ECG monitoring during the As2O3 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.

Acknowledgments

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