Unusual Effects of a QT-Prolonging Drug, Arsenic Trioxide, on Cardiac Potassium Currents

Benoit Drolet, PhD; Chantale Simard, PhD; Dan M. Roden, MD

Background—Cases of QT prolongation, torsades de pointes, and sudden death have been reported with arsenic trioxide (As$_2$O$_3$), a highly effective agent for acute promyelocytic leukemia. In this study, we evaluated the effects of As$_2$O$_3$ on repolarizing cardiac ion currents.

Methods and Results—In HERG- or KCNQ1+KCNE1-transfected CHO cells (n=32; total), As$_2$O$_3$ caused concentration-dependent block of both $I_{Ks}$ and $I_{Kr}$, with an IC$_{50}$ for tail current block of 0.14±0.01 μmol/L for $I_{Ks}$ and 1.13±0.06 μmol/L for $I_{Kr}$. In contrast to other QT-prolonging drugs, As$_2$O$_3$ also activated a time-independent current that additional experiments identified as $I_{K_{ATP}}$.

Conclusions—As$_2$O$_3$ blocks both $I_{Ks}$ and $I_{Kr}$ at clinically relevant concentrations. On the other hand, it also activates $I_{K_{ATP}}$, which maintains normal repolarization. We infer that variability in the extent of QT interval prolongation and onset of ventricular arrhythmias during arsenic therapy represents competing effects to block and activate multiple repolarizing potassium currents. (Circulation. 2004;109:26-29.)

Key Words: torsades de pointes ■ drugs ■ ion channels

Approximately 20% to 30% of patients with acute promyelocytic leukemia (APL) relapse with current standard all-trans retinoic acid and anthracycline-based chemotherapy regimen. In the mid-1990s, studies from China reported that arsenic trioxide (As$_2$O$_3$) achieved complete remission in as many as 90% of patients with APL; and additional trials have confirmed these results. However, treatment has also been associated with QT prolongation, torsades de pointes, and sudden death. Because most of the patients receiving As$_2$O$_3$ have been exposed to cardiotoxic chemotherapy, cardiac dysfunction is thought to be universal before As$_2$O$_3$ therapy begins. In addition, hypokalemia and hypomagnesemia are among the most common As$_2$O$_3$-related side effects. Thus, it has been proposed that QT prolongation and ventricular arrhythmias associated with arsenic could be exacerbated by concurrent electrolyte disturbances or previous chemotherapy-induced cardiac damage.

Recently, clinically relevant concentrations of As$_2$O$_3$ (1 to 10 μmol/L) have been reported to prolong the action potential duration in guinea pig papillary muscle. In another study using rabbit hearts, polymorphic ventricular tachycardia was observed with As$_2$O$_3$ 30 μmol/L. In this study, we therefore investigated the effects of As$_2$O$_3$ on cardiac repolarizing currents and identified an unexpectedly complex profile that may underlie variability in the arrhythmogenic potential of arsenic.

Methods

Experiments were performed in CHO cells transfected with 2 μg (each) of HERG, KCNQ1+KCNE1, or Kir6.2+SUR2A cDNAs. Kir6.2 and SUR2A cDNAs were kindly provided by Dr Joseph Bryan, Baylor College of Medicine, Houston, Tex. Cells were transfected using FuGENE 6 (Roche Applied Science). Green fluorescent protein (GFP) was coexpressed to identify transfected cells. All whole cell currents were recorded at 22°C to 23°C. Cells were held at −80 mV and pulsed to −40 to +60 mV for 1 second (HERG) or 5 seconds (KCNQ1+KCNE1), and tail currents were measured at −40 mV. The maximal tail current amplitudes were sampled after the capacitive transient in a 20-ms window. The composition of extracellular and internal pipette solutions for the HERG and KCNQ1+KCNE1 experiments were as described previously. For the Kir6.2+SUR2A experiments, cells were held at 0 mV and pulsed to −100 to +50 mV for 100 ms. The composition of solutions for these experiments was as described previously. As$_2$O$_3$ solutions of the extracellular buffer (30 mM/μL to 10 μmol/L) were prepared daily. Glibenclamide and pinacidil solutions in DMSO were prepared daily. The same concentration of DMSO (0.1% vol/vol) was also present in baseline and washout buffer solutions. As$_2$O$_3$, glibenclamide, and pinacidil were obtained from Sigma.

Statistical Analysis

Data are presented as mean±SEM. Concentration-dependent block of HERG or KCNQ1+KCNE1 tail current was tested by the Hotelling $t^2$ test. $P<0.05$ was considered statistically significant.

Results

Figure 1A shows currents elicited in a HERG-transfected cell under baseline conditions and after 20 minutes of As$_2$O$_3$ 100 nmol/L (Figure 1B). In this cell, As$_2$O$_3$ 100 nmol/L caused a 60% reduction of the tail current. Unexpectedly, how-
ever, there was also an increase in time-independent outward current (Figure 1B, arrow). Figures 1C and 1D show currents elicited before and during As$_2$O$_3$ 1 μmol/L. In this cell, As$_2$O$_3$ 1 μmol/L caused a 92% reduction of the tail current and clear activation of this time-independent outward current (Figure 1D).

Figure 1E shows concentration dependence of the effect on $I_{Kr}$ tail current, with an IC$_{50}$ of 0.14±0.01 μmol/L. Figures 1F through 1J show a similar concentration-dependent block of $I_{Ks}$ (assessed by reduction of tail currents) and, again, activation of a time-independent outward current. $I_{Ks}$ was ≈1 order of magnitude less sensitive to block, with an IC$_{50}$ of 1.13±0.06 μmol/L. Nearly all drugs that cause QT prolongation and torsades de pointes block $I_{Kr}$, and some (eg, quinidine and azimilide) also block $I_{Ks}$, often with somewhat higher IC$_{50}$ values. However, activation of an outward current, as in Figure 1, has not been reported previously. Because this effect may reflect activation of a current endogenous to CHO cells, we next step-studied cells transfected with GFP only. Figure 2A shows currents elicited in a GFP-transfected cell under baseline conditions, and Figure 2B shows currents elicited after 20 minutes of As$_2$O$_3$ 1 μmol/L. This gating pattern is reminiscent of $I_{K_{ATP}}$, and Figure 2C shows that the current was blocked by subsequent exposure to the $I_{K_{ATP}}$ blocker glibenclamide 10 μmol/L. Figures 2D through 2F show near-identical behaviors with the $I_{K_{ATP}}$ activator pinacidil 10 μmol/L (Figure 2E) and 10 minutes after the addition of glibenclamide 10 μmol/L (Figure 2F). To additionally test the hypothesis that As$_2$O$_3$ activates $I_{K_{ATP}}$, we studied Kir6.2+SUR2A-transfected CHO cells. Figures 2G through 2I show results similar to those in Figures 2A through 2C, demonstrating activation of $I_{K_{ATP}}$ by As$_2$O$_3$ 1 μmol/L. Figures 2J through 2L show that pinacidil 10 μmol/L activates a similar current and glibenclamide 10 μmol/L blocks this current, as expected.

**Discussion**

Our results show that As$_2$O$_3$ is a potent blocker of $I_{Kr}$ (IC$_{50}$: 0.14±0.01 μmol/L) and $I_{Ks}$ (IC$_{50}$: 1.13±0.06 μmol/L). It has been shown that $I_{Kr}$ block may lead to triggered
Figure 2. A, B, and C, Currents elicited by 1-second steps in a GFP-transfected CHO cell under baseline conditions, after 20 minutes of As$_2$O$_3$ 1 μmol/L, and 20 minutes after the addition of glibenclamide 10 μmol/L, respectively. D, E, and F, Currents elicited by 1-second steps in a GFP-transfected CHO cell under baseline conditions, after 20 minutes of pinacidil 10 μmol/L, and 20 minutes after the addition of glibenclamide 10 μmol/L. G, H, and I, Currents elicited by 100-ms steps in a Kir6.2 SUR2A-transfected CHO cell under baseline conditions, after 20 minutes of As$_2$O$_3$ 1 μmol/L, and 20 minutes after the addition of glibenclamide 10 μmol/L. J, K, and L, Currents elicited by 100-ms steps in a Kir6.2 SUR2A-transfected CHO cell under baseline conditions, after 20 minutes of pinacidil 10 μmol/L, and 20 minutes after the addition of glibenclamide 10 μmol/L.
tachyarrhythmias and sudden death. Moreover, adding the effect of an $I_{K_r}$ blocker on an already-compromised $I_{K_s}$ has been shown to additionally decrease the repolarization reserve, potentiating the action potential–prolonging effect of the $I_{K_s}$ blocker.16 These concentrations are well within the therapeutically relevant range. In one study of 8 patients with relapsed APL, mean peak plasma arsenic concentration was 6.85 μmol/L (range, 5.54 to 7.30 μmol/L), and after 10 hours, it was 1 μmol/L.2

Unexpectedly, our data also showed that exposure of HERG- or KCNQ1+KCNE1-transfected CHO cells to $As_2O_3$ activates a time-independent outward current. This $I_{K_{ATP}}$-like current was also activated to a similar extent in GFP-transfected CHO cells exposed to $As_2O_3$ and could be reversed by adding glibenclamide 10 μmol/L, an $I_{K_{ATP}}$ blocker.17 Indeed, the effects of $As_2O_3$ in GFP-transfected CHO cells were comparable to those of the $I_{K_{ATP}}$ activator pinacidil.17 Interestingly, one of the most common non–life-threatening side effects of $As_2O_3$ is hyperglycemia, observed in up to 45% of patients.9 This effect has also been associated with other $I_{K_{ATP}}$ activators, such as diazoxide.17 Moreover, depletion of intracellular ATP, as seen for example during cardiac ischemia, has been shown to activate $I_{K_{ATP}}$.17 Interestingly, arsenic is also recognized to uncouple cardiac mitochondrial oxidative phosphorylation.18 The mechanism is thought to be related to competitive substitution of arsenic for inorganic phosphate in the formation of ATP.18 As a result, arsenic-induced reduction of cardiac phosphorylation likely causes depletion of intracellular ATP and thus activation of cardiac $I_{K_{ATP}}$.

Therefore, while blocking both $I_{K_r}$ and $I_{K_s}$ at therapeutic concentrations, thereby producing a severe lesion in repolarization reserve, $As_2O_3$ also activates $I_{K_{ATP}}$, which may partially restore repolarization reserve and thus contribute to variability in the extent of QT-interval prolongation and onset of ventricular arrhythmias during arsenic therapy.

Conclusions
$As_2O_3$ is a potent blocker of both $I_{K_r}$ and $I_{K_s}$. On the other hand, it also activates cardiac $I_{K_{ATP}}$, which may blunt QT prolongation and arrhythmia risk by restoring repolarization reserve. The risk of torsades de pointes can be reduced by adherence to guidelines for safe use of the drug. In addition, variability in the extent of QT effects among patients may reflect this unusual combination of potassium channel actions.

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References
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