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α-Lipoic acid protects against arsenic trioxide-induced acute QT prolongation in anesthetized guinea pigs

Masafumi Kumazaki [a], Hitoshi Ando [a], Masafumi Kakei [b], Kentarou Ushijima [a], Yosuke Taniguchi [c], Masashi Yoshida [b], Shiho Yamato [b], Satoshi Washino [a], Taka-aki Koshimizu [d], Akio Fujimura [a,*]

[a] Division of Clinical Pharmacology, Department of Pharmacology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan
[b] First Department of Comprehensive Medicine, Saitama Medical Center, Jichi Medical University, 1-847 Amanuma-cho, Omiya-ku, Saitama-shi 330-8503, Japan
[c] Division of Bioorganic and Synthetic Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[d] Division of Molecular Pharmacology, Department of Pharmacology, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan

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ABSTRACT

Clinical use of arsenic trioxide (As_2O_3), which can induce the remission of relapsed or refractory acute promyelocytic leukemia, is often limited because of its cardiotoxicity. Symptoms of cardiotoxicity include acute cardiac conduction disturbances, such as QT prolongation. The present study was undertaken to evaluate the effects of α-lipoic acid (LA) on acute As_2O_3-induced ECG abnormalities (QTc interval prolongation) in anesthetized guinea pigs. Intravenous injection of As_2O_3 in guinea pigs caused QTc interval prolongation, which was significantly attenuated by co-treatment with LA (0.35, 3.5 and 35 mg/kg) in a dose-dependent manner. In isolated guinea pig cardiomyocytes, the decrease in I_{Ks} current induced by As_2O_3 (1 μM) was rapidly restored to the basal level by the addition of LA (10 μM). Consistent with this finding, the As_2O_3-induced QTc interval prolongation was also improved rapidly by post-treatment with LA in guinea pigs. Electrospray ionization time-of-flight mass spectrometry analysis detected an expected peak of arsenic-LA complex in vitro, indicating that LA and As_2O_3 form a new compound in vivo. In addition, pre-treatment with a chelating agent, British anti-Lewisite (BAL, 3.5 or 35 mg/kg), also attenuated the As_2O_3-induced QTc interval prolongation. In this study, co- and post-treatments with LA and pre-treatment with BAL ameliorated As_2O_3-induced acute QT prolongation in anesthetized guinea pigs. Because LA and probably BAL may bind to As_2O_3, these agents may exert protective effects through their chelating activity. Further studies are needed to determine whether LA is beneficial as a prophylactic or rescue agent for acute promyelocytic leukemia patients treated with As_2O_3.

1. Introduction

Arsenic trioxide (As_2O_3) is highly effective in the treatment of relapsed or refractory acute promyelocytic leukemia (Niu et al., 1999; Sanz et al., 2009; Shen et al., 1997; Soignet et al., 1998). However, As_2O_3 has frequently been reported to cause QT prolongation, T-wave changes, torsades de pointes, and sudden cardiac death as a result of the electrophysiological properties of As_2O_3 (Barbey and Soignet, 2001; Unnikrishnan et al., 2001; Westervelt et al., 2001).

Based on the previous reports, As_2O_3 might have two different short- and long-term adverse effects on the "human *ether-a-go-go*-related gene" (hERG) channel by which it induces QT prolongation (van der Heyden et al., 2008). Acute exposure (20 min) to As_2O_3 exerted direct inhibition of the rapidly activating delayed rectifier K^+ current (I_{Kr}) and slowly activating delayed rectifier K^+ current (I_{Ks}) in hERG- and KCNQ1+KCNE1-transfected CHO cells, respectively (Drolet et al., 2004). Long-term exposure to As_2O_3 reduced surface expression of the hERG channel itself (inhibition of hERG channel trafficking) in stably hERG-expressing HEK293 cells at clinically relevant concentrations (Dennis et al., 2007; Ficker et al., 2004; Katchman et al., 2006). Consistent with these findings, acute promyelocytic leukemia patients exhibit cardiac toxicity during acute as well as chronic treatment with As_2O_3. In some patients, QT interval prolongation is seen immediately after initiation of As_2O_3 treatment and, in other patients, As_2O_3-induced QT prolongation appears during chronic treatment (from a few days to a few months) (Ohnishi et al., 2002, 2000; Siu et al., 2006).

It is well-accepted that dithiol compounds (*e.g.*, BAL, British anti-Lewisite, DL-2,3-dimercaptopropanol) chelate trivalent arsenic (arsenite, As^{3+}) (Gurr et al., 1999; Huang et al., 2008; Kreppel et al.,

* Corresponding author. Tel.: +81 285 58 7387; fax: +81 285 44 7562.
E-mail address: akiofuji@jichi.ac.jp (A. Fujimura).

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1990) and BAL is approved for use as an antidote in the treatment of arsenic poisoning in clinical practice (Blanusa et al., 2005). However, the use of BAL as an antidote for heavy metal poisoning is not likely to be very safe partly because of its low solubility in water and high toxicity (Jones, 1991).

Previously, we showed that α-lipoic acid (LA), a naturally occurring dithiol compound (Smith et al., 2004), ameliorated As_2O_3-induced cytotoxicity through its antioxidant effects in renal cells (Sasaki et al., 2007). Furthermore, we recently demonstrated using a rat model that LA protects against As_2O_3-induced acute ECG abnormalities (ST–T wave change and PQ interval prolongation), and chronic treatment with LA prevented sudden death (Kumazaki et al., 2011). However, because As_2O_3 did not cause QT interval prolongation in rats, it is unclear whether LA might prevent As_2O_3-induced QT interval prolongation.

As_2O_3 has been reported to cause QT interval prolongation in guinea pigs (Chiang et al., 2002), and thus, in this study, we examined the potential protective effect of LA against As_2O_3-induced QT interval prolongation using this species. Additionally, because our previous study showed that the cardiac protective effect of LA was exerted without reducing As_2O_3-induced oxidative stress in rats (Kumazaki et al., 2011), we considered that LA protected against the As_2O_3-induced adverse cardiac effects through a mechanism other than its well-known antioxidant action. Thus, as a second purpose of this study, we attempted to clarify the mechanism underlying the effect of LA on the QT interval prolongation caused by As_2O_3.

2. Materials and methods

2.1. Chemicals

Arsenic trioxide (As_2O_3) was obtained from Nippon Shinyaku Co., Ltd. (Trisenox® injection, Kyoto, Japan). (±)-α-Lipoic acid (LA; Sigma-Aldrich, St. Louis, MO) for intravenous injection was prepared as described previously (Kumazaki et al., 2011). Briefly, LA was mixed with distilled water in a dark bottle, and 0.1 N NaOH was added until the solid was dissolved. The pH of the solution was then brought to 7.4 with 1.0 N HCl. BAL (British anti-Lewisite, 2,3-dimercaptopropanol) was purchased from Daiichi-Sankyo Co., Ltd. (BAL intramuscular injection, Tokyo, Japan).

2.2. Animals

Male Hartley guinea pigs were obtained from Japan SLC (Shizuoka, Japan) at 6 weeks of age and maintained in specific pathogen-free conditions with controlled temperature and humidity and a 12/12-h light/dark cycle. They were given a standard laboratory diet and water ad libitum. The animals were acclimated for two weeks before being used in an experiment. All animal procedures were performed in accordance with the Guidelines for Animal Research at Jichi Medical University (Tochigi, Japan) and approved by the Use and Care of Experimental Animals Committee.

2.3. Treatments

Guinea pigs, weighing 400–500 g, were anesthetized with an intraperitoneal (i.p.) injection of urethane (1.3 g/kg), and the right jugular vein was catheterized for drug infusion. Lead I ECG findings were recorded continuously with an ECG recorder (PowerLab, AD Instruments, Colorado Springs, CO). Recording began several minutes before drug injection, as described previously (Kumazaki et al., 2011). The following experiments were performed in this study.

Experiment 1 (As_2O_3 and LA were co-administered): (1) saline alone, (2) 0.15 mg/kg As_2O_3 intravenously (i.v.), a dose commonly used in humans (Niu et al., 1999; Soignet et al., 2001, 1998), (3) 1.5 mg/kg As_2O_3, (4) 1.5 mg/kg As_2O_3 and 0.35 mg/kg LA (i.v.), (5) 1.5 mg/kg As_2O_3 and 3.5 mg/kg LA, (6) 1.5 mg/kg As_2O_3 and 35 mg/kg LA, and (7) 35 mg/kg LA.
Experiment 2 (LA was given at 1 h after As_2O_3): (1) saline alone, (2) 1.5 mg/kg As_2O_3, (3) 1.5 mg/kg As_2O_3 and 35 mg/kg LA, and (4) 35 mg/kg LA.
Experiment 3: (1) saline alone, (2) 1.5 mg/kg As_2O_3, (3) 1.5 mg/kg As_2O_3 and 3.5 mg/kg BAL (i.p.), (4) 1.5 mg/kg As_2O_3 and 35 mg/kg BAL, and (5) 35 mg/kg BAL.

In *Experiment* 3, BAL was given intraperitoneally 10 min before As_2O_3 infusion to allow for its absorption, because BAL, dissolved in peanut oil, could not be injected through the catheter. The same volume of saline was used in each infusion. Saline, As_2O_3, and LA were infused for 2 h using a continuous-infusion pump (Terumo, Tokyo, Japan).

2.4. Electrocardiographic recording

The PQ, QRS, and QT intervals were measured over a 2-h period (just before and at 10, 20, 30, 60, 90, and 120 min after drug infusion). The corrected QT interval (QTc) was calculated with the Bazett formula [$QTc = QT/(RR)^{1/2}$], a method for correcting QT interval for rate in guinea pigs (Chiang et al., 2002; Hayes et al., 1994). The QTc and changes in heart rate were calculated automatically by the ECG analysis tool (ML360, AD Instruments).

2.5. Isolation of ventricular myocytes and patch clamping

Male guinea pigs (300–350 g) were anesthetized with sodium pentobarbital (30 mg/kg, i.p.). Ventricular myocytes were then isolated from dissected hearts using enzymatic dissociation, as reported previously (Shibata et al., 2012). Briefly, the heart was perfused with Ca^{2+}-free Tyrode's solution for 5 min, following perfusion with normal Tyrode's solution (1.8 mM Ca^{2+}) using the Langendorff perfusion system after dissecting out the heart under artificial respiration. The perfusate was then switched to a Ca^{2+}-free Tyrode's solution containing 0.08% collagenase (Yakult, Tokyo, Japan), and the heart was digested for approximately 15 min at 37 °C. The heart was then rinsed in a high K^+, low Cl^- solution before the left ventricle was dissected from the digested heart and stored in the same solution at 4 °C for over 1 h. The high K^+, low Cl^- solution contained the following: glutamic acid 50 mM, KCl 40 mM, KH_2PO_4 20 mM, taurine 20 mM, $MgCl_2$ 3 mM, HEPES 10 mM, EGTA 0.5 mM, glucose 10 mM, with a pH adjusted to 7.4 with KOH. The left ventricle was cut into small pieces (2 mm in size) by scissors. Then these small tissues were mingled in the high K^+, low Cl^- solution to be dispersed and filtered through stainless-steel mesh (105 μm). A proper amount of isolated single ventricular cells were put in the recording chamber and superfused with Tyrode's solution.

Perforated whole-cell clamped currents were recorded using a pipette solution (composition; K_2SO_4 40 mM, KCl 50 mM, HEPES 10 mM, $MgCl_2$ 5 mM, EGTA 0.5 mM, with the pH adjusted to 7.2 with KOH) containing amphotericin B (150–200 μg/mL) dissolved in 0.1% DMSO, as described previously (Dezaki et al., 2004; Nakazaki et al., 1995; Yoshida et al., 2010). Membrane currents were recorded using an amplifier (Axopatch, 200B, Foster City, CA) and a computer using the pCLAMP10.2 software. The resistances of the patch pipettes were 3–5 MΩ. Membrane current measurements were performed at 22 °C.