Short Communication

Protective Effect of α -Lipoic Acid Against Arsenic Trioxide–Induced Acute Cardiac Toxicity in Rats

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Abstract. The clinical use of arsenic trioxide (ATO) is often limited because of its adverse effects. We examined whether α -lipoic acid (LA) protects against the ATO-induced cardiac toxicity. In the chronic study, two of four rats suddenly died by the repeated dosing of ATO, whereas no deaths were observed in combination with LA. In the acute study, continuous ECG recording revealed that intravenous injection of ATO caused transient ST-T change, whereas pretreatment with LA abolished the ATO-induced ECG abnormality in all animals. These results suggest that LA protects against the ATO-induced acute cardiac toxicity and subsequent sudden death in rats.

Keywords: α -lipoic acid, arsenic trioxide, cardiac toxicity

Arsenic trioxide (ATO) is used for the treatment of relapsed or refractory acute promyelocytic leukemia (1). However, the clinical use of ATO is often limited by its adverse effects. For instance, ATO is reported to cause renal injury (2, 3) and cardiac toxicity, including T-wave changes, QT prolongation, torsades de pointes, and sudden cardiac death in humans (4–6). We previously performed gene expression analysis using DNA microarrays in human primary renal cortical cells and found that the expression of heme oxygenase 1, which is induced by oxidative stress, was strongly related to the ATO-induced cytotoxicity (7). Moreover, we observed in vitro that α -lipoic acid (LA), a naturally occurring dithiol compound with an antioxidant property (8), ameliorated the ATO-induced cytotoxicity by reducing superoxide anion production, while it did not alter the effect of ATO on promyelocytic leukemia cells or myeloma cells (7). Thus, these observations provide the possibility that LA protects against the ATO-induced adverse effects without loss of therapeutic efficacy in vivo. To determine the characteristics of the ATO-related adverse effects and potential preventive effect of LA, we administered ATO with and without LA repeatedly to small number of Wistar rats. Because the chronic study suggested that

ATO caused sudden cardiac death, and this adverse effect was prevented by LA, we further evaluated the effects of ATO and LA on ECG findings in the acute study.

Arsenic trioxide (ATO) was obtained from Nippon Shinyaku Co., Ltd. (Trisenox[®] injection, Kyoto). ($\pm$)- α -Lipoic acid (LA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). For intravenous injection, 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.

Male Wistar rats were obtained from Japan SLC (Shizuoka) at eight weeks of age and maintained under a specific pathogen-free condition with controlled temperature and humidity and a 12-h light / 12-h dark cycle. They were given standard laboratory diet and water ad libitum. All animal procedures were performed in accordance with the Guidelines for Animal Research at Jichi Medical University (Tochigi) and approved by the Use and Care of Experimental Animals Committee.

In the chronic study, rats ($n = 16$) were divided into the following four groups ($n = 4$ in each): Group I (control group) received i.p. injection of saline ($5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and gavage administration of 0.5% carboxymethyl cellulose sodium salt (CMC) solution as a vehicle; Group II (ATO group) and Group III (ATO + LA group) received i.p. injection of ATO ($5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and gavage administration of vehicle or LA (35

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mg·kg⁻¹·day⁻¹), respectively; and Group IV (LA group) received i.p. injection of saline and oral administration of LA (35 mg·kg⁻¹·day⁻¹). After 8 weeks of treatment, the 24-h urine sample was collected after the last dosing. Blood samples were also obtained.

In the acute study, rats (n = 32) were anesthetized with i.p. injection of urethane (1.3 g/kg), and the right femoral vein was catheterized for drug infusion. The lead I ECG was continuously recorded by an ECG recorder (PowerLab; AD Instruments, Colorado Springs, CO, USA) from several minutes before drug injection as follows (n = 4 in each group): 1) saline alone; 2) 0.15 mg/kg ATO, a dose commonly used in humans (9, 10); 3) 1.5 mg/kg ATO; and 4) 5 mg/kg ATO. Effect of LA on the ATO-related ECG abnormality was also evaluated as follows (n = 4 in each group): 1) Control group: infusions of saline, twice; 2) ATO group: infusions of saline and ATO (5 mg/kg); 3) ATO + LA group: infusions of LA (70 mg/kg) and ATO (5 mg/kg); and 4) LA group: infusions of LA (70 mg/kg) and saline. ATO was given at 10 min after LA dosing. The same volume of saline was used in each infusion. The dose of LA (70 mg/kg) was selected by reference to a previous study (11) reporting that a single intravenous injection of LA produced an acute protective effect on oxidative stress in the heart.

The ECG parameters were measured during the 2-h period (immediately before and at 10, 20, 30, 60, 90, and 120 min after infusion). The corrected QT interval (QTc) was calculated with the Bazett formula and the Waterfall Plot (three-dimensional image of the stacked averaged ECGs) was created using the ECG Analysis tool (MLS360, AD Instruments).

Serum concentrations of aspartate transaminase (AST),

alanine transaminase (ALT), alkaline phosphatase (ALP), and creatinine (sCr) and urinary concentration of 8-hydroxy-2'-deoxyguanosine (8-OHDG), a marker of oxidative stress, were assayed using commercial kits.

All data are presented as the mean ± S.E.M. One-way ANOVA was used to compare means among groups. The Bonferroni/Dunn procedure was used as a post-hoc test. All statistical analyses were carried out with StatView 5.0 software (SAS Institute, Cary, NC, USA). Differences were considered to be significant at *P* < 0.05.

To determine the characteristics of the ATO-related adverse effects and potential preventive effect of LA, ATO, and LA were repeatedly given to Wistar rats for eight weeks. As a result, two of the four ATO-treated rats suddenly died without prior symptoms of infection and cerebrovascular disease on days 25 and 28, respectively. However, no rats died in the other three groups. ATO reduced the body weight and serum AST concentration and increased the urinary excretion of 8-OHDG in the surviving rats (Table 1). Interestingly, LA did not ameliorate these ATO-related changes (Table 1), although the agent prevented the ATO-induced death in this study. Therefore, the cause of death in rats with ATO alone might not be malnutrition, chronic organ damage, or bone marrow suppression.

LA is a potent biological antioxidant and is reported to scavenge free radicals, chelate metals, and restore intracellular glutathione level (12). We previously showed that ATO causes renal cell damage with the elevation of heme oxygenase 1, which is induced by oxidative stress, and LA ameliorates the ATO-induced cytotoxicity by reducing superoxide anion production (7). These data led us to speculate that the protective effect of LA against the

Table 1. Effects of ATO and LA on the laboratory parameters

Parameter	Control	ATO [§]	ATO + LA	LA
Body weight (g)	267.1 (245.5, 286.2)	231.8 (217.5, 243.9)	223.5* (205.4, 242.0)	253.1 (249.0, 257.6)
AST (IU/mL)	88 (83, 91)	66 (58, 74)	59* (50, 78)	81 (68, 93)
ALT (IU/mL)	36 (32, 38)	31 (28, 34)	38 (32, 53)	40 (32, 48)
ALP (IU/mL)	707 (649, 779)	598 (547, 649)	547 (444, 610)	669 (582, 848)
sCr (mg/dL)	0.29 (0.27, 0.33)	0.33 (0.27, 0.39)	0.27 (0.27, 0.27)	0.27 (0.25, 0.28)
8-OHDG (ng/day)	261.7 (177.9, 332.0)	462.7 (252.8, 672.6)	447.6* (340.6, 535.8)	307.0 (270.9, 369.0)

Values are reported as the mean (min, max) (n = 4, [§]n = 2). **P* < 0.01, compared to the control. Differences among three groups except for the ATO group were determined by ANOVA. ATO dosing reduced the body weight and serum AST concentration and increased the urinary excretion of 8-OHDG in the surviving rats.

ATO-induced adverse effect resides in its antioxidative effect. However, to our surprise, LA did not diminish the systemic ATO-induced oxidative stress, which was reflected in urinary 8-OHdG excretion. Therefore, it remains probable that LA prevents sudden death by a mechanism other than the protection against oxidative stress-associated organ damages.

Because ATO treatment can lead to QT prolongation and T-wave changes in clinical practice (13), we speculated that 1) ATO caused sudden death due to cardiac toxicities and 2) LA prevented ECG abnormalities induced by ATO in rats. Then, we examined whether ATO induces abnormal ECG findings. The previous study showed that intravenous ATO (0.15 and 1.5 mg/kg) acutely prolonged QT interval in guinea pigs (14). However, infusion of the same (0.15 and 1.5 mg/kg) and higher (5 mg/kg) doses of ATO in Wistar rats did not change the QT interval (QTc) in this study (data not shown). However, the higher dose (5 mg/kg) of ATO caused transient ST-T wave changes on the ECG, from approximately 5–30 min after infusion (Fig. 1). In addition, ATO treatment significantly ($P < 0.01$) prolonged PQ interval by about 6 ms from 10 to 120 min after infusion (Fig. 1). Next, we examined the effect of LA pretreatment on such ECG abnormalities and found that both the ST-T wave change and PQ-interval prolongation induced by ATO treatment were completely prevented by co-administration of LA (Fig. 2). No other abnormal

ECG findings, such as prolonged intervals of QT/QTc, PR, and QRS, were detected in the ATO or LA groups (Fig. 2).

The present study demonstrated for the first time that LA exerts a protective effect against the ATO-induced ST-T wave change and PQ-interval prolongation. Considering that ATO affects at least cardiac potassium currents (15), LA seems to prevent the ATO-induced electrical abnormalities. Because dithiol chelating compounds, such as DL-2,3-dimercaptopropanol (British Anti-Lewisite, BAL), DL-2,3-dimercaptopropanesulfonate (DMPS), and meso-2,3-dimercaptosuccinic acid (DMSA), are effective in reducing acute ATO poisoning by reacting with trivalent arsenic (16), it is possible that LA might reduce the ATO-induced acute cardiac toxicity via the same mechanism. Further studies are needed to reveal the underlying mechanism of the protective effect of the agent.

The dose of ATO that caused ECG abnormalities in rats was about 30 times higher than the therapeutic dose in the usual clinical setting. However, a phase I/II clinical trial provided data showing the long elimination half-life (approximately 100 h) of arsenic and gradual elevation in blood concentration of the agent during the repeated dosing (Product information from Nippon Shinyaku, 2010). Plasma arsenic concentration is reported to elevate to 2 to 5 times higher concentrations at one week after the initiation of treatment. Therefore, it is possible that

Fig. 1. Changes of ECG waveform after the intravenous infusion of ATO in rats. Representative three-dimensional images of the stacked averaged ECGs in rats treated with saline (A) or 0.15 (B), 1.5 (C), and 5 (D) mg/kg of ATO are presented. Continuous ECG monitoring revealed that only 5 mg/kg of ATO caused a transient ST-T change (arrow) from approximately 5 to 30 min after infusion.

Fig. 2. Changes of ECG waveform after intravenous infusion of ATO in rats pretreated with and without LA. A) Lead I ECG waveforms at 0, 10, and 30 min after saline or ATO infusion in one representative rat. B) Lead I ECG waveforms at 10 min in all animals. The ST-T change induced by 5 mg/kg of ATO alone was reproducibly detected in all animals (black arrows), and the pretreatment with LA completely abolished such an ECG abnormality (gray arrows).

the arsenic concentration after multiple doses of ATO was high enough to cause myocardial toxicity during the repeated treatment in the first study.

In summary, ATO acutely caused ECG abnormalities, and LA completely prevented them in Wistar rats. Chronic treatment with LA did not reduce the oxidative stress induced by ATO, but prevented sudden death. These results suggest that LA protects against the adverse effect caused by ATO through a mechanism other than the anti-oxidant action. Clinical trials are needed to confirm whether LA can prevent ATO-induced cardiac toxicity in patients with acute promyelocytic leukemia.

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