Original Paper

Genistein Ameliorates Adverse Cardiac Effects Induced by Arsenic Trioxide Through Preventing Cardiomyocytes Apoptosis

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Key Words

Genistein • Arsenic trioxide • QT interval prolongation • Apoptosis

Abstract

Background/Aims: Arsenic trioxide (As₂O₃) is a highly effective agent for treatment of acute promyelocytic leukemia (APL). However, consecutively administered As₂O₃ induces serious adverse cardiac effects, including long QT syndrome (LQTS) and even sudden cardiac death. Previous studies have shown that genistein (Gen) exerts anti-oxidant, anti-inflammatory, and anti-apoptotic effects. The present study aimed to explore the potential protective effects of Gen on As₂O₃-induced adverse cardiac effects, and to elucidate the underlying molecular mechanisms. **Methods:** A rat model of As₂O₃-induced QT prolongation was generated by intravenous injection with As₂O₃. Surface electrocardiogram (ECG) and hemodynamics were employed to assess the LQTS and cardiac function. Intracellular calcium concentration ([Ca²⁺]_i) and mitochondrial membrane potential (ΔΨ_m) were measured by confocal microscopy, and cardiomyocytes apoptosis were assessed by TUNEL assay. Western blot was applied to determine protein levels. **Results:** We found for the first time that treatment with Gen significantly reversed LQTS and dose-dependently improved As₂O₃-induced impairment of cardiac function. As₂O₃ elevated [Ca²⁺]_i and Gen mitigated this effect. Meanwhile, Gen significantly reversed As₂O₃-mediated cardiomyocytes apoptosis. Furthermore, Gen dose-dependently inhibited the phosphorylated JNK and p38-MAPK (pp38-MAPK), and blocked ΔΨ_m collapse, and further decreased cleaved caspase-3. **Conclusion:** Gen protects against the adverse cardiac effects of As₂O₃ partly by mitigating cardiomyocytes apoptosis induced by As₂O₃ through attenuating intracellular calcium overload and downregulating protein expression of p-JNK and pp38-MAPK to ameliorate the damage of ΔΨ_m leading to suppression of caspase-3 activation. Gen might be used as an adjunction therapy in APL patients receiving As₂O₃ treatment to avoid, at least to minimize, the adverse cardiac effects of As₂O₃.

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Introduction

Acute promyelocytic leukemia (APL) is a common hematological system disease with high morbidity and mortality. Arsenic trioxide (As₂O₃) was first applied to treat APL at the Harbin Medical University in 1970s [1], and have been well documented with high therapeutic efficacy with complete remission rate of up to 85% [2]. Strikingly, it has emerged as the front-line agent for treatment of relapsed or refractory APL [3], but has major limitations, such as severe ventricular arrhythmia including long QT syndrome (LQTS) and sudden cardiac death [4-6]. Due to adverse cardiac effects, some patients have to be excluded from treatment with As₂O₃. Therefore, it is highly desirable to develop measures against adverse cardiac effects induced by As₂O₃ and to explore its potential molecular mechanisms.

It has been reported that promoting release of calcium from sarcoplasmic reticulum (SR) and increasing in L-type Ca²⁺ current (I_{cal}) lead to intracellular calcium overload, which contributes to cardiomyocytes apoptosis [7, 8]. Apoptosis is a critical and severely conserved mode of cell death that is regulated by a number of signaling pathways, and it is vital to maintain occurrence of arrhythmia, normal cardiac function and development. Recently, cardiomyocytes apoptosis has been shown to lead to LQTS [9, 10] and cardiac dysfunction, which occurs due to intracellular calcium overload and mitochondrial membrane potential ($\Delta\Psi$ m) collapse [7]. Multiple studies have shown that high intracellular calcium concentration ([Ca²⁺]_i) induces phosphorylation of c-Jun NH2-terminal kinase (JNK) and p38 mitogen activated protein kinase (p38-MAPK) [11, 12]. Interestingly, the phosphorylated JNK and p38-MAPK mediate $\Delta\Psi$ m collapse [13], which plays a pivotal role in mediating apoptosis. Recently, the MAPK signaling cascade has been shown to be incorporated into cardiomyocytes apoptosis [14]. It has been reported that JNK agonist promotes apoptosis, while its inhibitor prevents apoptosis by suppressing expression of apoptotic-related proteins [15]. Similarly, upregulation of p38-MAPK expression initiates, while its inhibitor alleviates, apoptosis in cardiomyocytes [16]. Caspase-3 is a member of the caspase family that plays a central role in the apoptotic program [17]. Thus, strategies that attenuate intracellular calcium overload, target the MAPK signaling cascade and inhibit caspase-3 activation may represent a promising therapeutic approach for adverse cardiac effect induced by As₂O₃.

Genistein (Gen; 4',5,7-Trihydroxy isoflavone), a natural biologically active flavonoid found in soy, has been shown to possess multiple pharmacological actions, including anti-cancer, anti-inflammatory, and anti-oxidant effects [18, 19]. Intriguingly, in contrast to As₂O₃, epidemiological studies showed that its usage is closely associated with decreased risks of cardiovascular disease [20], with additional cellular effects such as attenuated intracellular Ca²⁺, and anti-apoptotic effects [18, 21, 22].

In view of these multiple beneficial effects of Gen, the present study was designed to investigate whether Gen could protect against As₂O₃-induced adverse cardiac effects. Furthermore, we attempted to explore whether Gen is able to prevent cardiomyocytes apoptosis induced by As₂O₃ and to elucidate its underlying molecular mechanisms.

Materials and Methods

Drugs and reagent

Gen (purity 95%) was purchased from Xi'an QingYue Biotechnology co., Ltd (Xi'an, Shaanxi, China). As₂O₃ was obtained from Harbin YI-DA Pharmaceutical Limited Company. Fluo-3-acetoxymethyl (Fluo-3/AM) (Molecular Probes, Eugene, Oregon, USA) was dissolved in 0.1% DMSO and stored at -20°C in the dark.

Animals

Healthy male Wistar rats (200–300 g) were provided by the Experimental Animal Center of Harbin Medical University (Grade II), China. The rats were kept at 23±1°C and humidity of 55–60%, with a 12 h day/night cycle for two weeks. The use of animals was approved by the Institutional Animal Care and Use Committee of the Harbin Medical University, China.

Measurements of QT interval and hemodynamic parameters

The rats were randomly divided into several groups: control, As₂O₃ (0.8 mg/kg/day), Gen (10 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day), and As₂O₃+Gen (10 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day) groups. As₂O₃ was administrated intravenously for consecutively 7 days. For As₂O₃ group, the rats were firstly treated with saline for 7 days, and subsequently As₂O₃ was administrated intravenously for another 7 days. In As₂O₃+Gen groups, rats were firstly administered with Gen for 7 days prior to injection of As₂O₃ for another 7 days. For Gen group and control group, the animals were respectively treated with Gen and saline for 14 days. At the end of treatments, rats were anaesthetized with sodium pentobarbital (40 mg/kg, intraperitoneally) and surface electrocardiogram (ECG) was recorded by penetrating electrodes into four limbs. The QT interval was measured from the onset of QRS complex to the end of the T wave, whereas RR interval was recognized the distance between the apexes of consecutive R waves. They were assessed in a blinded manner by one reader using the same lead for all tracings for rats. The measurements of QT interval and RR interval were used to acquire heart rate-corrected QT interval using the Friedericia's formula ($QT_c = QT / (RR)^{1/3}$) [23]. Two weeks after drug administration, hemodynamic parameters were recorded using a pressure volume control unit (Scisense Inc., London, Ontario, Canada). A pressure-sensing catheter (1.9F, Scisense Inc.) was inserted into the left ventricle of the rats via the right common carotid artery. Heart rate (HR), cardiac output (CO), ejection fraction (EF), mean arterial pressure (MAP), +dp/dt max, and -dp/dt max were also calculated. After the measurements, the rats were sacrificed, and hearts were quickly dissected and frozen at -80°C for subsequent experiments.

Isolation and culture of neonatal rat ventricular cells (NRVCs)

NRVCs were collected from hearts of 1-to 3-day-old rats. Briefly, neonatal rats were bathed in 75% alcohol and decapitated, and hearts were removed and placed into DMEM. Hearts were carefully cut into small pieces and treated with 0.25% trypsin solution. Then, the isolated cardiomyocytes were kept in DMEM with 10% foetal bovine serum (FBS). Subsequently, cardiomyocytes were centrifuged, and pellets were resuspended with fresh medium. The cellular suspensions were kept for 90 min at 37°C. Finally, the cardiomyocytes-enriched suspensions were removed from the culture flask and cultured with fresh medium.

Measurement of intracellular Ca²⁺ fluorescence

NRVCs were cultured with 6-well plate and allowed to grow for 48 h at 37°C. Briefly, NRVCs were deprived of serum and cultured in DMEM for 12 h prior to incubation with Gen (10 μM) for 1 h followed by incubating with As₂O₃ or with As₂O₃ alone for 6 h. The dynamic change of intracellular Ca²⁺ was measured as previously described [24]. Briefly, NRVCs were loaded with Fluo-3/AM (5 μM) for 20 min and fluorescent signals were detected by a confocal laser scanning microscope (Olympus FV-300) with 488 nm beam for excitation from an argon ion laser and 530 nm beam for emission. Acquisition rate of [Ca²⁺]_i was one frame per 5 s for about 150 s in normal Ca²⁺-containing Tyrode's solution and KCl (30 mM) was added at tenth scan. The relative magnitude of fluorescent signals was calculated as FI/F₀, which F₀ represents the basal level of fluorescence and FI the value was after drug treatment [25].

Measurement of mitochondrial membrane potential (ΔΨm)

The effects of As₂O₃ and Gen on apoptosis of NRVCs were investigated by measuring ΔΨm using cell-penetrating lipophilic cationic fluorochrome JC-1 (5,5', 6,6'-tetrachloro-1, 1', 3,3'-tetraethylbenzimidazole-carbocyanide iodine; Beyotime, China). Red emission of the dye represents a normal, high mitochondrial transmembrane potential and green fluorescence indicates early apoptosis of cardiomyocytes with depolarized ΔΨm. NRVCs were seeded into 6-well plate and allowed to grow for 48 h at 37°C. Cardiomyocytes were deprived of serum and cultured in DMEM for 12 h prior to incubation with different concentrations of Gen for 1 h followed by incubation with As₂O₃ or with As₂O₃ alone for 12 h. The ΔΨm was measured by a confocal laser scanning microscope.

TUNEL assay

DNA fragmentation in cardiomyocytes was detected by using the TUNEL assay (Cell Death Detection Kit, Roche Biochemicals, Mannheim, Germany). Briefly, air-dried slides were fixed with 4% paraformaldehyde for 30 min at room temperature and rinsed three times with phosphate-buffered saline (PBS) for 10 min, pH7.4, and then permeabilized with 1% Triton X-100 for 4 min at 4°C. Then, the TdT-labelled nucleotide

Fig. 1. Genistein (Gen) shortens As₂O₃-induced QT interval prolongation in rats. (A) Representative surface electrocardiogram (ECG) of rat hearts showing the QT intervals from control, Gen (100 mg/kg), As₂O₃ (0.8 mg/kg), and As₂O₃+Gen (10, 50, 100 mg/kg). (B) Corrected QT (QTc) was significantly prolonged after treatment with As₂O₃ alone, but not altered by Gen alone. Notably, Gen (10, 50, 100 mg/kg) prevented As₂O₃-induced QT interval prolongation (** $p < 0.01$, vs. control, † $p < 0.05$, †† $p < 0.01$ vs. As₂O₃ treated group; $n = 6$ rats).

mix was added to each slide and incubated at 37°C for 60 min in dark. Slides were washed twice with PBS and then counterstained with 10 mg/ml 4,6-diamidino-2-phenylindole (DAPI) for 5 min at 37°C. The cardiomyocytes were randomly assigned into five groups: control, As₂O₃ 5 μ M, As₂O₃ (5 μ M)+Gen of 10, 50, and 100 μ M. Cardiomyocytes were treated with As₂O₃ and Gen for 24 h. Three independent experiments were carried for each group.

Protein extraction and immunoblotting analysis

Total protein samples were prepared with adult and neonatal rats ventricular cells. The protein samples were subjected to 8%-15% SDS-PAGE and blotted to nitrocellulose membrane, then blocked by 5% non-fat milk, and washed in phosphate-buffered saline (PBS). Next, the membranes were probed with primary antibodies of phospho-JNK (p-JNK), JNK, phospho-p38-MAPK (pp38-MAPK), p38-MAPK (Cell Signaling Technology, Danvers, MA, USA), Cav1.2, cleaved caspase-3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and GAPDH (Kangcheng Inc., Shanghai, China) in PBS and incubated overnight at 4°C. Membranes were washed three times with 10 min each time in PBS containing 0.5% Tween-20, and incubated with secondary antibody (Alexa Fluor, Molecular Probes, Eugene, OR, USA) for 1 h. Western blot bands were recorded by Imaging System (LI-COR Biosciences, Lincoln, NE, USA) and quantified by Odyssey v3.0 software by measuring the band intensity (area $\times$ OD) in each group and normalized to GAPDH (anti-GAPDH antibody) as an internal control.

Statistical analysis

Data are expressed as mean $\pm$ SEM. The significance of statistical analysis was assessed by applying one-way ANOVA followed by Dunnett's t -test. Two-tailed $p < 0.05$ was considered to have statistical differences.

Results

Gen shortens As₂O₃-induced QT interval prolongation

The effect of As₂O₃ on QTc was first observed in rat hearts. The representative traces of ECG of the six groups are shown in Fig. 1A. The QTc interval was significantly prolonged from 145 ± 3 ms in untreated animals (control) compared to 169 ± 6 ms in As₂O₃ group with a dosage 0.8 mg/kg. This prolongation was significantly mitigated, in a dose-dependent

Group (n=6)	Control (n=6)	Gen (100mg/kg, n=6)	As ₂ O ₃ (0.8mg/kg, n=6)	As ₂ O ₃ +Gen (10mg/kg, n=6)	As ₂ O ₃ +Gen (50mg/kg, n=6)	As ₂ O ₃ +Gen (100mg/kg, n=6)
QT (ms)	76±2	79±2	87±4 **	79±3 †	73±1 ††	74±2 ††
RR (ms)	144±7	155±6	140±7	144±10	129±7	28±8

Table 1. Gen shortens As₂O₃-induced QT interval prolongation in rats. ***p*<0.01 vs. control; †*p*<0.05, ††*p*<0.01 vs. As₂O₃ treated group.

Group (n=6)	Control (n=6)	Gen (100mg/kg, n=6)	As ₂ O ₃ (0.8mg/kg, n=6)	As ₂ O ₃ +Gen (10mg/kg, n=6)	As ₂ O ₃ +Gen (50mg/kg, n=6)	As ₂ O ₃ +Gen (100mg/kg, n=6)
Heart rate (beat/min)	433±10	428±4	392±11**	406±12	435±17†	453±10††
CO (μl/min)	122377±966	108368±1324	70518±5348**	79662±16309	126180±10168††	124366±17789††
EF (%)	54±4	47±3	32±2**	32±2	40±6†	47±7††
dp/dt max (mm Hg/s)	10585±949	10164±628	7139±458*	8982±1095	11424±762††	12861±1362††
-dp/dt max (mm Hg/s)	8896±512	7942±432	5555±381*	7344±1086	8343±632††	8546±315††

Table 2. Gen restores impaired cardiac function caused by As₂O₃ in rats. **p*<0.05, ***p*<0.01 vs. control; †*p*<0.05, ††*p*<0.01 vs. As₂O₃ treated group. HR, Heart Rate; CO, Cardiac output; EF, Ejection fraction.

manner, to 151 ± 2 ms, 145 ± 1 ms, and 147 ± 3 ms in rats treated with Gen at dosages of 10, 50 and 100 mg/kg/day. Intriguingly, Gen (100 mg/kg) alone produced no effects on QTc interval of the normal hearts (Fig. 1B). In addition, QT and RR duration was acquired from the ECG at all heart rates (Table 1).

Gen improves As₂O₃-induced cardiac function impairment

In the absence of Gen, As₂O₃ produced deleterious effects on cardiac function as illustrated in Table 2. The heart rate was significantly decreased by As₂O₃ (*p*<0.01); meanwhile, cardiac output, ejection fraction, +dp/dt max, and -dp/dt max were also reduced by As₂O₃ (*p*<0.01). However, treatment with Gen significantly attenuated the detrimental effects of As₂O₃ in a dose-dependent manner, though Gen (100 mg/kg) alone failed to affect these hemodynamic parameters of the normal hearts. Neither As₂O₃ nor Gen alone affected MAP (mean arterial pressure) parameter (*p*>0.05, data not shown).

Gen attenuates intracellular Ca²⁺ overload and downregulates expression of Cav1.2 in As₂O₃ treated NRVCs

To investigate whether changed Ca²⁺ is involved in Gen produced cardioprotective effects. Gen (10 μM) was applied for 1 h before As₂O₃ (5 μM) administration. The representative fluorescent images of [Ca²⁺]_i in Fig. 2A showed that As₂O₃ provoked remarkable elevation of [Ca²⁺]_i when stimulated with KCl (30 mM) in NRVCs, and co-incubated with Gen attenuated this elevation (Fig. 2A). The averaged data were summarized in Fig. 2B. The averaged ratio

Fig. 2. Gen abrogates intracellular Ca²⁺ overload and downregulates Cav1.2 protein in As₂O₃ treated neonatal rat ventricular cells (NRVCs). (A) Representative images of Fluo-3/AM staining of NRVCs showing the changes of intracellular Ca²⁺ concentrations ([Ca²⁺]_i). The middle column represents As₂O₃ (5 μM) treated cells, the right column was Gen (10 μM) and As₂O₃ incubation, the left column indicated normal cardiomyocytes. (B) Mean values of FI/FI₀ ratio at varying time points in the presence of 30 mM KCl, showing that Gen produced instantaneous and time-dependent suppression of the elevated [Ca²⁺]_i induced by As₂O₃, where F₀ represents the basal level of fluorescence and F_I the value after drug treatment. (C) Mean data of the peak FI/FI₀ ratio (FI_{max}/FI₀), n=16 cardiac myocytes from each group. (D) Gen (10, 50, 100 μM) depressed the upregulation of Cav1.2 protein level in NRVCs. ***p*<0.01 vs. control, ††*p*<0.01 vs. As₂O₃ treated group; n=3 independent experiments.

of peak value (FI_{max} : FI₀) was significantly increased to 6.30 ± 0.71 in the As₂O₃ treated NRVCs compared with 2.97 ± 0.28 in normal group. However, the ratio of peak FI/FI₀ was significantly decreased to 3.01 ± 0.17 in Gen-administered group compared with As₂O₃ treated group (*p*<0.01, Fig. 2C). To investigate whether the attenuation of [Ca²⁺]_i was related to changes of I_{CaL}, we measured the protein level of Cav1.2, the α₁-subunit of L-type Ca²⁺ channel carrying I_{CaL}. The results showed that As₂O₃ alone upregulated Cav1.2 protein level, while co-treatment with Gen (10, 50, and 100 μM) for 24 h significantly attenuated this effect of As₂O₃ in a dose-dependent manner (Fig. 2D).

Gen improves As₂O₃-induced ΔΨm collapse in NRVCs

Evidence from multiple studies suggests that the altered [Ca²⁺]_i can mediate ΔΨm damage [26, 27]. Therefore, we next measured the ΔΨm following treatment with Gen and As₂O₃. As shown in control and As₂O₃ (5 μM) treated groups, normal NRVCs were stained in red and early apoptotic cells were in green (Fig. 3A). ΔΨm was reduced after exposure to As₂O₃, whereas co-incubation with Gen ameliorated the impaired ΔΨm in a dose-dependent manner (Fig. 3B).

Fig. 3. Gen prevents the impairment of mitochondrial membrane potential ($\Delta\Psi_m$) induced by As₂O₃. (A) Representative images showing the changes of $\Delta\Psi_m$ as an indicator of early apoptosis in the presence of As₂O₃ and Gen in NRVCs. (B) Averaged values of fluorescence density of JC-1 polymer were shown. ** $p < 0.01$ vs. control, †† $p < 0.01$ vs. As₂O₃ treated groups; $n = 3$ independent photographs shot for each group.

Fig. 4. Gen reduces As₂O₃-induced cardiomyocytes apoptosis. (A) Photograph of a TUNEL-positive cardiomyocytes. (B) Quantitative analysis of apoptotic nuclei. The number of apoptotic nuclei was significantly increased in As₂O₃-treated cardiomyocytes compared with control group. And fewer apoptotic nuclei were found in the As₂O₃+Gen groups compared with the As₂O₃-treated group. ** $p < 0.01$ vs. control, †† $p < 0.05$ or ††† $p < 0.01$ vs. As₂O₃; $n = 3$ independent experiments.

Gen decreases As₂O₃-mediated cardiomyocytes apoptosis

To determine the protective effects of Gen against As₂O₃-induced cardiomyocytes apoptosis, TUNEL assay was carried out. As shown in Fig. 4, TUNEL-positive cells were significantly increased in As₂O₃-treated cardiomyocytes, and this increase was prevented by Gen (10, 50, 100 μM) treatment for 24h.

Fig. 5. Gen represses the protein levels of phospho-JNK (p-JNK), phospho-p38-MAPK (pp38-MAPK) and cleaved caspase-3 in NRVCs. (A & B) The protein expression of p-JNK and JNK in NRVCs. (C & D) Representative images of immunoblot bands of pp38-MAPK and p38-MAPK in NRVCs. (E) Western blotting showing the inhibition of cleaved caspase-3 produced by Gen in As₂O₃-treated NRVCs. ***p* < 0.01 vs. control, †*p* < 0.05 or ††*p* < 0.01 vs. As₂O₃; n = 3 independent experiments.

Gen downregulates protein levels of p-JNK, pp38-MAPK and cleaved caspase-3 in NRVCs

In order to confirm the underlying molecular mechanisms of Gen on As₂O₃-induced cardiomyocytes apoptosis, we measured the levels of apoptosis-related proteins. We found that Gen significantly repressed the expression of p-JNK in a dose-dependent manner; however, the total level of JNK was not affected (Fig. 5A & 5B). The p38-MAPK signaling cascade has been reported to participate in cardiomyocytes apoptosis [15]; our results confirmed that As₂O₃ alone caused a robust increase in the pp38-MAPK expression and co-treatment with Gen abolished the activation of pp38-MAPK. The protein level of total p38-MAPK was not altered by As₂O₃ and Gen (Fig. 5C & 5D). Cleaved caspase-3, the activated form of caspase-3, is known to be the executor of cardiomyocytes apoptosis [28]. Indeed, in our study, As₂O₃ alone significantly increased the level of cleaved caspase-3 in NRVCs, and more importantly, co-treatment with Gen prevented caspase-3 activation (Fig. 5E).

Gen downregulates the levels of Cav1.2 and p-JNK and pp38-MAPK in As₂O₃-treated rats

To further explore the beneficial effects of Gen against the adverse cardiac effects of As₂O₃, we measured the protein levels of Cav1.2, p-JNK and pp38-MAPK in As₂O₃ treated heart. We found that administration of As₂O₃ for seven days significantly increased Cav1.2 protein levels, and this upregulation was abrogated following treatment with Gen at varying doses (10, 50, and 100 mg/kg/day). Consistent with this observation, the levels of p-JNK

Fig. 6. Gen downregulates the protein levels of Cav1.2, and p-JNK and pp38-MAPK in As₂O₃ treated animals. (A) Gen reduced the protein expression of Cav1.2 in the left ventricle tissues. (B & D) Gen reduced the protein expression of p-JNK, pp38-MAPK in As₂O₃ treated hearts. (C & E) Protein levels of JNK and p38-MAPK were not altered in rat hearts. ***p*<0.01 vs. control, ††*p*<0.01 vs. As₂O₃; n=3 independent experiments.

and pp38-MAPK was reduced by Gen in As₂O₃-treated rats, while their total proteins were unaltered (Fig. 6).

Discussion

The present study demonstrates that treatment with Gen is beneficial against the As₂O₃-induced adverse cardiac effects. The main findings of our study are: (1) Gen significantly shortens As₂O₃-induced LQTs and dose-dependently ameliorates the impaired cardiac function. (2) Gen remarkably reduces protein expression of Cav1.2 and [Ca²⁺]_i overload, improves ΔΨ_m and reverses As₂O₃-induced cardiomyocytes apoptosis. (3) Gen effectively inhibits phosphorylation of JNK and p38-MAPK, and inhibits caspase-3 activation as indicated by the decreased level of cleaved caspase-3. These results suggest that the protective role of Gen against As₂O₃-induced adverse cardiac effects is, at least in part, attributable to an inhibition of cardiomyocytes apoptosis.

As₂O₃ has been found to be an effective agent for refractory and relapsed APL. Recently, clinical studies indicated that some APL patients were accompanied with QT interval prolongation and sudden cardiac death during As₂O₃ treatment [6]. These adverse effects of As₂O₃ were reproduced in our model (Fig. 1 & Table 1). Moreover, previous studies have elucidated that the adverse cardiac effects produced by As₂O₃ is closely related to intracellular calcium overload and cardiomyocytes apoptosis [7, 29]. Recently, Narula et al. revealed that

apoptosis is closely associated with ventricular dysfunction [30, 31]. In addition, Bennett suggested that aberrant apoptosis of the cardiomyocyte is involved in the LQT syndrome and congenital heart block [32]. James et al. demonstrated that apoptosis may be implicated in LQT syndrome by monitoring sinus nodes by light and electron microscopy [33]. Thus, attenuation of intracellular calcium overload and elimination of cardiomyocytes apoptosis might be a new strategy to prevent As₂O₃-induced adverse cardiac effects, despite that it is not fully understood how exactly apoptosis causes LQTS.

The herbal compounds such as sodium tanshinone IIA [34] and resveratrol [29] exhibit beneficial effects on injured heart. Previous studies suggested that Gen protects against myocardial ischemia/reperfusion injury [22]. However, it was unclear whether Gen can ameliorate the adverse cardiac effect produced by As₂O₃. The present study sheds the first light on this issue, by demonstrating that treatment with Gen for two weeks significantly shortened QT intervals and ameliorated the impaired cardiac function caused by As₂O₃, in a dose-dependent manner.

Intracellular calcium overload is a causative factor and an upstream initiator of cardiomyocytes apoptosis. The voltage-activated L-type Ca²⁺ channels (Cav1.2) are heteromeric proteins composed of a pore-forming α_1 subunit and accessory $\alpha_2\delta$ and β subunits. Recently, multiple studies have demonstrated that genistein has inhibitory effects on I_{CaL} [21, 27]. We found here that As₂O₃-induced upregulation of Cav1.2 was significantly decreased by Gen. Furthermore, in accordance with previous study [8], we found that treatment with As₂O₃ increased [Ca²⁺]_i, which was prevented by Gen. A central facet of calcium mediated apoptosis was closed associated with $\Delta\Psi_m$ [35]. Our data support this notion: As₂O₃ caused severe damage of $\Delta\Psi_m$ whereas pretreatment with Gen rescued this deleterious change. $\Delta\Psi_m$ collapse can be caused by activation of the intracellular mitogen-activated protein kinase (MAPK) signaling cascade [13]. Moreover, calcium-mediated activation or overexpression of JNK and p38-MAPK has been known to result in cardiomyocyte apoptosis [12, 36-38]. We showed here that Gen prevented the activation of JNK and p38-MAPK induced by As₂O₃ in both *in vitro* and *in vivo* models (Fig. 5 & 6). Caspase-3, a member of the family of specific cysteine proteases which is recognized as the executioner of apoptosis induced by various proapoptotic stimuli, can be activated by p38-MAPK and JNK [39, 40]. On the other hand, overexpression of inactivated JNK or application of p38 inhibitor blocks caspase-3 activation [41-43]. In the present study, Gen was found to reduce the cleaved caspase-3 in As₂O₃-treated NRVCs.

Taken together, it appears that Gen protects against the cardiac adverse effects of As₂O₃ by mitigating cardiomyocytes apoptosis induced by As₂O₃ through attenuating intracellular calcium overload and downregulating protein expression of p-JNK and pp38-MAPK to ameliorate the damage of $\Delta\Psi_m$ leading to suppression of caspase-3 activation. Though it is yet to be determined how Gen participates in this anti-apoptotic signaling pathway, our study suggests that Gen can be used as an adjunction therapy in APL patients receiving As₂O₃ treatment to avoid, at least to minimize, the cardiac adverse effects of As₂O₃.

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Errata

In the article by Lauf et al., entitled “Canonical Bcl-2 Motifs of the Na⁺/K⁺ Pump Revealed by the BH3 Mimetic Chelerythrine: Early Signal Transducers of Apoptosis?” [Cell Physiol Biochem 2013;31(2-3):257-276 (DOI: 10.1159/000343366)], is a printing error in Table 1 on page 267. The peptide sequence of PBD # 3B8E on the sixth line/fourth column, has erroneously one L in excess. Instead it should read: ARAAEILARDGPN.

In the article by de Carvalho Leite et al., entitled “Glycolytic and Mitochondrial Metabolism in Pancreatic Islets from MSG-Treated Obese Rats Subjected to Swimming Training” [Cell Physiol Biochem 2013;31(2-3):242-256 (DOI: 10.1159/000343365)], in the page 247 the percentage of increase on hypertrophy on pancreatic islets is 60% instead of 2400%, compared MSG-SED with CON-SED.

Therefore the sentence should be as following:

Pancreatic islets became hypertrophic in the MSG-SED group in relation to the CON-SED group (Fig. 2K), with 60% larger islet diameters in the MSG-SED group (p<0.05).

In the article by Fan et al., entitled “Genistein Ameliorates Adverse Cardiac Effects Induced by Arsenic Trioxide Through Preventing Cardiomyocytes Apoptosis” [Cell Physiol Biochem 2013;31(1):80-91 (DOI: 10.1159/000343351)], is a printing error in Table 1. The second line from As₂O₃+Gen(100mg/kg)-RR (ms) interval is 28±8, and it should read: 128±8.