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 (ΔΨm) 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 ΔΨm 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 ΔΨm 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.
Introduction

Acute promyelocytic leukemia (APL) is a common hematological system disease with high morbidity and mortality. Arsenic trioxide (As$_2$O$_3$) 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 frontline 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 As$_2$O$_3$. Therefore, it is highly desirable to develop measures against adverse cardiac effects induced by As$_2$O$_3$ 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 Ca$^{2+}$ 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 ([Ca$^{2+}]_i$) 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 As$_2$O$_3$.

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 As$_2$O$_3$, epidemiological studies showed that its usage is closely associated with decreased risks of cardiovascular disease [20], with additional cellular effects such as attenuated intracellular Ca$^{2+}$, 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 As$_2$O$_3$-induced adverse cardiac effects. Furthermore, we attempted to explore whether Gen is able to prevent cardiomyocytes apoptosis induced by As$_2$O$_3$ 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). As$_2$O$_3$ 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, As$_2$O$_3$ (0.8 mg/kg/day), Gen (10 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day), and As$_2$O$_3$+Gen (10 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day) groups. As$_2$O$_3$ was administrated intravenously for consecutively 7 days. For As$_2$O$_3$ group, the rats were firstly treated with saline for 7 days, and subsequently As$_2$O$_3$ was administrated intravenously for another 7 days. In As$_2$O$_3$+Gen groups, rats were firstly administered with Gen for 7 days prior to injection of As$_2$O$_3$ 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 (\(QTc=QT/(RR)^{1/3}\)) [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 Ca$^{2+}$ 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 incubation with As$_2$O$_3$ or with As$_2$O$_3$ alone for 6 h. The dynamic change of intracellular Ca$^{2+}$ 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 [Ca$^{2+}$]$^+$ was one frame per 5 s for about 150 s in normal Ca$^{2+}$-containing Tyrode’s solution and KCl (30 mM) was added at tenth scan. The relative magnitude of fluorescent signals was calculated as F1/F0, which F0 represents the basal level of fluorescence and F1 the value was after drug treatment [25].

Measurement of mitochondrial membrane potential (ΔΨm)

The effects of As$_2$O$_3$ 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’-tetraethylbenzimidazolcarbocyanide iodide; 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 As$_2$O$_3$ or with As$_2$O$_3$ 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
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 μM), As₂O₃ (5 μM)+Gen of 10, 50, and 100 μM. 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×OD) in each group and normalized to GAPDH (anti-GAPDH antibody) as an internal control.

Statistical analysis
Data are expressed as mean±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
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).

### Table 1. Gen shortens As$_2$O$_3$-induced QT interval prolongation in rats. **p<0.01 vs. control; †p<0.05, ††p<0.01 vs. As$_2$O$_3$ treated group.

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Control (100mg/kg, n=6)</th>
<th>Gen (0.8mg/kg, n=6)</th>
<th>As$_2$O$_3$ (10mg/kg, n=6)</th>
<th>As$_2$O$_3$+Gen</th>
<th>As$_2$O$_3$+Gen (50mg/kg, n=6)</th>
<th>As$_2$O$_3$+Gen (100mg/kg, n=6)</th>
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<tr>
<td>QT (ms)</td>
<td>76±2</td>
<td>79±2</td>
<td>87±4 **</td>
<td>79±3 †</td>
<td>73±1 ††</td>
<td>74±2 ††</td>
</tr>
<tr>
<td>RR (ms)</td>
<td>144±7</td>
<td>155±6</td>
<td>140±7</td>
<td>144±10</td>
<td>129±7</td>
<td>28±8</td>
</tr>
</tbody>
</table>

**Gen improves As$_2$O$_3$-induced cardiac function impairment**

In the absence of Gen, As$_2$O$_3$ produced deleterious effects on cardiac function as illustrated in Table 2. The heart rate was significantly decreased by As$_2$O$_3$ (p<0.01); meanwhile, cardiac output, ejection fraction, +dp/dt max, and –dp/dt max were also reduced by As$_2$O$_3$ (p<0.01). However, treatment with Gen significantly attenuated the detrimental effects of As$_2$O$_3$ in a dose-dependent manner, though Gen (100 mg/kg) alone failed to affect these hemodynamic parameters of the normal hearts. Neither As$_2$O$_3$ nor Gen alone affected MAP (mean arterial pressure) parameter (p>0.05, data not shown).

### Table 2. Gen restores impaired cardiac function caused by As$_2$O$_3$ in rats. *p<0.05, **p<0.01 vs. control; †p<0.05, ††p<0.01 vs. As$_2$O$_3$ treated group. HR, Heart Rate; CO, Cardiac output; EF, Ejection fraction.

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Control (100mg/kg, n=6)</th>
<th>Gen (0.8mg/kg, n=6)</th>
<th>As$_2$O$_3$ (10mg/kg, n=6)</th>
<th>As$_2$O$_3$+Gen</th>
<th>As$_2$O$_3$+Gen (50mg/kg, n=6)</th>
<th>As$_2$O$_3$+Gen (100mg/kg, n=6)</th>
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<tr>
<td>Heart rate (beat/min)</td>
<td>43±3±10</td>
<td>42±8±4</td>
<td>39±2±11 **</td>
<td>40±12</td>
<td>43±5±17†</td>
<td>45±3±10††</td>
</tr>
<tr>
<td>CO (pl/min)</td>
<td>122377±966</td>
<td>108368±1324</td>
<td>70518±5348**</td>
<td>79662±16309</td>
<td>126180±10168††</td>
<td>124366±17709††</td>
</tr>
<tr>
<td>EF (%)</td>
<td>54±4</td>
<td>47±3</td>
<td>32±2**</td>
<td>32±2</td>
<td>40±6†</td>
<td>47±7††</td>
</tr>
<tr>
<td>dp/dt max (mm Hg/s)</td>
<td>10585±949</td>
<td>10164±628</td>
<td>7139±458†</td>
<td>8982±1095</td>
<td>11422±762††</td>
<td>12861±1362††</td>
</tr>
<tr>
<td>-dp/dt max (mm Hg/s)</td>
<td>8896±512</td>
<td>7942±432</td>
<td>5555±981†</td>
<td>7344±1086</td>
<td>8343±632††</td>
<td>8546±315††</td>
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</table>

**Gen attenuates intracellular Ca$^{2+}$ overload and downregulates expression of Cav1.2 in As$_2$O$_3$ treated NRVCs**

To investigate whether changed Ca$^{2+}$ is involved in Gen produced cardioprotective effects. Gen (10 μM) was applied for 1 h before As$_2$O$_3$ (5 μM) administration. The representative fluorescent images of [Ca$^{2+}$] in Fig. 2A showed that As$_2$O$_3$ provoked remarkable elevation of [Ca$^{2+}$], 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
of peak value (FImax : 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/F0 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 IcaL, we measured the protein level of Cav1.2, the α1-subunit of L-type Ca2+ channel carrying IcaL. 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).

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**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) incubation, the left column indicated normal cardiomyocytes. (B) Mean values of FI/F0 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 F1 the value after drug treatment. (C) Mean data of the peak FI/F0 ratio (FImax/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.
Gen decreases As$_2$O$_3$-mediated cardiomyocytes apoptosis

To determine the protective effects of Gen against As$_2$O$_3$-induced cardiomyocytes apoptosis, TUNEL assay was carried out. As shown in Fig. 4, TUNEL-positive cells were significantly increased in As$_2$O$_3$-treated cardiomyocytes, and this increase was prevented by Gen (10, 50, 100 μM) treatment for 24 h.
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 As$_2$O$_3$-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 As$_2$O$_3$ 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 As$_2$O$_3$ and Gen (Fig. 5C & 5D). Cleaved caspase-3, the activated form of caspase-3, is known to be the executer of cardiomyocytes apoptosis [28]. Indeed, in our study, As$_2$O$_3$ 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 As$_2$O$_3$-treated rats

To further explore the beneficial effects of Gen against the adverse cardiac effects of As$_2$O$_3$, we measured the protein levels of Cav1.2, p-JNK and pp38-MAPK in As$_2$O$_3$ treated heart. We found that administration of As$_2$O$_3$ 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
and pp38-MAPK was reduced by Gen in As$_2$O$_3$-treated rats, while their total proteins were unaltered (Fig. 6).

**Discussion**

The present study demonstrates that treatment with Gen is beneficial against the As$_2$O$_3$-induced adverse cardiac effects. The main findings of our study are: (1) Gen significantly shortens As$_2$O$_3$-induced LQTs and dose-dependently ameliorates the impaired cardiac function. (2) Gen remarkably reduces protein expression of Cav1.2 and [Ca$^{2+}$]_i overload, improves $\Delta$Ψ_m and reverses As$_2$O$_3$-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 As$_2$O$_3$-induced adverse cardiac effects is, at least in part, attributable to an inhibition of cardiomyocytes apoptosis.

As$_2$O$_3$ 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 As$_2$O$_3$ treatment [6]. These adverse effects of As$_2$O$_3$ were reproduced in our model (Fig. 1 & Table 1). Moreover, previous studies have elucidated that the adverse cardiac effects produced by As$_2$O$_3$ 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 \( \text{As}_2\text{O}_3 \)-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 \( \text{As}_2\text{O}_3 \). 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 \( \text{As}_2\text{O}_3 \), in a dose-dependent manner.

Intracellular calcium overload is a causative factor and an upstream initiator of cardiomyocytes apoptosis. The voltage-activated L-type \( \text{Ca}^{2+} \) channels (Cav1.2) are heteromeric proteins composed of a pore-forming \( \alpha \) subunit and accessory \( \alpha, \delta \) and \( \beta \) subunits. Recently, multiple studies have demonstrated that genistein has inhibitory effects on \( I_{\text{cal}} \) [21, 27]. We found here that \( \text{As}_2\text{O}_3 \)-induced upregulation of Cav1.2 was significantly decreased by Gen. Furthermore, in accordance with previous study [8], we found that treatment with \( \text{As}_2\text{O}_3 \) increased \( [\text{Ca}^{2+}]_i \), 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: \( \text{As}_2\text{O}_3 \) 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 \( \text{As}_2\text{O}_3 \) in both \textit{in vitro} and \textit{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 \( \text{As}_2\text{O}_3 \)-treated NRVCs.

Taken together, it appears that Gen protects against the cardiac adverse effects of \( \text{As}_2\text{O}_3 \) by mitigating cardiomyocytes apoptosis induced by \( \text{As}_2\text{O}_3 \) 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 adjuvant therapy in APL patients receiving \( \text{As}_2\text{O}_3 \) treatment to avoid, at least to minimize, the cardiac adverse effects of \( \text{As}_2\text{O}_3 \).

Acknowledgements

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References


 Fan/Wang/Zhang et al.: Genistein Prevents As₂O₃-Induced Cardiotoxicity


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 As$_2$O$_3$+Gen(100mg/kg)-RR (ms) interval is 28±8, and it should read: 128±8.