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Original Article

Exogenous taurine attenuates mitochondrial oxidative stress and endoplasmic reticulum stress in rat cardiomyocytes

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Taurine, a conditionally essential amino acid, plays a critical role in cardiovascular function. Here we examined the effect of taurine on mitochondria and endoplasmic reticulum in rat cardiomyocytes during glucose deprivation (GD). Data showed that cell viability, intracellular taurine contents, and taurine transporter expression were decreased during GD. In contrast, an increase in reactive oxygen species and intracellular Ca^{2+} contents was observed. GD also caused disrupted mitochondrial membrane potential, apoptotic cell death, and dissociation of unfolded protein response (UPR)-relative proteins in cardiomyocytes. Signal transduction analysis showed that Bcl-2 family protein balance was disturbed, caspase-12 was activated and UPR-relative protein levels were up-regulated. Moreover, pre-treatment with 80 mM exogenous taurine attenuated GD effect in cardiomyocytes. Our results suggest that taurine have beneficial effects on inhibiting mitochondria-dependent cell apoptosis and UPR-associated cell apoptosis and might have clinical implications on acute myocardial infarction in future.

Keywords taurine; taurine transporter; glucose deprivation; mitochondrial oxidative stress; endoplasmic reticulum stress

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Introduction

Taurine is an abundant β-amino acid which is found in heart at high concentrations. It is functionally involved in calcium modulation, membrane stabilization, osmoregulation, antioxidation, protein phosphorylation, and ion flux [1–6], and plays central roles in ischemia injury in cardiomyocytes [7].

Endogenous taurine is originally derived from cysteine. The synthesis of taurine mainly occurs in liver, whereas only a limited amount taurine is synthesized in heart. The maintenance of the large intracellular taurine pool in cardiomyocytes may depend on the uptake of β-amino acid from plasma through taurine transporter (TauT). Previous studies have showed that TauT knockout mice exhibit a deficiency of taurine content in myocardial and skeletal muscle when compared with their wild-type littermate [8]. It is supposed that taurine deprivation causes cardiomyocyte atrophy, mitochondrial and myofiber damage [9]. Furthermore, cardiac dysfunction may be closely related to the actions of taurine [1,9]. Increasing evidence has showed that taurine plays a crucial role in cardiac protection [1,4,7,8,10–18].

Numerous studies suggested that ischemic oxidative stress acts by reducing myocardial antioxidants, breaking up mitochondrial membrane potential ($\Delta\psi_m$), and releasing superoxide [9,19]. The role of taurine in antioxidant is shown in attenuating mitochondrial oxidative stress. Intracellular taurine provides protection against oxidative stress by converting HOCl to *N*-chlorotaurine [20] and by regulating the rate of reactive oxygen species (ROS) generation in mitochondria [21]. The endoplasmic reticulum (ER), also known as sarcoplasmic reticulum (SR) in cardiac, regulates protein synthesis, protein folding and trafficking, cellular responses to stress, and intracellular calcium (Ca^{2+}) levels. Alterations in Ca^{2+} homeostasis and accumulation of unfolded/misfolded proteins in the ER cause ER stress and finally lead to cell apoptosis [22]. In heart muscle cell, SR is recognized as an intracellular organelle specializing in the regulation of Ca^{2+} fluxes and in the control of excitation–concentration coupling [23].

The morphology and respiratory functions of cardiomyocytes significantly change in TauT knockout rats [8], indicating the profound role of taurine in cardiomyocytes. However, the function and expression of TauT in acute myocardial ischemia were still elusive. It i's still not clear whether TauT could uptake extrinsic taurine into cardiomyocytes and ultimately protect heart from mitochondrial

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oxidative stress and ER stress injury. The current work is designed to evaluate the effect of taurine on rat cardiomyocyte funciton under glucose deprivation (GD) and ultimately make contributions to cardiac protection.

Materials and Methods

Materials

Rat H9c2 cell line was purchased from American Type Culture Collection (ATCC; Manassas, USA). Cell counting kit (CCK-8) and Fluo-4 AM were purchased from Dojindo (Dojindo, Japan). Dolbecco's modified Eagle's medium (DMEM), Dolbecco's modified Eagle's medium-no glucose, fetal bovine serum, TRIzol reagent, bovine serum albumin, Bradford reagent and 5-(and-6)-carboxy-2′,7′-dichlorofluorescein diacetate (DCF-DA) were purchased from Invitrogen (Carlsbad, USA). RevertAid first-strand cDNA synthesis kit was purchased from Thermo Fisher (Madison, USA). Taurine, anti-GAPDH primary antibody, perchloric acid, methanol and all other reagents were purchased from Sigma (St Louis, USA). All other antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, USA).

Cell culture

Cardiomyocytes were cultured in DMEM at 37°C with 5% CO_2, supplemented with 10% (*v*/*v*) heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin.

Measurement of intracellular taurine level

Intracellular taurine level was measured by reversed-phase high-performance liquid chromatography. Approximately 1×10^6 cardiomyocytes were dissolved in 0.4 M perchloric acid ($HClO_4$) and stored at −80°C until later use. Supernatants of samples were purified in a dual-bed ion-exchange column, eluted with 2 ml water and then lyophilized. Samples were dissolved in 100 μl ultrapure water with a Nova-Pak C18 column (Bio-Rad). Glutamine was used as authentical standard. Standard curve of taurine content was linear and the recovery of taurine was >90%. The value of taurine was expressed as nanomoles per milligram protein.

Determination of the dose-dependent activity of taurine

Cardiomyocytes viability was measured by using CCK-8. Briefly, cells were plated in 96-well plates at a density of 4×10^4 cells per well. After being pre-treated with taurine (0, 20, 40, 80, 120, 160, or 200 mM) for 12 h, 10 μl of CCK-8 reagent was added into each well and plates were incubated for another 1 h until media turned yellow. The optical density was determined at 450 nm using a Multiskan MK3 microplate reader (Thermo Electron, Waltham, USA). Each experiment was performed in triplicate and repeated at least three times.

Assessment of cardiomyocyte viability with GD treatment at different time

Cardiomyocyte viability was measured by using CCK-8. Briefly, cells were plated in 96-well plates at a density of 4×10^4 cells per well. After being pre-treated with 80 mM taurine for 12 h, cells were maintained under GD condition for different time (0, 15, 30, 60, 120, and 180 min), then 10 μl of CCK-8 reagent was added into each well and the plates were incubated for another 1 h until media turned yellow. The optical density was determined at 450 nm using a Multiskan MK3 microplate reader (Thermo Electron). Each experiment was performed in triplicate and repeated at least three times.

Measurement of intracellular ROS production

Cardiomyocytes were incubated with ROS detection reagents (Invitrogen) and DCF-DA (10 mM) for 60 min at 37°C in dark, followed by washing with PBS immediately. Fluorescence emission was measured by using a DM2000 fluorescent microscope (Leica Microsystems, Wetzlar, Germany) using a 525-nm band pass filter.

$\Delta\psi_m$ determination

Intracellular $\Delta\psi_m$ was evaluated by using fluorescent probe 5,5′-6,6′-tetrachloro-1,1′,3,3′-iodide (JC-1). Briefly, plates were kept in staining solution for 20 min at 37°C in dark and washed with JC-1 staining buffer. The fluorescence intensity was measured sing a DM2000 fluorescent microscope (Leica Microsystems) with a mercury laser excitation at 514 nm and a 585-nm band pass filter.

Measurement of intracellular free Ca^{2+}

Intracellular Ca^{2+} levels were determined using intracellular Ca^{2+} probe Fluo-4 AM, which binds Ca^{2+} with a 1 : 1 stoichiometry. Cardiomyocytes were incubated in dark with Fluo-4 AM for 30 min at 37°C. Fluorescence emission was measured by using a confocal microscope (FV1000; Olympus, Tokyo, Japan) with an excitation at 494 nm and emission at 516 nm. LAS AF Lite software (Leica, Germany) was employed to analyze data.

RNA isolation and real-time polymerase chain reaction

Total RNA was isolated from cells using Trizol reagent (Invitrogen) according to manufacturer's instructions. After quantification and determination of the quality of total RNA, the first-strand cDNA was synthesized with a ReverAid first strand cDNA synthesis kit (Thermo Scientific). mRNA level was determined by real-time (polymerase chain reaction) PCR using SYBR Premix Ex Taq II (TaKaRa). PCR amplification cycles were

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Table 1 Primers used in real-time PCR

Primer	Sequence	Tm (°C)
β-Actin-rt-Fw	AGTGTGACGTTGACATCCGT	59.02
β-Actin-rt-Rv	GGGCAGTAATCTCCTTCTGC	58.89
CDO-rt-Fw	GACGAAGTCAATGTGGAGGA	60.03
CDO-rt-Rv	TTGATCCACAAGGTTTCGAG	60.01
CSD-rt-Fw	GGAGTGCCACTACTCCATCA	57.98
CSD-rt-Rv	CAGACTGATCTGCCTCTCCA	58.02
TauT-rt-Fw	ATGCTCCTCTTGCTTGGACT	59.04
TauT-rt-Rv	GGAAGGACGGGTAAAGATCA	58.98
Grp78/Bip-rt-Fw	CCCTGTCTTCTCAGCATCAA	59.07
Grp78/Bip-rt-Rv	GTAGAGCGGAACAGGTCCAT	60.01
PERK-rt-Fw	CCCTGTCTTCTCAGCATCAA	58.91
PERK-rt-Rv	GTAGAGCGGAACAGGTCCAT	58.88
IRE1-rt-Fw	CGATGGACTGGTGGTAACTG	59.01
IRE1-rt-Rv	GTTGATGTGCACCACCTTTC	58.99
ATF6-rt-Fw	AAGAAGGAGAATGGGTCCCT	58.96
ATF6-rt-Rv	GGACTTTGAGCCTCTGGTTC	59.01

programmed at 95°C for 30 s, followed by 40 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 40 s. *GAPDH* was used as an endogenous control. Relative expression of genes was calculated and expressed as $2^{-\Delta\Delta Ct}$. The primers used here were listed in **Table 1**.

Immunobloting

Cardiomyocytes were harvested into 1 × RIPA lysis buffer (Thermo Pierce, Rockford, USA) supplemented with complete protease inhibitor cocktail (Roche, Basel, Switzerland). Lysates were centrifuged at 12 000 rpm for 20 min at 4°C and the total protein content of supernatant fraction was measured by using BCA protein assay kit (Thermo Pierce). Equal amounts of protein (40 μg/lane) were separated by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a 0.45-μm poly(vinylidene difluoride) membrane (Millipore, Billerica, USA). The membrane was blocked with 5% skimmed milk in TBS-T, then incubated with affinity-purified antibodies against GAPDH (1 : 30 000), TauT (1 : 1800), Bax (1 : 6000), Bcl-2 (1 : 1000), nuclear factor-κB (1 : 6000), and Grp78 (1 : 2000), and incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (1 : 6000). Finally, membranes were detected with Immobilon Western chemiluminescent HRP substrate (Bio-Rad, Hercules, USA) and images were captured with Gel Doc XR System (Bio-Rad).

Statistical analysis

Data were expressed as the mean ± SEM. One-way analysis of variance and SPSS 13.0 (SPSS, Chicago, USA) were used to analyze data. All experiments were repeated at least three times.

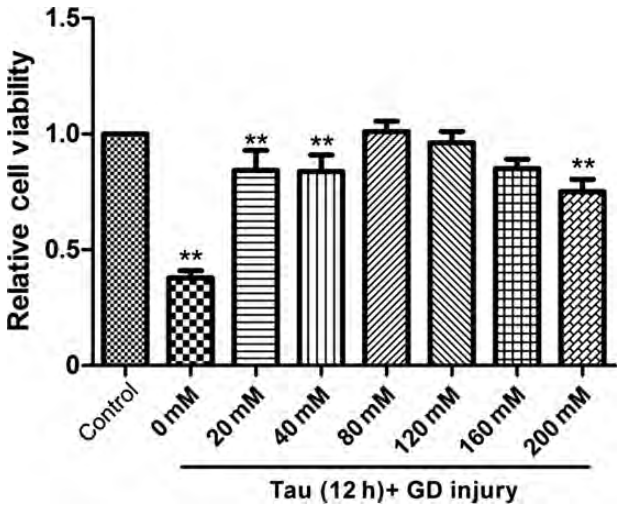

Figure 1 Dose-dependent effect of exogenous taurine under GD Cells were pre-incubated in taurine for 12 h at a dose of 0, 20, 40, 80, 120, 160, and 200 μM, and then incubated in no-glucose DMEM for 1 h. Cells in the control group were normal cardiomyocytes. $^{**}P < 0.05$ vs. control group, $n = 6$.

Results

Optimum dose of taurine determined by CCK-8 assay

CCK-8 was used to determine the optimum dose necessary for taurine to protect cardiomyocytes against GD-induced cell apoptosis. Results showed that taurine at different concentrations (0, 120, and 160 mM) had protective effects on cells from GD-induced injury, and higher doses had cytotoxicity in cardiomyocytes (**Fig. 1**). A lower-dose of 80 mM was chosen for subsequent experiments.

Taurine attenuated oxidative stress and enhanced cell viability depressed by GD

GD is a potential incentive of ROS [**Fig. 2(A)**]. The formation of ROS is considered to be a rate-limiting step in lipid peroxidation. The biochemical determination of malondialdehyde (MDA) indicates the lipid peroxide formation. We observed that MDA level in cardiomyocyte increased ~2.2-fold with GD treatment for 30 min when compared with normal group (**Table 2**). Pre-treatment with taurine reduced MDA level ~1.7 folds compared with control (**Table 3**).

To determine the effects of GD on cardiomyocytes viability, cells were treated for different time (15~180 min) under GD and the number of cell was counted by using CCK-8 assay. Results showed that GD reduced the viability of cardiomyocytes by ~40% in a time-dependent manner when compared with the control [**Fig. 2(B)**]. Pre-treatment with 80 mM taurine for 12 h dramatically suppressed GD-induced reductions in cell viability, maintaining the same level as the control group [**Fig. 2(B)**]. Taurine under

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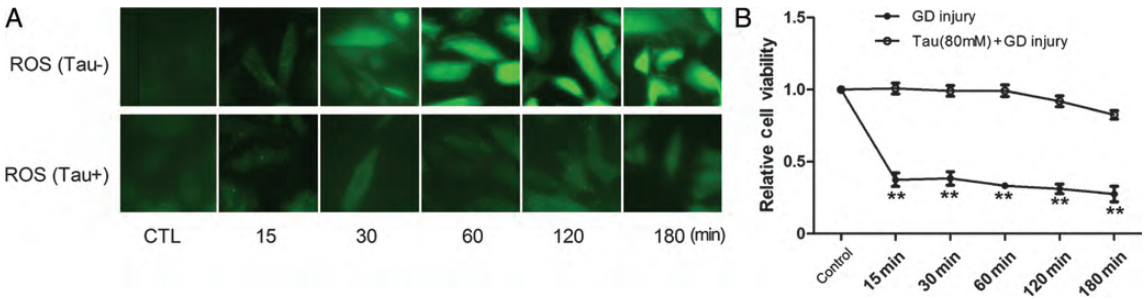

Figure 2 Taurine attenuated oxidative stress and increased cell viability against GD (A) Impact of taurine (80 mM) on ROS production. (B) Impact of taurine on cell viability under GD. Normal cardiomyocytes were used in control group. $^{**}P < 0.05$ vs. control group, $n = 6$.

Table 2 Effect of GD on lipid peroxidation at different time

MDA (nmol/mg protein)					
Control	15 min	30 min	60 min	120 min	180 min
0.621 ± 0.049	0.813 ± 0.015	1.377 ± 0.052[a]	2.081 ± 0.090[a]	2.409 ± 0.127[a]	2.563 ± 0.175[a]

[a]$P < 0.05$ vs. control group, $n = 3$.

Table 3 Effect of taurine (80 mM) pre-treatment on lipid peroxidation during GD at different time

MDA (nmol/mg protein)					
Control	15 min	30 min	60 min	120 min	180 min
0.625 ± 0.058	0.722 ± 0.053	1.086 ± 0.071[a]	1.943 ± 0.132[a]	2.099 ± 0.085[a]	2.521 ± 0.123[a]

[a]$P < 0.05$ vs. control group, $n = 3$.

Table 4 Taurine contents in cardiomyocytes during GD at different time

Taurine (nmol/mg protein)					
Control	15 min	30 min	60 min	120 min	180 min
178.152 ± 0.084	171.921 ± 0.411	133.020 ± 10.629[a]	103.286 ± 1.736[a]	95.909 ± 20.009[a]	71.576 ± 10.418[a]

[a]$P < 0.05$ vs. control group, $n = 3$.

normal condition showed no effect on cardiomyocyte viability.

Effect of GD on intracellular taurine level

Table 4 showed the level of taurine in cardiomyocytes under GD. GD caused a significant reduction in taurine content in cardiomyocytes, which was slightly prevented by taurine treatment (**Fig. 3**).

Decreased *TauT*, *CDO*, *CSD* mRNA, and TauT protein expression under GD

Higher intracellular taurine contents were maintained by biosynthesizing or uptaking taurine. We detected the relative gene expression levels of *CDO* and *CSD* under GD. The results showed that *CDO* mRNA level decreased approximately by 47% and CSD about 39% with GD for 180 min when compared with the controls, indicating that the expressions of both *CDO* and *CSD* were down-regulated under GD [**Fig. 4(A)**]. Gene and protein expressions of TauT were also down-regulated during GD, which was reversed by exogenous taurine [**Fig. 4(B,C)**].

Taurine suppressed disruption of $\Delta\psi_m$ induced by GD

Maintenance of intact $\Delta\psi_m$ is important to cell survival, and the loss of $\Delta\psi_m$ triggers a cascade of reactions leading to cell apoptosis. To determine whether exogenous taurine

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could maintain $\Delta\psi_m$ integrity, $\Delta\psi_m$ was measured by using a fluorescent microscope. The results showed that GD significantly reduced $\Delta\psi_m$ in cardiomyocytes [**Fig. 5(A)**], whereas taurine pre-treatment increased it [**Fig. 5(B)**], confirming the disruptive effect of GD and preservative effect of taurine on $\Delta\psi_m$.

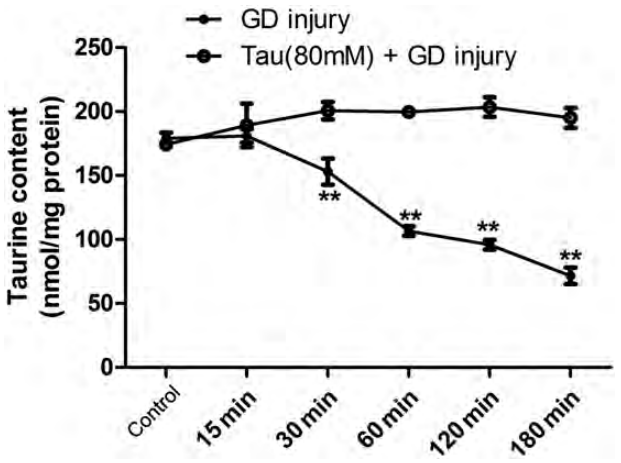

Figure 3 Impact of intracellular taurine level under GD $^{**}P < 0.05$ vs. control group, $n = 6$.

Taurine inhibited GD-induced intrinsic apoptotic pathways in cardiomyocytes

Because taurine could suppress GD-induced apoptosis, we evaluated the effects of taurine on the levels of Bcl-2 family proteins in cardiomyocytes under GD. Immunoblotting studies showed that GD induced the down-regulation of anti-apoptotic Bcl-2 and the up-regulation of pro-apoptotic Bax in cardiomyocytes (**Fig. 6**). The Bax/Bcl-2 ratio increased 2 folds compared with the control, which was effectively repressed by taurine treatment.

Taurine attenuated the increase in intracellular Ca^{2+} induced by GD and the SR-mediated apoptotic pathway

To investigate the involvement of ER/SR-mediated pathway of apoptosis, intracellular Ca^{2+} and caspase-12 expression levels were analyzed. The accumulation of intracellular Ca^{2+} could significantly increase apoptosis susceptibility by the activation of caspase-12. **Figure 7** showed that an increase in Ca^{2+} fluorescence was detected in cardiomyocytes when cells were exposed to GD. Relative concentration of intracellular Ca^{2+} increased ~1.8 folds in 15-min group when compared with the control and further induced caspase-12 protein expression [**Fig. 7(B)**], whereas taurine

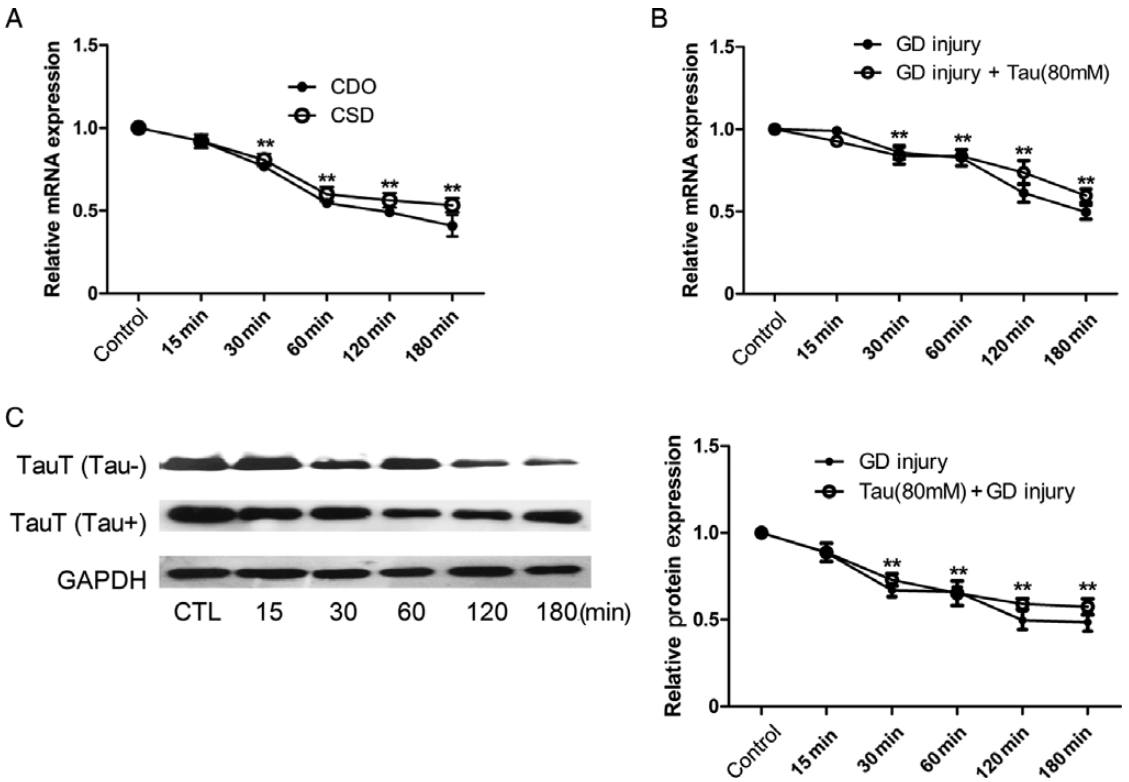

Figure 4 Biosynthesis pathway and uptaking pathway of taurine were inhibited under GD (A) Key enzymes of taurine biosynthesizing, CDO and CSD, were down-regulated under GD. (B, C) Taurine transporter expressed at both mRNA level and protein level in the presence and absence of taurine under GD. $^{**}P < 0.05$ vs. control group.

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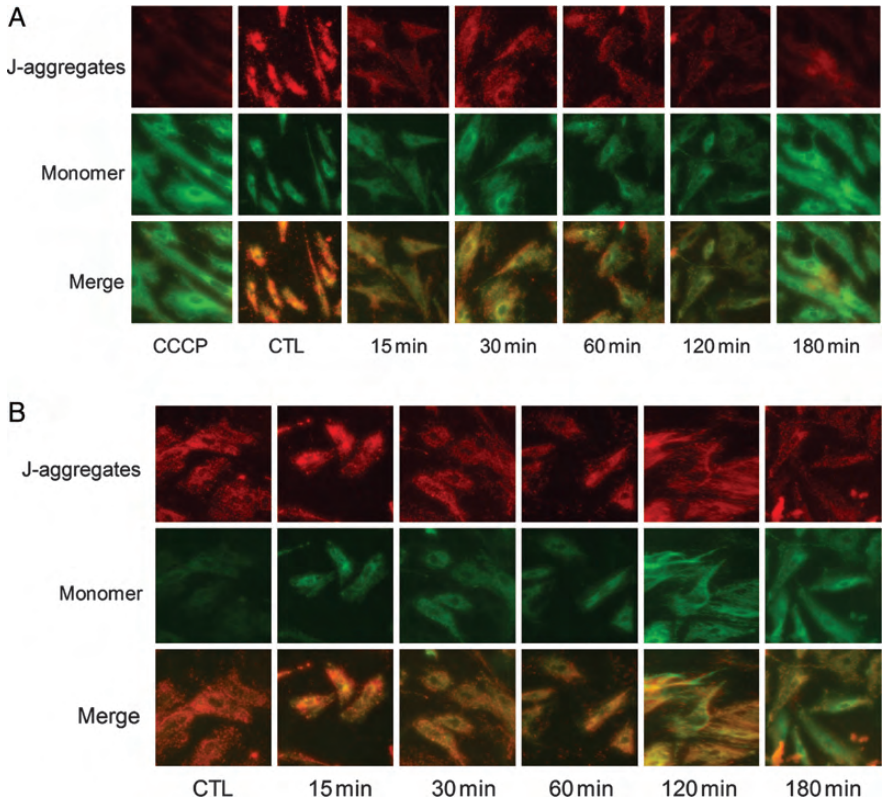

Figure 5 Δψm observed using fluorescent microscope (A) C-1 aggregates in the mitochondria matrix when MMP is high, and it can produce red fluorescent. (B) Under GD, MMP decreased significantly. Under this condition, JC-1 can not aggregates in mitochondria matrix and stay in monomer that produce green fluorescent. CCCP (caibonyl cyanide 3-chlotophenylhydrazone) is a positive control which can induce the decreasing of Δψm and produce green fluorescent.

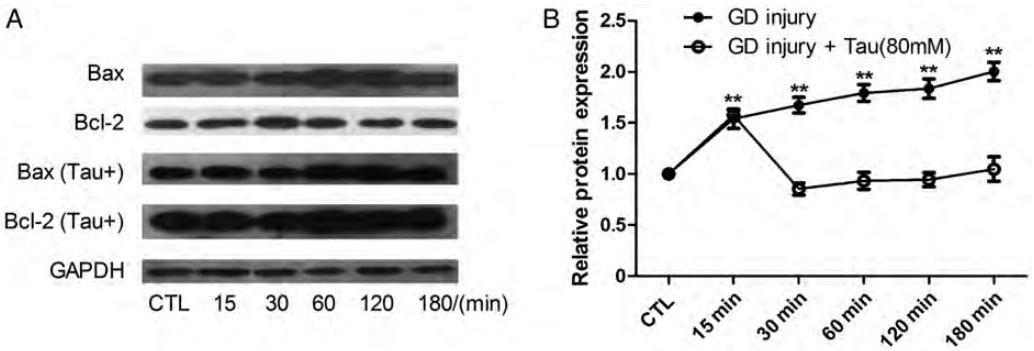

Figure 6 Immunoblot analysis on mitochondrion-dependent pathway in the presence and absence of taurine under GD in cardiomyocytes GAPDH was used as an internal control. CTL: protein expression in normal cardiomyocytes; Tau (+): cardiomyocytes incubated with 80 mM taurine, and then by glucose deprivation; Tau (−): cardiomyocytes incubated in absence with taurine and under GD. $^{**}P < 0.05$ vs. control group.

pre-treatment reduced intracellular Ca^{2+} concentration and caspase-12 activation [**Fig. 7(A,B)**].

UPR-relative proteins were up-regulated under GD while taurine attenuated the process

To determine the effect of taurine on ER stress induced by GD, cells were divided into two groups: GD group and taurine pre-treatment group. Gene expressions of unfolded protein response (UPR)-relative proteins, *Grp78/Bip*, *ATF6*, *IRE1*, and *PERK*, were determined by real time-PCR. The results showed that all of these four genes were up-regulated under GD [**Fig. 8(A–D)**]. Taurine significantly reduced mRNA expressions of *Grp78/Bip*, *ATF6*, and *PERK* [**Fig. 8(A,C,D)**]. Exogenous taurine (80 mM) inhibited *IRE1* expression at the time point of 15 min, whereas it had no significant effects on IRE1 pathway [**Fig. 8(B)**].

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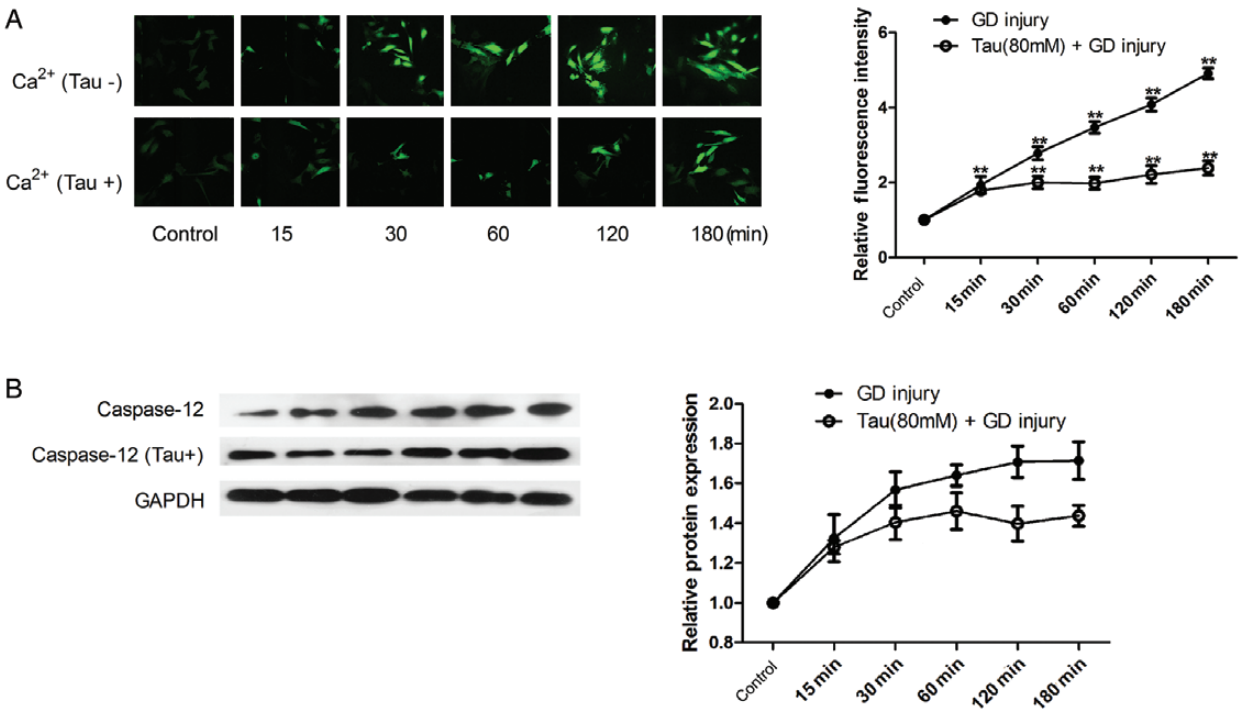

Figure 7 Investigation of ER/SR-mediated pathways in the presence and absence of Tau (80 mM) in cardiomyocytes (A) Intracellular Ca^{2+} levels were monitored at different time with or without taurine pre-treatment and by confocal microscopy; fluorescence intensity were analyzed using ImageJ software. (B) Protein level of caspase-12. GAPDH was used as an internal control. $^{**}P < 0.05$ vs. control group.

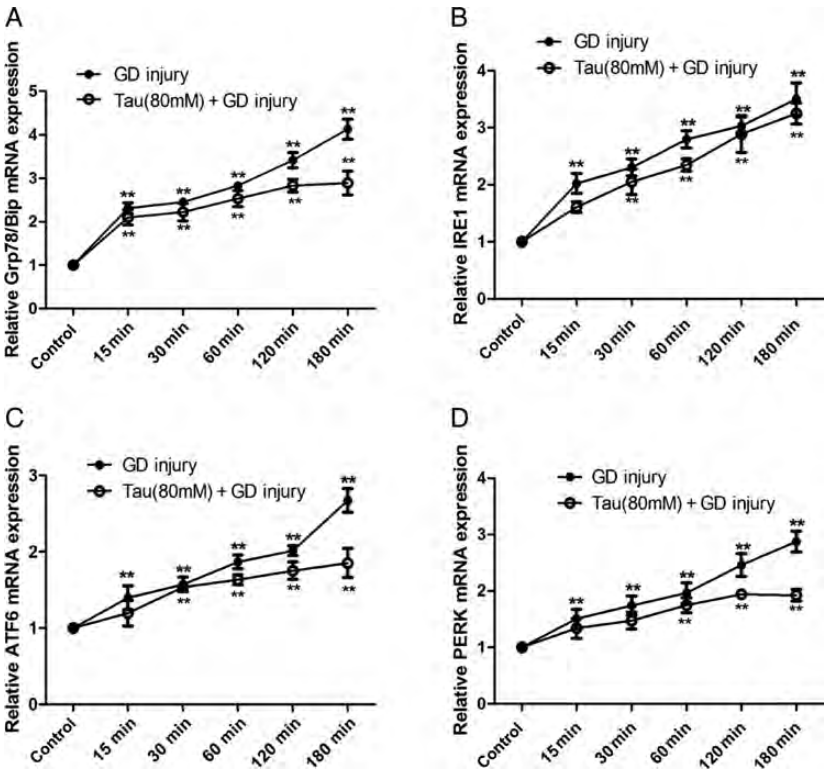

Figure 8 Real time-PCR analysis of UPR-relative protein expression in mRNA level β-actin was used as an internal control. $^{**}P < 0.05$ vs. control group.

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Discussion

Myocardial infarction is the leading cause of death [24–26]. Taurine is a kind of conditionally essential amino acid which is found in human and rodent hearts at high concentrations and is an ideal modulator of basic processes, such as calcium modulation, membrane stabilization, osmoregulation, antioxidation, regulation of protein phosphorylation, and ion flux [7,24]. *TauT* knockout mice showed cardiac atrophy and heart failure [8]. We found that intracellular taurine decreased rapidly and significantly under GD and that cell viability dropped, indicating that taurine plays crucial roles in cardiomyocytes against GD-induced apoptosis. However, exogenous taurine at 80 mM significantly elevated the viability of cardiomyocytes.

Many biological effects of taurine rely upon its intracellular concentration, which primarily controls CDO and CSD [25,27] and TauT. The plasma taurine concentration is 40–100 μM, but in myocardium taurine concentration is demonstrated to be as high as 30 mM, as heart has limited ability to synthesize taurine to overcome [28]. This phenomenon may mainly rely upon uptaking taurine by TauT, which is a high-affinity and low-capacity sodium- and chloride-dependent transporter [29,30]. The stoichiometry of TauT is 2 Na^+:1 Cl^-:1 taurine, showing narrow specificity for β-amino acids [31]. In the present study, we found that CDO, CSD, and TauT were all down-regulated during GD. However, when pre-incubated with 80 mM taurine, both gene and protein levels of TauT were up-regulated when compared with the expression under GD, indicating that it plays physiological roles in cardiomyocytes by uptaking exogenous taurine.

Taurine has been proposed to play a vital role in mitochondria in animal cells. Taurine depletion which was caused by *TauT* knockout, induced ultrastructural damage to myofilaments and mitochondria [8]. Previous studies have showed that taurine could regulate the rate of ROS generation that in turn triggers the apoptotic cascade [21,32]. In the present study, we observed that cell viability decreased by 40% under GD, pro-apoptotic protein (Bax) was up-regulated and anti-apoptotic protein (Bcl-2) down-regulated, and $\Delta\psi_m$ reduced in cardiomyocytes, indicating that taurine deprivation caused by GD resulted in seriously mitochondrion-dependent apoptosis in cardiomyocytes, while taurine could antagonize all of these pro-apoptotic events.

The increased intracellular Ca^{2+} in apoptotic cells induced ROS production, which in turn ensued the release of Ca^{2+} from ER/SR, through Ca^{2+}-sensitive enzymes [33,34]. Caspase-12 is activated by ER stress, implicated in cell apoptosis, and executed the mechanism of ER stress [35,36]. Our research firstly discussed the importance of taurine in cardiomyocytes under early GD insult, as cardiomyocytes were sensitive to GD with decreased taurine content. We also observed that GD increased intracellular Ca^{2+} concentration and activated caspase-12. However, with pre-treatment of 80 mM taurine, the rates of Ca^{2+} overload were notably attenuated. We further measured caspase-12 expression levels in the presence or absence of taurine under GD and found that caspase-12 was significantly reduced by taurine pre-incubation. These results showed that taurine can make a contribution to an effective inhibition of ER stress induced by GD.

Ischemia triggers the accumulation of unfoled protein in the ER, leading to the UPR- and ER-associated protein degradation [37]. In ER, the proximal signaling pathways that are initiated in response to the UPR include activation of transcription factor 6 (ATF6), serine/threonine-protein kinase/endoribonuclease 1 (IRE1), and double-stranded RNA-dependent protein kinase-like endoplasmic reticulum kinase (PERK), which in turn activate distinct signaling cascades mediating the response to the ER stress [38–40]. We found that GD resulted in a strong increase in *ATF6*, *IRE1*, and *PERK* expression, indicating that all of these three pathways were activated. However, there were no significant alternations in *IRE1* mRNA level with taurine pre-treatment. In short-term under GD (15 min), taurine appeared to delay the activation of IRE1 pathway [**Fig. 8(B)**]. This phenomenon may be caused by taurine delaying ER stress induction over the short-term in *ATF6*, *IRE1*, and *PERK* pathways. For a long-term exposure to GD conditions, at the later stage, taurine significantly attenuated mRNA expression of *ATF6* and *PERK* but had little effect on *IRE1* pathway. In summary, it was suggested that taurine can make contribution to both *ATF6* and *PERK* pathways. Although it may delay the initiation of IRE1 pathway at a relatively earlier time point, it can not change the activation of *IRE1* pathway under chronic stress induced by GD.

Our research demonstrated that GD impaired cardiomyocytes survival at both mitochondria and ER levels, which ultimately led to the mitochondrial-dependent cell apoptosis and UPR-associated cell apoptosis. Further studies are needed to explore how taurine up-regulates TauT during GD and which transcription factor in cardiomyocytes contributes to TauT regulation.

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