

Tauroursodeoxycholic Acid Alleviates H₂O₂-Induced Oxidative Stress and Apoptosis via Suppressing Endoplasmic Reticulum Stress in Neonatal Rat Cardiomyocytes

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Dose-Response:
An International Journal
July-September 2018:1-9
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DOI: 10.1177/1559325818782631
journals.sagepub.com/home/dos



Abstract

Introduction: We aimed to test the mechanism of protective effects of tauroursodeoxycholic acid (TUDCA) on cardiovascular disease using cultured cardiomyocytes.

Methods: Neonatal rat cardiomyocytes (NRCMs) were isolated and cultured and then the cells were divided into 4 groups based on the treatments: control group (cells treated with culture medium), H₂O₂/thapsigargin (TG) group (cells treated with oxidative stress and endoplasmic reticulum [ER] stress inducer), TUDCA group, and H₂O₂/TG + TUDCA group. The treated NRCMs were then subjected to serial analyses including flow cytometry, enzyme-linked immunosorbent assay, and Western blotting.

Results: Tauroursodeoxycholic acid significantly attenuated H₂O₂-induced reactive oxygen species generation and lactate dehydrogenase release and restored H₂O₂-induced reductions of glutathione and superoxide dismutase levels in NRCMs. Tauroursodeoxycholic acid also alleviated H₂O₂-induced cardiomyocytes apoptosis, as well as the Bax/Bcl2 ratio compared with that of H₂O₂ treated alone. In addition, TUDCA suppressed TG-induced ER stress as reflected by inversing cell viability and the expression levels of glucose-regulated protein 78 kDa and C/enhancer-binding protein homologous protein.

Conclusion: Our data indicated that TUDCA-mediated inhibition on H₂O₂-induced oxidative stress and cardiomyocytes apoptosis was through suppressing ER stress, and TUDCA possesses the potential to be developed as therapeutic tool in clinical use for cardiovascular diseases.

Keywords

tauroursodeoxycholic acid, H₂O₂-induced oxidative stress, cell apoptosis, ER stress, neonatal mouse cardiomyocytes

Introduction

Oxidative stress is one of the major molecular denominators in cardiovascular disease, and during myocardial oxidative stress, the generation of reactive oxygen species (ROS) is enhanced and the defense mechanisms of myocytes are altered.¹ In acute myocardial infarction (MI), oxidative stress induces the generation of large amounts of ROS and increases blood vessel permeability, severely damaging vessel tissues and cardiomyocytes.² Acute MI remains a leading cause of morbidity and mortality worldwide, despite substantial improvements in prognosis over the past decade.³ Thus, understanding the intricate underlying mechanisms and exploring new treatment

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Received 24 October 2017; received revised 28 March 2018; accepted 15 May 2018

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targets of oxidative stress-induced acute MI are pivotal to lower the burden of cardiovascular disease.

Bile acids, such as ursodeoxycholic acid (UDCA) and its conjugated derivative tauroursodeoxycholic acid (TUDCA), have been shown to reduce inflammation and infarct area in ischemia/reperfusion animal models.^{4,5} The *in vivo* data suggested that the neuroprotection of TUDCA was mediated by inhibition of mitochondrial perturbation and subsequent caspase activation which lead to apoptotic cell death.⁴ Changes in the mitochondrial morphology of cardiac myocytes have been reported in several heart diseases.⁶ The mitochondrion is the primary organelle involved in mediating the intrinsic apoptotic pathway.⁷ Cardiomyocyte apoptosis is an important mechanism of myocardial injury, remodeling, and cardiac dysfunction. The mitochondria-dependent apoptosis signaling pathway is one of the important processes of cardiomyocyte injury and loss and is triggered by intracellular stress such as oxidative stress and calcium overload.⁸ Apoptosis occurs in the heart during processes associated with the production and release of ROS, including ischemia and reperfusion,⁹ and cell culture model of isolated cardiomyocytes and different ROS-generating systems including H₂O₂ or superoxide anion (O₂⁻) induction identified ROS as potential inducers of cardiomyocyte apoptosis.¹⁰ Nonspecific antioxidant approaches (such as vitamin supplementation) have generally proven unsuccessful in clinical trials that aimed to reduce cardiovascular morbidity and mortality, and more target-specific strategies against ROS sources and downstream pathways could be a potential approach.¹¹ Bile acids have gained the attention to be used as therapeutic tool because the administration of the drug could be orally, intravenously, or intraperitoneally and the compound is blood-brain barrier permeable.⁴ The related drug, UDCA—also known as Actigall, Urso, or Ursodiol—reduces liver damage in the setting of cholestasis and has been approved by Food and Drug Administration for the treatment of primary biliary cirrhosis.¹² Recent studies have described that TUDCA has beneficial effects on patients with amyotrophic lateral sclerosis.¹³ In this study, we aimed to test the cardioprotective effects of TUDCA using neonatal rat cardiomyocytes (NRCMs), which have been used to demonstrate the protection of antioxidants, such as propofol and lycopene, on H₂O₂-induced oxidative stress and apoptosis successfully.^{14,15}

Materials and Methods

Reagents

Tauroursodeoxycholic acid was purchased from Gibbstown, New Jersey (catalogue #580549). H₂O₂ solution and thapsigargin (TG) and all other reagents were purchased from (Sigma-Aldrich St. Louis, MO) unless specified otherwise. The primary antibodies against Bax, Bcl-2, glucose-regulated protein 78 kDa (GRP78), CCAAT/enhancer-binding protein-homologous protein (CHOP), and β -actin were purchased from Santa Cruz Biotechnology (Dallas, TX).

Isolation of NRC and Cell Treatment

Neonatal rat cardiomyocytes were isolated and cultured as previously described.¹⁶ In brief, the neonatal rat heart was removed within 30 minutes after birth. Cardiomyocytes were digested with 0.10% trypsin (Lonza, Guangzhou, China) for 10 minutes at 37°C under agitation, and trypsin was removed by centrifugation at 300g for 5 minutes. After 6 repeats of digestion, isolated cells were plated into a 100-mm petri dish for 2 hours in Dulbecco modified Eagle medium (DMEM; Gibco, Bethesda, Maryland) with 10% fetal calf serum (Gibco, Grand Island, New York) in a 37°C incubator, 5% CO₂. Nonattached NRCM cells were cultured in DMEM with 10% fetal bovine serum supplemented with penicillin/streptomycin (Gibco, Grand Island, NY). To exclude the interference of the reagents cytotoxicity to the assay, the concentrations of H₂O₂ and TUDCA were optimized by adding different doses of H₂O₂ (0.1, 0.2, 0.3, 0.4, 0.5, 1, and 2 mM) and TUDCA (0.1, 0.15, 0.2, 0.25, 0.3, and 0.35 mM), followed by the cell viability assay. Further, cells were divided into 4 groups based on the treatments: control group (cells treated with culture medium), H₂O₂ group (cells treated with H₂O₂ for 6 hours), TUDCA group (cells treated with TUDCA for 2 hours), and H₂O₂ + TUDCA group (cells pretreated with TUDCA at optimized concentration of 0.2 mM for 2 hours and then exposed to H₂O₂ at optimized concentration of 0.3 mM for 6 hours). Neonatal rat cardiomyocytes underwent different treatments were then subjected to serial analyses including flow cytometry (FCM), enzyme-linked immunosorbent assay (ELISA), and Western blotting. All study protocols were approved by the ethics committee of Daqing Oilfield general Hospital.

Cell Viability Assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. In brief, cells were seeded at 3×10^5 cells per well into 96-well plates in triplicate. After the treatment with H₂O₂ and TUDCA at the indicated concentrations, 10 μ L CCK-8 solution was added to the cells in each well and then the cells were incubated at 37°C for 4 hours. The cell viability in each well was determined by reading the absorbance of the culture medium at 450 nm on microplate reader (Molecular Device, Downingtown, Pennsylvania).

Measurement of Intracellular ROS Generation

Intracellular ROS production was determined using FCM. Briefly, 5×10^5 cells per well were seeded into the 12-well plate; after the indicated treatment, the cells were incubated with freshly prepared dichloro-dihydro-fluorescein diacetate (Abcam, Cambridge, United Kingdom) at 37°C for 30 minutes in the dark, and the fluorescence of cells was analyzed immediately by FCM FACSCalibur (Becton Dickinson, California) at 485 nm for excitation and 535 nm for emission. The data were analyzed using the CellQuest software (Becton Dickinson, San Jose, California). These experiments were repeated for

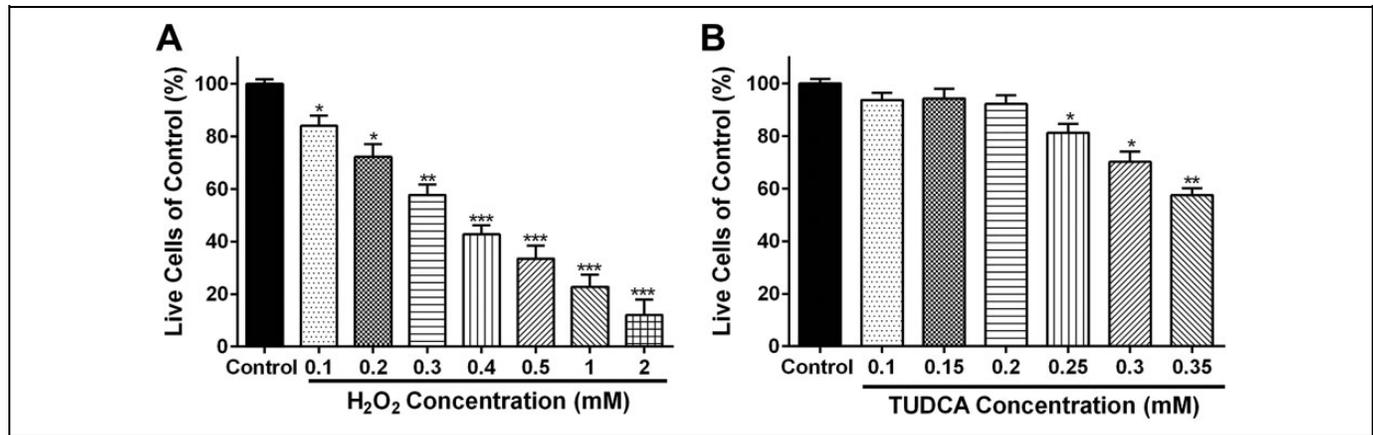


Figure 1. Concentration-dependent toxicity of H₂O₂ and TUDCA. Effect of A, H₂O₂ (0.1, 0.2, 0.3, 0.4, 0.5, 1, and 2 mM) and B, TUDCA (0.1, 0.15, 0.2, 0.25, 0.3, and 0.35 mM) on the cell viability of NRCMs. Data are presented as mean \pm SEM. n = 3, *P < .05, **P < .01, and ***P < .001 compared to control. NRCMs indicates neonatal rat cardiomyocytes; SEM, standard error of the mean; TUDCA, tauroursodeoxycholic acid.

3 times, and cellular fluorescence intensity was expressed as the fold change to the control group.

Enzyme-Linked Immunosorbent Assay

The isolated NRCMs were treated with H₂O₂ and TUDCA, and the levels or activities of lactic dehydrogenase (LDH), glutathione (GSH), and superoxide dismutase (SOD) in NRCMs were measured by ELISA kits (Nanjing Jiancheng Biotechnology, Nanjing, China) according to the manufacturer's instruction.

Assessment of Apoptosis

Early apoptosis and late apoptosis/necrosis were detected using an annexin V fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Thermo Fisher Scientific, Rockville, Maryland) according to the manufacturer's instruction. Briefly, cardiomyocytes were seeded into a 12-well plate at a density of 5×10^5 cells/well and adherent cells were digested for 50 second with 0.25% trypsin and collected together with floating dead cells. Approximately 1×10^6 cells were washed twice with cold phosphate-buffered saline and then resuspended in 200 μ L of 1 \times binding buffer containing 5 μ L of annexin V-FITC and 5 μ L of PI. Cells were incubated at room temperature for 15 minutes in the dark and fluorescence was analyzed using flow cytometer. At least 10 000 events were recorded. Apoptotic cells were expressed as a percentage of the total number of cells.

Western Blotting Analysis for Mitochondria and Endoplasmic Reticulum Stress Pathways

Protein extraction and Western blotting analysis were performed as previously described.¹⁵ Twenty microgram of total protein were applied and separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred to polyvinylidene difluoride membranes (Millipore, Billerica,

Massachusetts). Nonspecific binding was blocked for 2 hours at 37°C in Tris-buffered saline, 0.1% Tween 20 (TBST) with 5% fat-free milk. The membranes were incubated with primary antibodies against Bax (1:1000), Bcl-2 (1:1000), GRP78 (1:1000), CHOP (1:500), and β -actin antibody (1:5000) at 4°C overnight and then incubated with an anti-mouse or anti-rabbit IgG antibody (1:5000) for 1.5 hours. The membrane was washed 7 times using TBST and was developed by enhanced chemiluminescence using Super Signal West Pico blotting detection reagents (Fisher Scientific, Rockville, Maryland). Each experiment was repeated at least 3 times.

Thapsigargin-Induced Endoplasmic Reticulum Stress and Injury in Cardiomyocytes

To further investigate the effects of TUDCA on endoplasmic reticulum (ER) stress-induced injury, TG was used to establish an ER stress model, and cardiomyocytes were pretreated with TG (0.1, 0.3, 0.5, 0.7, 0.9, and 1.2 μ M) for 10 hours to optimize dose. After that cardiomyocytes were divided into dimethyl sulfoxide control (0.05%), TG, TUDCA, and TUDCA + TG groups, similar to the groups described earlier for H₂O₂ treatment.

Statistical Analysis

Data are expressed as mean \pm standard error of the mean (SEM). Statistical analyses were performed with GraphPad Prism 5.0 software (GraphPad Software, San Diego, California). Data were analyzed with 1-way analysis of variance followed by post hoc least significant difference tests. Statistical significance was set at P-value < .05.

Results

Dose-Dependent Toxicity of H₂O₂ and TUDCA on NRC

The rat cardiomyocytes were exposed to different concentrations of H₂O₂ in a range of 0.1 to 2 mM for 6 hours, followed

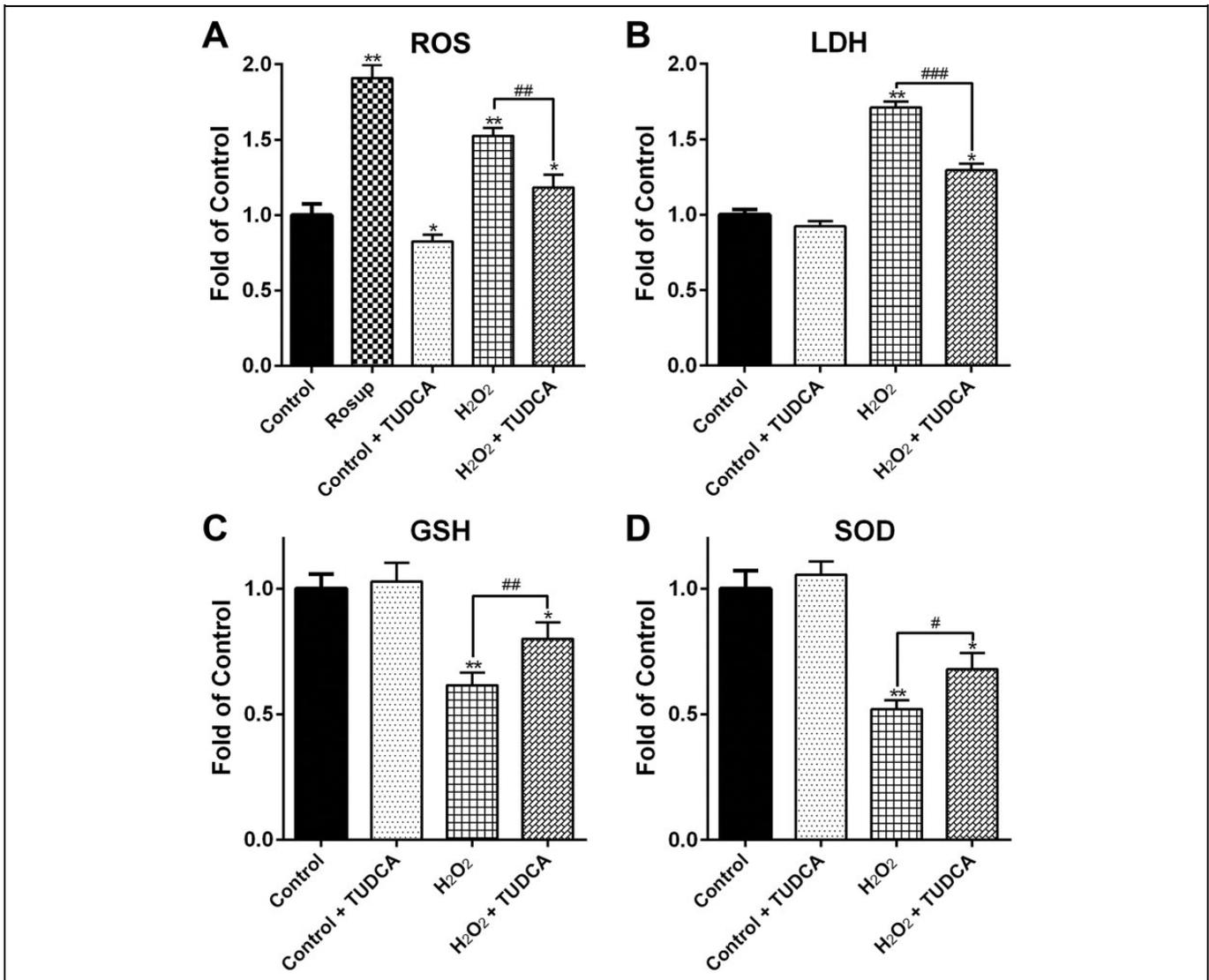


Figure 2. The effects of TUDCA on ROS, LDH, GSH, and SOD in H_2O_2 -induced changes in NRCMs. A, The ROS levels indicated by the intensity of DCFH fluorescence in NRCMs. Rosup was a compound mixture in the ROS assay kit which was used as positive control in inducing ROS generation. B, The LDH release from NRCMs. C, Intracellular GSH level of NRCMs. D, Intracellular SOD level of NRCMs. Data are presented as mean \pm SEM. $n = 3$, * $P < .05$, ** $P < .01$ compared to control. # $P < .05$, ## $P < .01$, ### $P < .001$. GSH indicates glutathione; LDH, lactic dehydrogenase; NRCM, Neonatal rat cardiomyocytes; ROS, reactive oxygen species; SEM, standard error of the mean; SOD, superoxide dismutase; TUDCA, tauroursodeoxycholic acid; DCFH, dichloro-dihydro-fluorescein.

by the cell viability assay using the CCK-8 kit. As demonstrated in Figure 1A, the survival of rat cardiomyocytes declined from 82% at 0.1 mM H_2O_2 to 12% as the concentration of H_2O_2 increased to 1.2 mM. At 0.3 mM of H_2O_2 , approximately 58% of the cardiomyocytes remained viable; therefore, this concentration was selected for the subsequent oxidative stress induction of NRCMs. To further confirm the appropriate working concentration of TUDCA on NRCMs, cardiomyocytes were exposed to a range of TUDCA concentrations from 0.1 to 0.35 mM for 10 hours to find an optimal dose (Figure 1B). Tauroursodeoxycholic acid presented a dose-dependent decrease in the cell viability of cardiomyocytes, among which treatment with 0.25 mM of TUDCA reduced cell viability to 82.67% of control, while 0.35 mM

of TUDCA decreased cell viability to 58.21% of control. In contrast, cells treated with 0.1, 0.15, and 0.2 mM of TUDCA demonstrated similar cell viabilities as control. Based on these results, 0.2 mM of TUDCA was used for the subsequent assays.

Tauroursodeoxycholic Acid Attenuates H_2O_2 -Induced Oxidative Stress in NRC

The levels of ROS, LDH, GSH, and SOD are important indices of oxidative stress status. To further evaluate the effects of TUDCA on H_2O_2 -induced oxidative stress, we measured the level of intracellular ROS by FCM and the levels of LDH release and intracellular GSH and SOD by ELISA. As shown

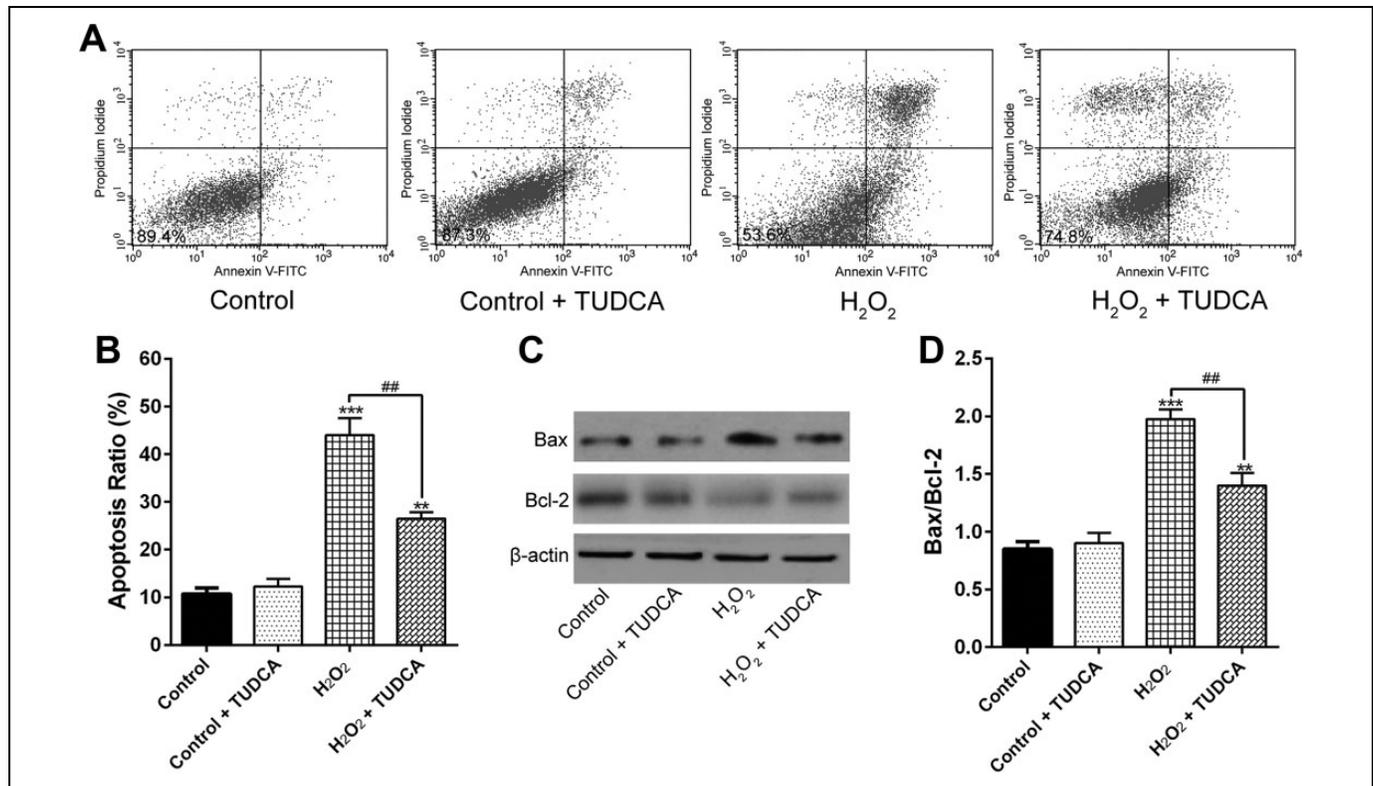


Figure 3. The effects of TUDCA on H₂O₂-induced apoptosis of NRCMs. A, Typical original FCM plots. NRCMs were stained with annexin V/propidium iodide and analyzed by flow cytometry. B, Statistical bar graph showing the apoptosis ratio ($n = 4$). Western blots showing the protein levels of Bax and Bcl-2. C, The Bax/Bcl-2 ratio. D, Data are presented as mean \pm SEM. $n = 4$, $**P < .01$, $***P < .001$ compared to control. $###P < .01$. FCM indicates flow cytometry; NRCM, Neonatal rat cardiomyocytes; SEM, standard error of the mean; TUDCA, tauroursodeoxycholic acid.

in Figure 2A, H₂O₂ treatment elevated ROS generation approximately 1.5-fold (Figure 2A), increased LDH release to 1.7-fold (Figure 2B), and reduced the GSH and SOD (Figure 2C and D) significantly to half levels of those in the control group treated with cultural medium. Further, TUDCA significantly attenuated H₂O₂-induced ROS generation (Figure 2A) and LDH release (Figure 2B) and significantly restored H₂O₂-induced reductions of GSH (Figure 2C) and SOD (Figure 2B). Notably, the levels of all these detected indicators in the TUDCA-only group remained the same as medium control (Figure 2A-D), indicating the minimum cell toxicity of the chosen dose of TUDCA.

Tauroursodeoxycholic Acid Alleviates H₂O₂-Induced Apoptosis of NRC

To evaluate the effects of TUDCA on H₂O₂-induced apoptosis of NRCMs, cells were treated either with H₂O₂ or H₂O₂ in combination with TUDCA as described in the Methods. H₂O₂ at 300 μ M increased the percentage of apoptotic cells significantly (Figure 3A and B), whereas the addition of TUDCA at 200 μ M decreased the percentage of apoptotic cells. In consistent with the FCM results, H₂O₂ increased the Bax/Bcl2 ratio compared with that of the ratio in the control group,

while TUDCA decreased the Bax/Bcl2 ratio compared with that of H₂O₂ treated alone (Figure 3C and D). These results indicate that TUDCA could alleviate H₂O₂-induced apoptosis of NRCMs.

Tauroursodeoxycholic Acid Suppresses Cardiomyocyte Oxidative Stress and Apoptosis via Endoplasmic Reticulum Signaling Pathways

To explore the underlying mechanisms of the suppression of TUDCA on the cardiomyocytes oxidative stress and apoptosis, the expression levels of GRP78 and CHOP, 2 of the most common molecules involved in ER stress, were measured in NRCMs. H₂O₂ significantly elevated the levels of GRP78, and these effects of H₂O₂ were decreased by TUDCA. Tauroursodeoxycholic acid itself did not significantly affect the expression levels of GRP78 compared with the control (Figure 4A and B).

Tauroursodeoxycholic Acid Alleviates TG-Induced ER Stress and Injury in Cardiomyocytes

To further confirm whether TUDCA could alleviate ER stress and relevant injury, TG was used to stimulate the

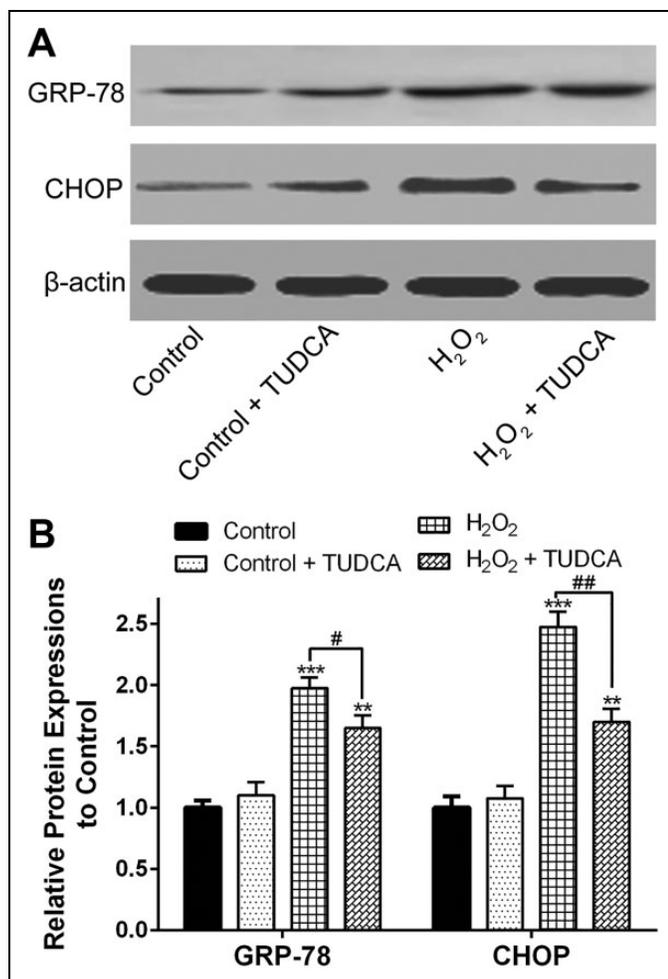


Figure 4. The TUDCA treatment (0.2 mM) affected the expression of glucose-regulated protein 78 (GRP78) and C/CHOP in NRCMs. Western blotting was used to assay the protein expressions; β -actin was used as a loading control A, and relative expressions from the Western blotting B. Data are presented as mean \pm SEM. $n = 3$, $^{***}P < .01$ and $^{***}P < .001$ compared to control. $^{\#}P < .05$ and $^{\#\#}P < .01$. CHOP indicates CCAAT/enhancer-binding protein-homologous protein; GRP, glucose-regulated protein 78 kDa; NRCM, neonatal rat cardiomyocytes; SEM, standard error of the mean; TUDCA, tauroursodeoxycholic acid.

cardiomyocytes. In order to select suitable concentration of TG, the cell toxicity of TG at different doses (0.1-1.2 μ M) was detected. As illustrated in Figure 5A, 10 hours treatment with 0.1 and 1.2 μ M of TG markedly reduced cell viability compared to that of control, respectively, whereas 0.5 μ M treatment reduced the cell viability further. According to these results, treatment with 0.5 μ M TG for 10 hours was chosen for the following assay. As shown in Figure 5B, compared with control treatment, TUDCA pretreatment decreased the TG-induced loss of cell viability. Similarly, exposure of cardiomyocytes with 0.5 μ M TG pretreatment caused an increase in the protein expression levels of GRP78, and CHOP and TUDCA at 200 μ M remarkably decreased these protein levels in cardiomyocytes (Figure 5C, D).

Discussion

The ER is an organelle that has essential roles in multiple cellular processes that are required for cell survival and normal cellular functions. Because of its role in protein folding and transport in the secretory pathway, the ER is also rich in calcium-dependent molecular chaperones such as GRP78.¹⁷ The accumulation of the unfolded proteins represents a cellular stress induced by multiple stimuli and pathological conditions including hypoxia and oxidative injury.¹⁸ In homeostasis, cellular processes are in a dynamic equilibrium. Perturbation of homeostasis causes stress. Disruption of the internal ER micro-environment leads to ER dysfunction, which is designated as ER stress.¹⁹ The ER stress response is a highly conserved signaling system that has been studied in many different cell and tissue types, which involve numerous pathophysiological processes, including aging, inflammation, diabetes, neurodegenerative diseases, and cardiovascular diseases.²⁰

Tauroursodeoxycholic acid is detected in human bile and has a long history in Chinese medicine as a remedy for biliary and liver diseases. Tauroursodeoxycholic acid has been reported to reduce ER stress in the heart of type 2 diabetic rats.²¹ Treatment of obese and diabetic mice with TUDCA reversed hyperglycemia and insulin sensitivity, reduced fatty liver, and enhanced the action of insulin in the liver, muscle, and adipose tissues.²² Overall, accumulating evidence indicates that inhibition of ER stress is crucial for the protection of TUDCA. In this study, we are more interested in the protection role of TUDCA in the cardiovascular system. From the literatures, we understood that TUDCA treatment reduces infarct size in mice by preventing cyclophilin D translocation to the inner mitochondrial membrane,²³ and the effect of TUDCA on reducing the number of apoptotic cells in a model of MI model entail a potential therapeutic application in a clinical setting for the treatment of acute MI or in revascularization procedures.²⁴ However, there was no comprehensive proof to show the direct mechanisms of cardioprotection role of TUDCA.

In this study, we elected to investigate the protective effect of TUDCA in NRCMs rather than other cells, because cardiomyocytes isolated from neonatal rats have several advantages over cultures of adult cardiomyocytes and other cell lines. Foremost, the isolation procedure for neonatal rat hearts is simpler and less expensive when compared to the isolation of cardiomyocytes from adult mouse or rat.²⁵ In addition, the *in vitro* effects of TUDCA on the cultured cells reflected the indirect action of TUDCA. That is to say, TUDCA shows the protective roles when there is inflammation. The cell toxicity assay implied that TUDCA itself at the optimized concentration (200 μ M) did not affect the levels of ROS, LDH, GSH, and SOD in NRCMs, but the TUDCA treatment effectively alleviated H₂O₂-induced oxidative stress status, suggesting the inhibition of TUDCA on oxidative stress markers. To be noted, the *in vivo* effects of TUDCA could be different since there is always oxidative reaction in the complicated system. For instance, previous study showed that the chronic administration of TUDCA significantly reduced the expression of ER stress

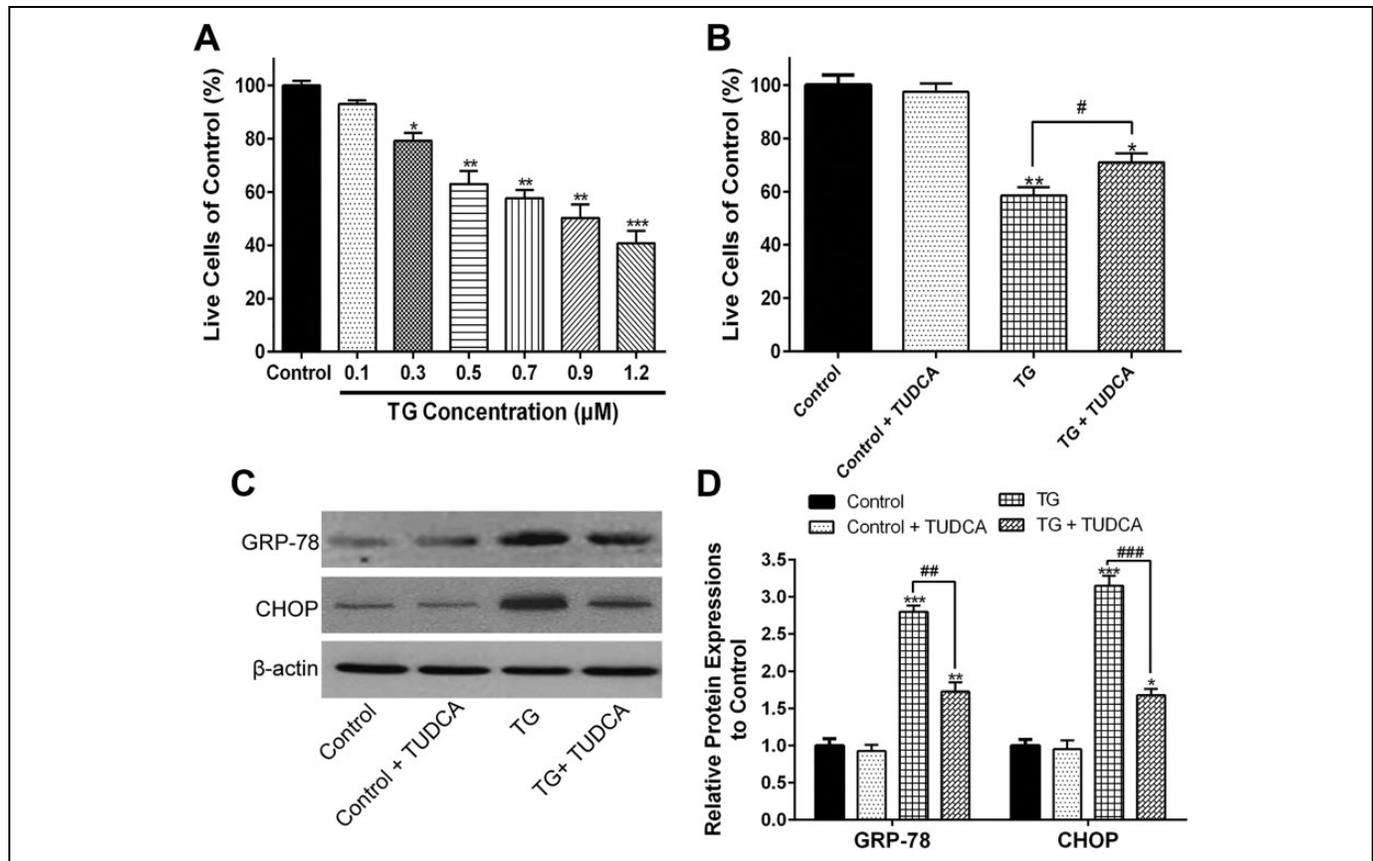


Figure 5. Effects of TUDCA on ER stress inducer (thapsigargin)-induced changes in NRCMs. **A**, effect of TG (0.1, 0.3, 0.5, 0.7, 0.9, and 1.2 μM) on the cell viability of NRCMs. **B**, The TUDCA reversed TG-caused loss of cell viability. Cells were pretreated with or without 0.5 μM TG for 10 hours after the TUDCA pretreatment. Western blotting was used to assay the expression of GRP78 and CHOP, β-actin was used as a loading control **C**, and relative expressions from the Western blotting. **D**, Data are presented as mean ± SEM. $N = 3$, * $P < .05$, ** $P < .01$, and *** $P < .001$ compared to control. # $P < .05$, ### $P < .01$, and #### $P < .001$. CHOP indicates CCAAT/enhancer-binding protein-homologous protein; ER, endoplasmic reticulum; GRP, glucose-regulated protein 78 kDa; NRCM, neonatal rat cardiomyocytes; SEM, standard error of the mean; TG, thapsigargin; TUDCA, tauroursodeoxycholic acid.

markers and attenuated the development of aortic lesions in the high-fat diet-enhanced atherosclerosis.²⁶ In fact, exogenous addition of H₂O₂ in the cultured cells has been used to induce acute oxidative stress,^{27,28} and thus, our study is limited to neglect role of TUDCA in the chronic inflammation system. It has been well known that these oxidative stress markers are commonly involved in mitochondria-mediated apoptosis pathway, annexin V/PI-based FCM was used to detect the apoptosis of NRCMs, and our results demonstrated that TUDCA alleviated H₂O₂-induced cardiomyocyte apoptosis. As general consensus for FCM, staining with annexin V is typically used in conjunction with a vital dye such as PI for identification of early and late apoptotic cells, and the cells in the upper left quadrant which shows only PI-positive cells are indicating the necrotic cells. To be noted, in the H₂O₂ and TUDCA combined group, the necrotic cells demonstrated higher absolute number when compared with all the other 3 groups, and it would be interesting to investigate the external factors which result in the unregulated digestion of cell components behind cell necrosis in further study.

In consistent with the apoptosis results by FCM, H₂O₂ increased the Bax/Bcl-2 ratio compared with that of the ratio in the control group, while TUDCA decreased the Bax/Bcl-2 ratio compared with that of H₂O₂ treated alone. Bcl-2 family has been discovered to play a key role in promoting or inhibiting intrinsic apoptotic pathway triggered by mitochondrial dysfunction.²⁹ Therefore, the balance between pro- and antiapoptotic members of this family can determine the cellular fate. Our results indicate that TUDCA could alleviate H₂O₂-induced apoptosis of NRCMs via mitochondria-mediated apoptosis signaling pathway.

Bcl-2 family proteins, along with the transcription factor CHOP, have been implicated in the regulation mechanisms that mediate ER stress-induced apoptosis.³⁰ In addition, GRP 78 is a sensor of ER stress. Endoplasmic reticulum stress is associated with cardiomyocyte apoptosis^{31,32} and the loss of cell viability following ischemia/reperfusion.^{33,34} Endoplasmic reticulum stress represents a therapeutic target for a number of cardioprotective signaling molecules such as adenosine monophosphate-activated protein kinase.³² In the present

study, we found decreased CHOP and GRP 78 expression in H₂O₂-induced cardiomyocytes. These results support our hypothesis that mitochondrial pathway is one of the signaling pathways by which TUDCA exerts its protective effect on cardiomyocytes.

Endoplasmic reticulum stress can be elicited by the ER stress inducer TG, which depletes Ca²⁺ from ER.³⁵ Our results showed that TUDCA was able to prevent TG-induced ER stress as reflected by improving the cell viability of TG-treated cardiomyocytes and by attenuating the protein expression of GRP78 and CHOP compared to TG group. These results suggest that the protective effects of TUDCA on H₂O₂-induced oxidative injury are through alleviating ER stress and ER stress-induced apoptosis in neonatal mouse cardiomyocytes.

Taken together, our data indicated that TUDCA inhibited H₂O₂-induced oxidative stress and cardiomyocytes apoptosis via suppressing ER stress, providing comprehensive understanding of the mechanism of the cardioprotection role of TUDCA.

Conclusion

Our data indicated that TUDCA-mediated inhibition on H₂O₂-induced oxidative stress and cardiomyocytes apoptosis was through suppressing ER stress, and TUDCA possesses the potential to be developed as therapeutic tool in clinical use for cardiovascular diseases.

Authors' Note

Lin Zhang and Yanmin Wang contributed equally to this study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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