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Original Article

Tert-butylhydroquinone ameliorates doxorubicin-induced cardiotoxicity by activating Nrf2 and inducing the expression of its target genes

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Abstract: Oxidative stress plays an important role in doxorubicin (DOX)-induced cardiotoxicity. Nuclear factor E2-related factor-2 (Nrf2) is a transcription factor that orchestrates the antioxidant and cytoprotective responses to oxidative stress. In the present study, we tested whether tert-butylhydroquinone (tBHQ) could protect against DOX-induced cardiotoxicity *in vivo* and, if so, whether the protection was associated with the up-regulation of the Nrf2 pathway. The results showed that treatment with tBHQ significantly decreased the DOX-induced cardiac injury in wild-type mice. Moreover, tBHQ ameliorated the DOX-induced oxidative stress and apoptosis. Further studies suggested that tBHQ increased the nuclear accumulation of Nrf2 and the Nrf2-regulated gene expression, including heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxido-reductase-1 (NQO-1) expression. Knocking out Nrf2 in mice abolished the protective effect of tBHQ on the DOX-induced cardiotoxicity. These results indicate that tBHQ has a beneficial effect on DOX-induced cardiotoxicity, and this effect was associated with the enhanced expression of Nrf2 and its downstream antioxidant genes, HO-1 and NQO-1.

Keywords: Doxorubicin, nuclear factor erythroid 2-related factor 2, tert-butylhydroquinone, cardiotoxicity, heme oxygenase-1, NAD(P)H:quinone oxido-reductase-1

Introduction

The anthracyclin drug doxorubicin (DOX) is a quinine-containing antitumor antibiotic with a broad antitumor spectrum. It is commonly used to treat various cancers, including severe leukemias, lymphomas, and a few solid tumors [1, 2]. However, its clinical usage and efficacy are limited by a dose-dependent cardiotoxicity [3, 4]. Therefore, the search for an effective and safe antagonist of the DOX-induced cardiac toxicity remains a critical issue in both cardiology and oncology.

DOX-induced oxidative stress has been the prevailing hypothesis for its cardiotoxicity. The evidence indicates that the levels of reactive oxygen species (ROS) in the cardiomyocytes increase soon after exposure to DOX and that this contributes significantly to the cardiotoxic effects of DOX [5, 6]. However, cells have natural mechanisms for resisting intercellular oxidant stress [7-9]. Endogenous antioxidant proteins and related regulators of the antioxidant responsive element (ARE), a cis-acting element within the regulatory region of the antioxidant and phase II detoxicant genes, can actively antagonize the action of ROS [10]. Notably, nuclear factor erythroid 2-related factor 2 (Nrf2) is an activator of ARE. Nrf2 activation can induce the expression of antioxidants such as heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxido-reductase-1 (NQO1) as well as phase II detoxicants [11-13]. Li et al. have reported that Nrf2 acts as a critical negative regulator of DOX-induced cardiomyopathy, which highlights the

potential that targeting Nrf2 may be a novel therapeutic strategy to attenuate the DOX-induced cardiotoxicity and cardiomyopathy [14]. Based on these findings, chemicals that are able to activate Nrf2 will be potential candidate drugs with which to treat DOX-induced cardiotoxicity.

Tert-butylhydroquinone (tBHQ) is a synthetic phenolic antioxidant that exerts its antioxidant function via the Nrf2 pathway. tBHQ can inhibit oxidative stress-induced injury [15-17]. Accordingly, we hypothesized that treatment with tBHQ would induce Nrf2 and downstream antioxidant expression, which would attenuate the DOX-induced cardiotoxicity. In this study, we evaluated the effects of tBHQ on the DOX-induced cardiotoxicity and determined the underlying mechanisms.

Materials and methods

Animals and treatments

Wild-type (Nrf2+/+) and Nrf2-deficient (Nrf2-/-) CD1/ICR mice were obtained from Dr Thomas W. Kensler (Johns Hopkins University, Baltimore, MD). The mice were bred and genotyped for Nrf2 expression by PCR amplification of genomic DNA using the following primers. NRF5: TGG ACG GGA CTA TTG AAG GCT G; NLACZ: GCG GAT TGA CCG TAA TGG GAT AGG; NAS: GCC GCC TTT TCA GTA GAT GGA GG. The mice were housed under temperature- and humidity-controlled conditions with a 12 h light/dark cycle. They were fed a standard rodent diet and provided with water ad libitum. Age-matched wild-type and Nrf2-/- male mice were subjected to the treatments described below. A total of 40 wild type mice were randomly divided into 4 groups: (1) control; (2) tBHQ; (3) DOX; (4) tBHQ+DOX. tBHQ (25 mg/kg) was administered for 3 consecutive days, and DOX (20 mg/kg) was administered once on day 2. Because the tBHQ was dissolved in 3% ethanol/isotonic saline and the DOX was dissolved in a saline solution, the mice serving as the vehicle controls were given the same volume of 3% ethanol/isotonic saline solution or saline solution. All chemicals were given by intraperitoneal injection. The dosing volume was 0.1 ml/10 g body weight. Forty-eight hours after the single intraperitoneal injection of DOX, the mice were anesthetized with thiopentone (35 mg/kg; i.p.). The abdomen of each mouse was opened, and the hearts were rapidly removed and washed in ice-cold isotonic saline. Half of each heart was stored at -80°C, and the other half of the heart was placed in a fixative solution (4% paraformaldehyde). To explore the role of Nrf2, the same procedures were also performed using 40 Nrf2-/- male mice.

Serum biochemical assays

A blood sample was collected from the inner canthus of each mouse prior to sacrifice. The blood was centrifuged at 3000 g for 15 min to separate the sera, which were stored at -80°C for the biochemical analyses. Creatine phosphokinase (CK) and creatine kinase isoenzyme-MB (CK-MB) activities were determined according to standard methods using diagnostic kits from BioSystems S.A. (Barcelona, Spain) and a CHEMIX-180 automatic biochemistry analyzer (Sysmex).

Histopathological examination of the heart tissue

The paraformaldehyde-fixed heart tissue samples were embedded in paraffin wax, serially sectioned (5 μm thickness) and stained with hematoxylin and eosin for the assessment of the histopathological changes.

Immunohistochemistry

The expression of 3-nitrotyrosine (3-NT) and 4-hydroxy-2-nonenal (4-HNE) in the heart was characterized by immunohistochemistry. Tissue sections were dewaxed and then incubated with 1 × target retrieval solution in a microwave oven for 15 min at 98°C for antigen retrieval, followed by sequential treatments with 3% hydrogen peroxide for 15 min at room temperature and 5% bovine serum albumin for 30 min. These sections were incubated with primary antibodies overnight at 4°C. The primary antibodies were anti-3-nitrotyrosine (3-NT, YSLY8W, Beijing Biosynthesis Biotechnology CO., LTD) at a 1:400 dilution and anti-4-hydroxy-2-nonenal (4-HNE, Abcam, Cambridge, MA) at a 1:400 dilution. The sections were then washed with PBS and incubated with horseradish peroxidise-conjugated secondary antibodies (1:300-400 dilutions in PBS) for 2 h at room temperature. To develop the color, the sections were treated with the peroxidase substrate DAB and counterstained with hematoxylin. Five sections

Table 1. Primers for real-time PCR

Genes	Gene access #	Forward	Reverse	Product length (bp)
Nrf2	NM_010902	CAGTGCTCCTATGCGTGAA	GCGGCTTGAATGTTTGTC	109
HO-1	NM_010442	ACAGATGGCGTCACTTCG	TGAGGACCCACTGGAGGA	128
NQO-1	NM_008706	CTTTAGGGTCGTCTTGGCAACCAGC	CAATCAGGGCTCTTCTCG	102
β-actin	NM_007393	CACTGTGCCCATCTACGA	ATGTCACGCACGATTTCCCTCTCAG	155

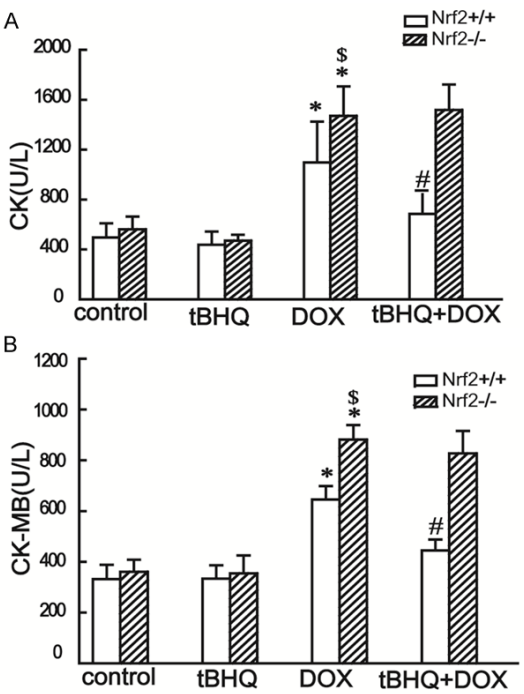

Figure 1. Effects of DOX alone or after pretreatment with tBHQ on the activity of the cardiac enzymes CPK and CK-MB in wild-type or Nrf2-deficient mice. Nrf2+/+, wild-type mice; Nrf2-/-, Nrf2-deficient mice. Each column represents the mean ± SD, n = 5. *P < 0.01 *vs.* corresponding control; #P < 0.01 *vs*. corresponding DOX; $$P$ < 0.01 *vs*. DOX in wild-type mice.

from each group were evaluated in a blinded manner.

Determination of lipid peroxides (measured as MDA)

These measurements were performed as previously described [18]. The fresh heart tissue was rinsed and then homogenized in a buffer [10 mM Tris-HCl, 137 mM NaCl, 1 mM Na_2EDTA, and 0.5 mM dithiotreitol (DTT)] with 250 mM sucrose at pH 7.4 using a homogenizer (T 18 basic Ultra-Turrax®; Mandel Scientific Company Inc., Guelph, Canada). The homogenate was centrifuged at 1,000 × g for 15 min at 4°C. The supernatants were removed, and their total protein concentration was measured using a protein assay kit. The supernatants were used for the biochemical assay and western blot analysis. The malondialdehyde (MDA) content in the heart tissue was used as an index of the lipid superoxide level. The measurements were conducted using a spectrophotometer (Perkin-Elmer, Norwalk, Conn., USA) and a commercially available kit (Jiancheng Bioengineering Institute, Nanjing, China).

Apoptosis assessment using TUNEL assay

The TUNEL (terminal deoxynucleotide transferase-mediated dUTP nick end-labelling) staining was performed using an in situ cell apoptosis detection kit (Boster Biological Technology, Wuhan, Hubei, China). According to the manufacturer's instructions, paraffin-embedded sections of samples were deparaffinized and hydrated, and then incubated in 20 g/ml protease K at room temperature for 5 min. After being washed twice, the samples were transferred to sodium citrate buffer (2 mmol/L citric acid and 10 mmol/L trisodium citrate, pH 6.0) at 37.8°C for 5 min. After two more 5-min washes with phosphate-buffered saline (PBS), the samples were transferred to 20 ml of the TUNEL reaction mixture (1 ml terminal deoxynucleotidyl transferase, 1 ml digoxin-labelled d-UTP and 18 ml Labelling Buffer) and incubated at 37.8°C for 60 min. After rinsing, the sections were incubated with a biotinylated anti-digoxin antibody for 30 min at 37.8°C and developed with DAB substrate kit. The slides were lightly counterstained with hematoxylin and then dehydrated and mounted. For each myocardial specimen, the tissue sections were examined microscopically at × 400 magnification, and 10 random fields per section were counted. The percentage of apoptotic cells was calculated as the apoptotic index, i.e., the ratio of the number of positively stained myocyte nuclei to the total number of myocyte nuclei.

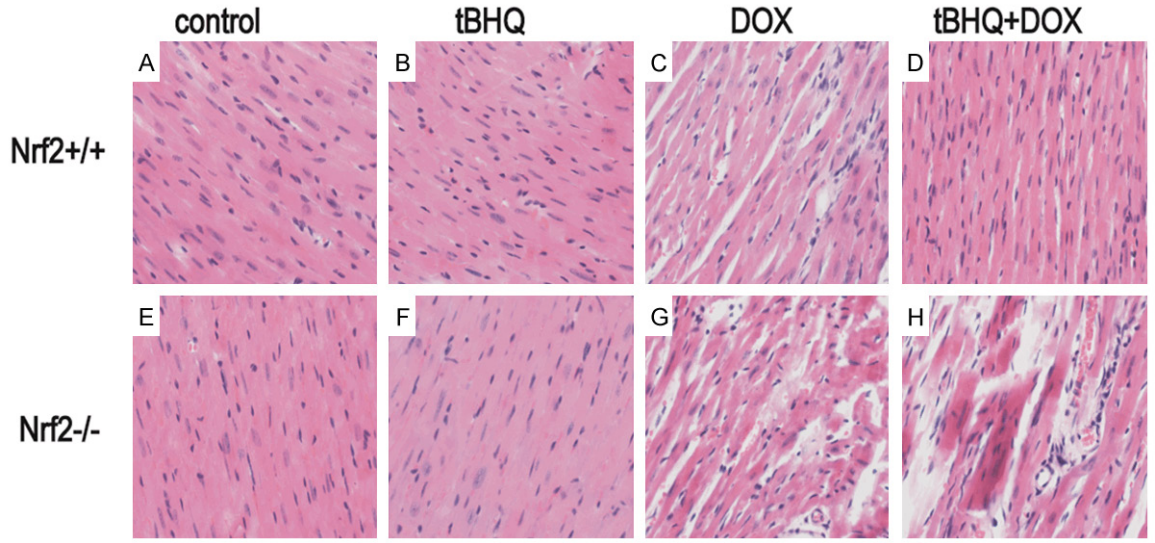

Figure 2. Histopathological changes in the cardiac tissue after DOX treatment in wild-type and Nrf2-deficient mice. Light micrographs of heart sections from the control (A and E), tBHQ-treated (B and F), DOX-treated (C and G) and tBHQ+DOX-treated (D and H). Original magnification, × 400.

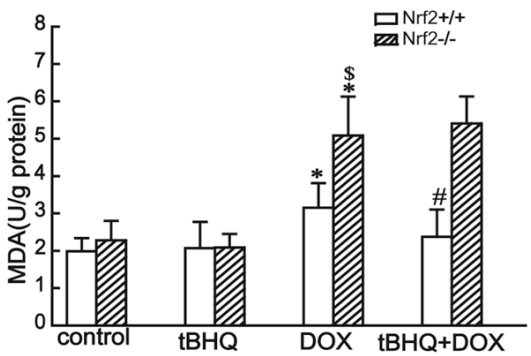

Figure 3. Effects of DOX alone and after pretreatment with tBHQ on the MDA levels in the heart homogenates. Nrf2+/+, wild-type mice; Nrf2-/-, Nrf2-deficient mice. Each column represents the mean ± SD, n = 5. *$P < 0.01$ *vs.* corresponding control; $^{\#}P < 0.01$ *vs*. corresponding DOX; $^{\$}P < 0.01$ *vs*. DOX in wild-type mice.

Reverse transcriptase PCR

Total RNA was prepared from the LV tissue using the Trizol reagent (Qiagen, Hilden, Germany). Complementary DNAs were synthesized by standard techniques using a QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany). Real-time PCR was performed, recorded, and analyzed using a Line-Gene K Real Time PCR System (Bioer Technology CO., LTD, Hangzhou, China) with SYBR Green I detection. The cDNA was amplified using a TransStart Top Green qPCR SuperMix kit (TransGen Biotech, Beijing, China) with specific primers (**Table 1**). To confirm the specificity of the PCR, the products from each primer pair were subjected to a melting curve analysis.

Western blotting examination of Nrf2

The heart nuclear proteins were extracted using a nuclear and cytoplasm protein extraction kit (KeyGen Biotech, Nanjing, PR China). After measuring the protein concentrations, both the cytoplasmic and nuclear protein extracts were separated using 10% SDS-PAGE and transferred to PVDF membranes. The membranes were then blocked with 5% nonfat dry milk in TBST (50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween, pH 7.4) for 1 h at room temperature. The membranes were next incubated with the polyclonal IgG for Nrf2 (1:200, Abcam, Cambridge, MA), β-actin (1:500, Santa Cruz Biotechnology) or histone H1 (1:200, Santa Cruz Biotechnology) overnight at 4°C. The blots were then washed three times for 10 min each with TBST, and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000) for 1 h at 37°C. After washing, the targets were detected using an enhanced chemiluminescence plus system (Zhong-Shan Bioengineering Institute, Beijing, China). The western blot signals were quantified based on the densitometric measurements.

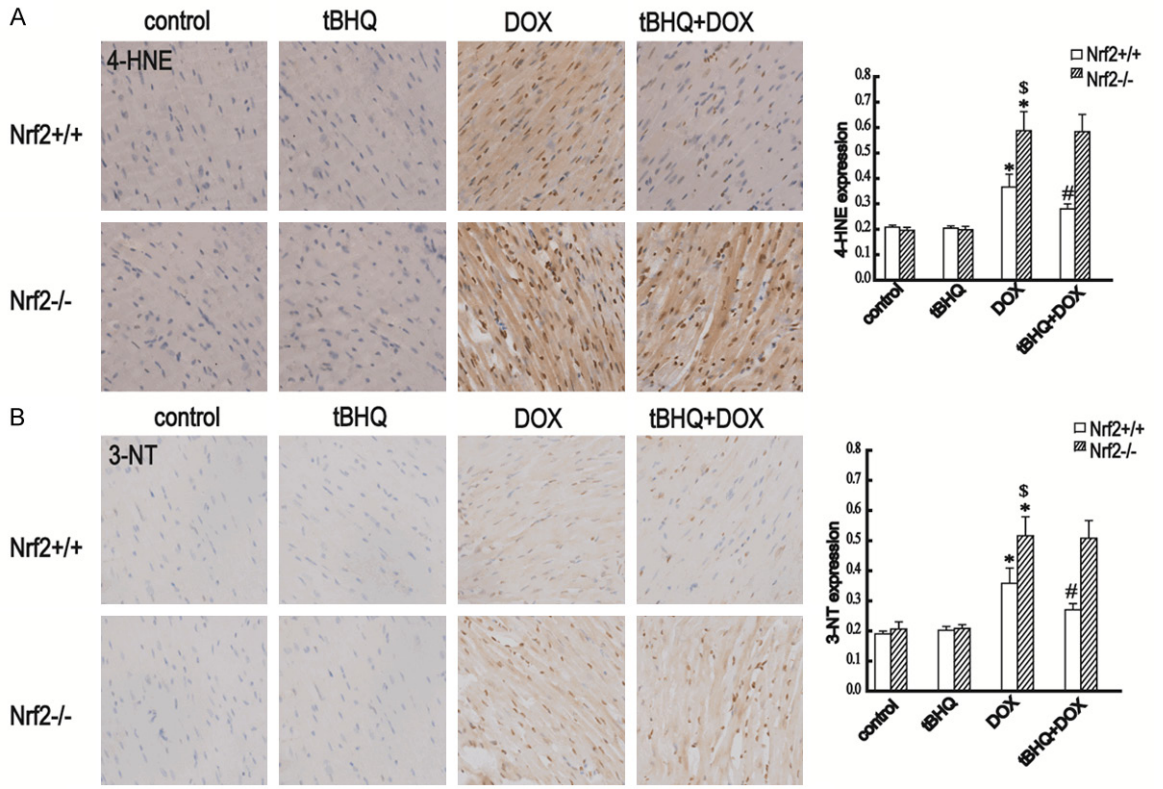

Figure 4. The protective effect of tBHQ on the myocardial oxidative damage induced by DOX. Immunohistochemical staining and a subsequent semi-quantitative analysis of positive staining for 3-NT (A) and 4-HNE (B) were performed to measure the oxidative damage. The data are presented as the means ± SD (n = 5). **P* < 0.01 *vs*. corresponding control; #*P* < 0.01 *vs*. corresponding DOX; $*P* < 0.01 *vs*. DOX in wild-type mice.

Statistical analyses

All results are expressed as the means ± SD, and the Statistical Package for Social Science 11.0 (SPSS/PC) was used for all analysis. A one-way analysis of variance (ANOVA) with LSD post hoc analysis was used to identify the significant differences. The level of significance was defined as $P < 0.05$.

Results

tBHQ alleviated DOX-induced cardiotoxicity in wild-type mice

To demonstrate the tBHQ-induced protection towards DOX-induced cardiotoxicity, wild-type mice were treated with a single dose of DOX (20 mg/kg) with and without pretreatment with tBHQ (25 mg/kg). The serum markers for myocardial injury, CPK and CK-MB, were significantly ($P < 0.05$) elevated in the DOX-only-treated group compared with the control and tBHQ-only-treated groups. Pretreatment with tBHQ significantly reduced the CPK and CK-MB levels compared with the DOX-only-treated group in the wild-type mice (**Figure 1**). Light microscopic examination of the heart sections 48 h after the DOX treatment revealed vascular congestion, edema of myocardial tissue and cells and loss of striations, as well as an increase in inflammatory cells (**Figure 2C**). In contrast, the DOX-induced histopathological changes were partially attenuated in the tBHQ+DOX group of wild-type mice (**Figure 2D**).

tBHQ alleviated the DOX-induced oxidative damage in wild type mice

On the basis that oxidative stress is considered to be the primary cause of DOX-induced cardiomyopathy, we further evaluated the myocardial oxidative stress induced by DOX. MDA and 4-HNE accumulation were measured as indices of lipid peroxidation. 3-NT accumulation is an index of nitrosative damage. **Figure 3** shows the effects of the tBHQ pretreatment on the levels of MDA in the heart homogenates. A single

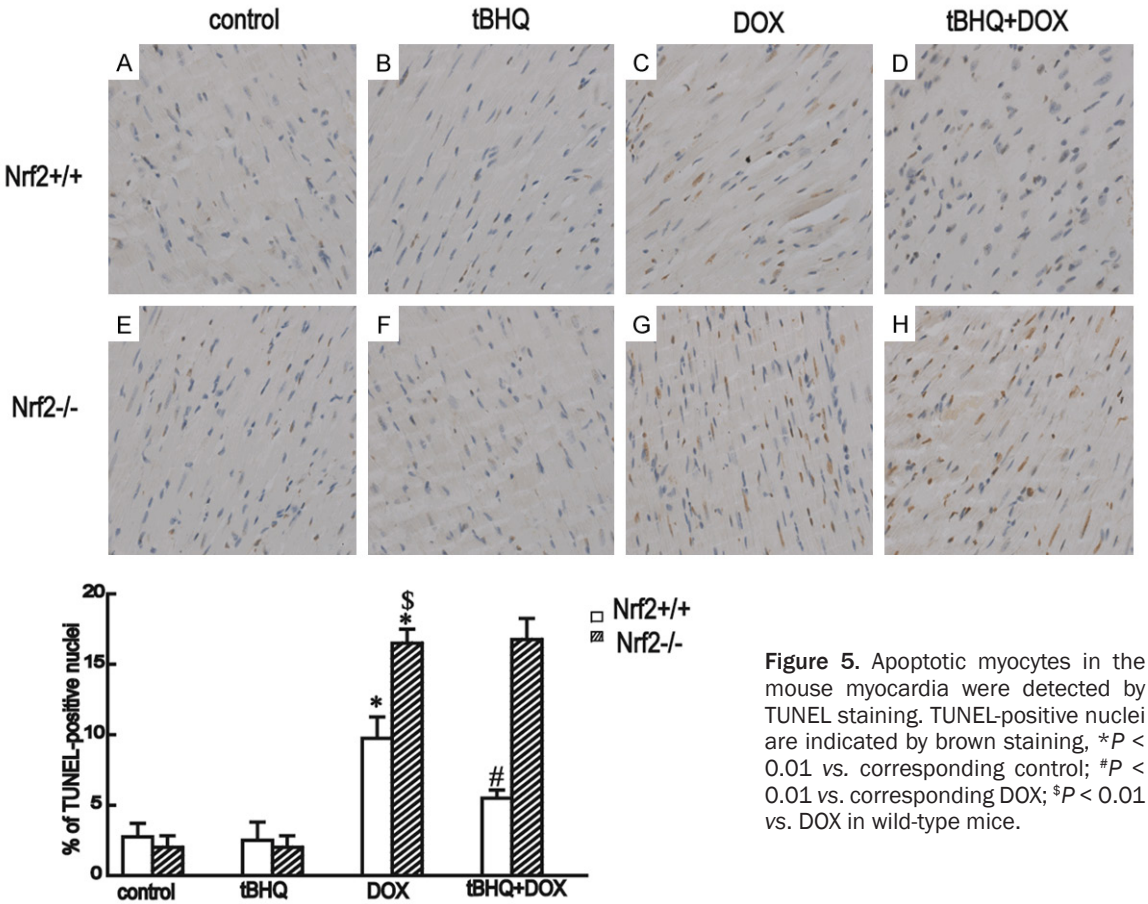

Figure 5. Apoptotic myocytes in the mouse myocardia were detected by TUNEL staining. TUNEL-positive nuclei are indicated by brown staining, $^{*}P < 0.01$ *vs.* corresponding control; $^{\#}P < 0.01$ *vs*. corresponding DOX; $^{\$}P < 0.01$ *vs*. DOX in wild-type mice.

dose of DOX induced a 59% increase in the MDA levels in the heart homogenates, whereas there was only a 36% increase in the tBHQ-pretreated group. The immunohistochemical staining showed that 4-HNE and 3-NT were both significantly increased in the cardiac myocytes after DOX treatment in the wild-type mice (**Figure 4A** and **4B**), an effect that was significantly attenuated by the tBHQ pretreatment.

tBHQ attenuated the DOX-induced myocardial apoptosis in wild-type mice

Because oxidative stress can induce apoptosis and myocardial apoptosis is also a causative factor in cardiomyopathy, we examined the apoptotic changes using the TUNEL assay. As shown in **Figure 5**, the apoptotic index (TUNEL-positive cells) following DOX treatment was significantly increased from 4% to 9.7% (**Figure 5C**), but the apoptotic index was decreased to 5.5% by the combined treatment with tBHQ (**Figure 5D**).

tBHQ upregulated the expression of Nrf2 and its downstream genes in wild-type mice

The results described above showed that tBHQ can protect against the DOX-induced myocardial injury, oxidative damage and apoptosis. tBHQ is an activator of Nrf2. Therefore, to determine whether tBHQ protected the heart from DOX-induced injury by activating Nrf2, we first measured the Nrf2 expression and the transcription of its target genes in the heart. By real-time PCR and western blotting, Nrf2 expression was found to be significantly increased in the hearts of the tBHQ-treated control mice (**Figure 6A** and **6B**). tBHQ also stimulated the translocation of Nrf2 into the nuclei (**Figure 6C**). Treatment with DOX alone enhanced the myocardial Nrf2 mRNA and protein levels and triggered the Nrf2 nuclear translocation 48 h after the treatment. However, more remarkable increases in the Nrf2 mRNA and protein levels were detected in the tBHQ+DOX group ($P < 0.01$). In the next study, we fur-

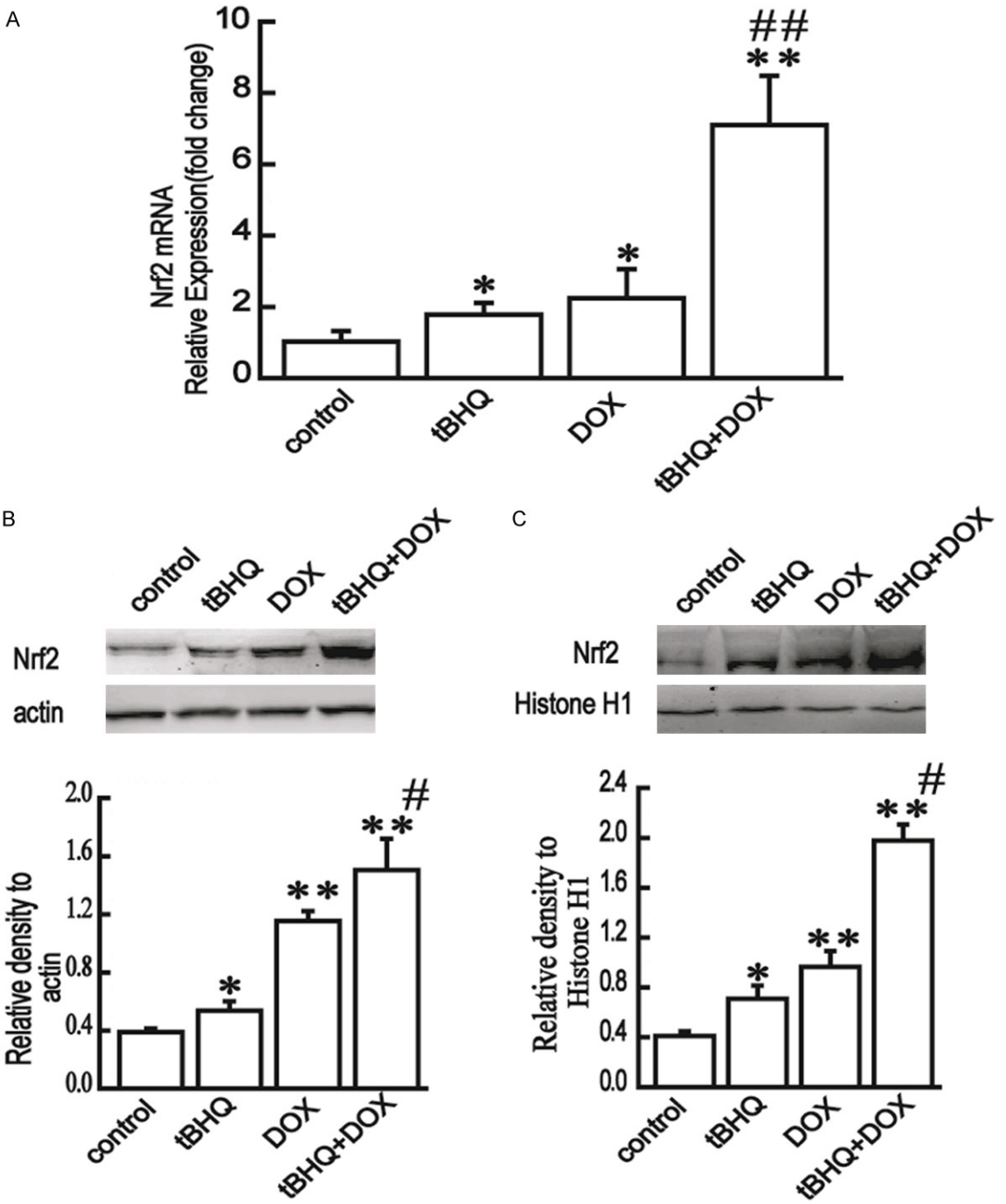

Figure 6. Expression of Nrf2 in the hearts of wild-type mice. The hearts were isolated from the experimental and control mice 28 h after the single intraperitoneal injection of DOX. Subsequently, quantitative analysis of the levels of Nrf2 mRNA transcripts relative to those for GAPDH and of Nrf2 protein relative to β-actin in the hearts of the individual wild-type mice was performed using RT-PCR (A) and Western blotting (B), respectively. The nuclear proteins of the individual mouse hearts were extracted and the levels of Nrf2 relative to those for histone H1 were characterized using western blotting (C). The data are presented as the means ± SD (n = 5). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* DOX.

ther examined the Nrf2 transcriptional activity by measuring the expression of its downstream anti-oxidative genes. Exposure to tBHQ or DOX alone increased the mRNA expression of HO-1 and NQO1; more remarkable increases in the HO-1 and NQO1 levels were detected in the

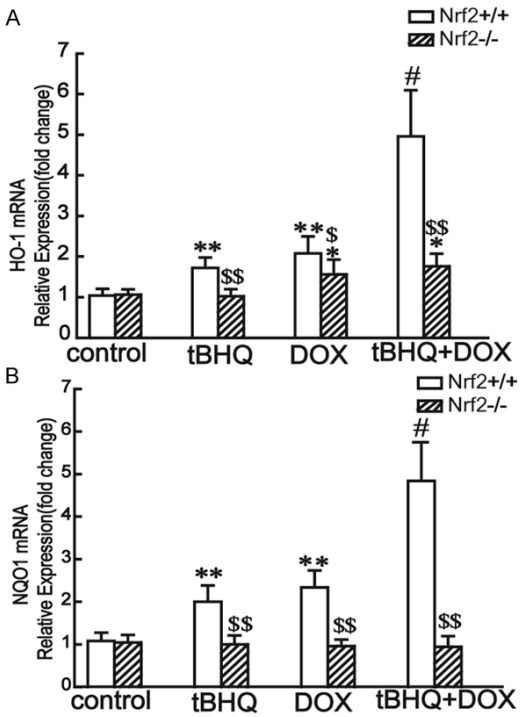

Figure 7. Relative mRNA expression of the Nrf2-regulated genes in the hearts of wild-type or Nrf2-deficient mice. The mRNA expression of the Nrf2-regulated genes HO-1 (A) and NQO1 (B). The data are presented as the means ± SD (n = 5). $^{*}P < 0.01$, $^{**}P < 0.01$ *vs.* corresponding control; $^{\#}P < 0.01$ *vs.* corresponding DOX; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ *vs.* DOX in wild-type mice.

tBHQ+DOX group. The changes in these Nrf2 downstream genes (**Figure 7**) were consistent with the Nrf2 levels in the nuclei. This finding clearly suggested the possible contribution of tBHQ-upregulated Nrf2 expression and function in the protection against DOX-induced cardiotoxicity.

The protective effect of tBHQ on DOX-induced cardiotoxicity was abolished by knocking out Nrf2

Nrf2-deficient mice were used to further explore the role of Nrf2 in the cardioprotective effect of tBHQ. PCR and agarose gel electrophoresis of DNA from the tails were used to confirm the wild type (700 bp) and Nrf2-/- (400 bp) genotypes (**Figure 8A**). The protein and RNA expression analyses confirmed disruption of Nrf2 in the knock-out mouse hearts (**Figure 8B** and **8C**). There were no differences in the basal conditions between the hearts of the Nrf2-knockout mice and those of the wild-type control mice. As shown in **Figures 1**-**5**, knockout of Nrf2 exaggerated the DOX-induced myocardial injury, oxidative stress and apoptosis. tBHQ pretreatment did not reduce the levels of CK and CK-MB or the histopathological changes induced by DOX in the Nrf2-knockout mouse hearts. Moreover, the tBHQ pretreatment did not attenuate the oxidative damage and apoptosis in the Nrf2-deficient mice (**Figures 4H**, **5H**). These results indicate that the Nrf2 pathway plays a key role in the tBHQ-induced protection.

Knocking out Nrf2 abolished the upregulation of the antioxidant genes

Notably, although treatment with either tBHQ or DOX increased the expression levels of HO-1 and NQO1 in the hearts of the Nrf2 wild-type mice, more remarkable increases in both HO-1 and NQO1 levels were detected in the tBHQ+DOX group. However, the upregulation of the antioxidant genes was not observed in the hearts of the Nrf2-knockout mice (**Figure 7**). These results suggested that the upregulation of the antioxidant genes by tBHQ or DOX was Nrf2-dependent. Therefore, the inability of tBHQ to alleviate the DOX-induced cardiotoxicity in the Nrf2-deficient mice is probably attributable to the loss of the Nrf2-dependent defensive response.

Discussion

DOX is a commonly used broad-spectrum chemotherapeutic agent. However, its clinical use is limited due to a serious dose-dependent cardiotoxicity that leads to irreversible degenerative cardiomyopathy and heart failure. Several mechanisms have been hypothesized in the etiology of DOX-induced cardiotoxicity including increased oxidative stress in cardiomyocytes. In the present study, we demonstrated for the first time that tBHQ, a widely used Nrf2 activator, protects against DOX-induced cardiotoxicity through activation of Nrf2. These results suggest that the cardiotoxicity induced by DOX can be prevented by tBHQ and that this effect is associated with the up-regulation of Nrf2 expression and activity.

A rich body of evidence has demonstrated that tBHQ is effective in protecting against cellular dysfunction induced by oxidative stressors such as alcohol, dopamine, hydrogen peroxide and glutamate in various cell types [19-23]. It

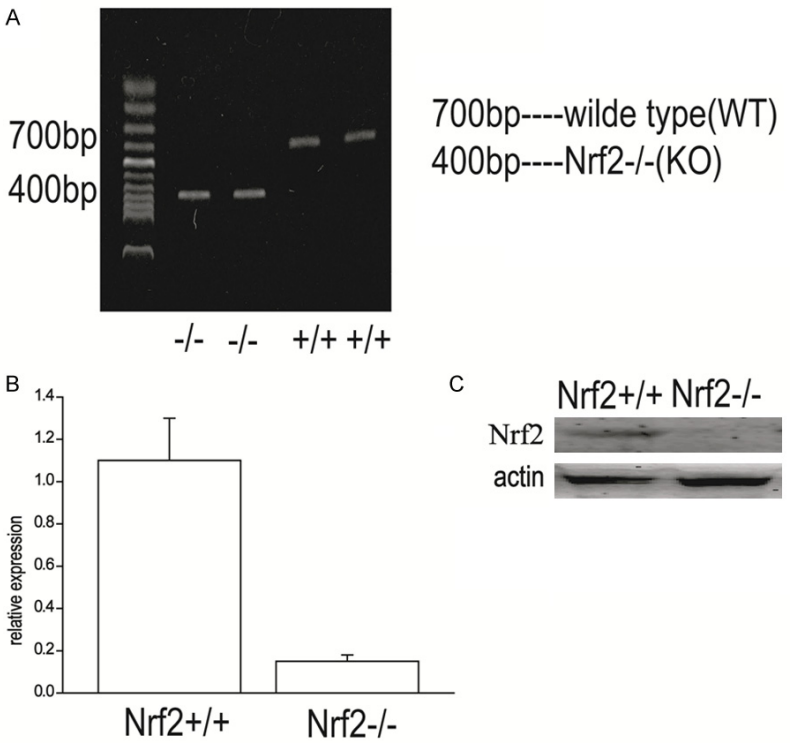

Figure 8. Identification of gene knock-out Nrf2 mice. A. PCR and agarose electrophoresis of the DNA from the tails of the WT (700 bp) and Nrf2-/- (400 bp) genotype mice. B. Nrf2 mRNA expression in the heart. Real-time qPCR analysis showing basal or no expression of Nrf2 gene in the Nrf2-/- mouse myocardium (n = 4). C. Western blotting analysis of Nrf2 protein expression.

has been well-established that tBHQ exerts its antioxidant function through a mechanism whereby it increases Nrf2 protein stability by inhibiting the Kelch-like ECH-associated protein 1 (Keap1)-mediated ubiquitination [23, 24]. Based on these observations, tBHQ has become a widely employed Nrf2 activator in various experimental settings. Recently, some studies have demonstrated that tBHQ is not only an Nrf2 activator. It can also oppose stress-induced activation of FoxO3a and therefore may have potential protective utility in neurodegeneration [25]. Li et al. also reported that tBHQ protects hepatocytes against lipotoxicity by inducing autophagy, but the induction of autophagy was not required for tBHQ-induced activation of Nrf2 [26]. These data provide strong evidence that the protective effect of tBHQ against lipotoxicity in hepatocytes was independent of its effect on Nrf2. In our study, we found that the Nrf2, HO-1 and NQO-1 expression and the nuclear Nrf2 levels were significantly elevated in the hearts of the tBHQ-treated mice, which indicates that tBHQ enhanced the expression of Nrf2 and related antioxidants as well as the nuclear accumulation of Nrf2 in the hearts of the wild-type mice. To further confirm the role of Nrf2, Nrf2-deficient mice were used. We found that the protective effect of tBHQ on the DOX-induced cardiotoxicity was abolished in the Nrf2-/- mice, and knocking out Nrf2 abrogated the upregulation of the antioxidant genes. The results demonstrate that tBHQ attenuated the DOX-induced cardiotoxicity through Nrf2-dependent antioxidant gene activation.

Some studies have demonstrated that Nrf2 is a critical endogenous inhibitor of maladaptive cardiac remodeling and dysfunction that acts by suppressing oxidative stress in the heart [27]. Nordgren et al. reported that the Nrf2 pathway is an important initial response to acute DOX-induced oxidative injury [28]. In the present study, DOX treatment increased the myocardial Nrf2 mRNA and protein levels and triggered the nuclear translocation of Nrf2. In addition, DOX treatment led to increases in the myocardial mRNA for NQO1 and HO-1, the downstream antioxidant genes of Nrf2. The Nrf2 deficiency exaggerated the DOX-induced myocardial oxidative stress and cardiac toxicity. Our results are consistent with a previous report [14]. These results demonstrated that DOX treatment activates Nrf2 in the heart, thus supporting the concept that Nrf2 activation serves as a defense mechanism to suppress DOX-induced cardiomyopathy by suppressing oxidative stress and apoptosis.

Nrf2 transactivates the expression of a group of cytoprotective enzymes including heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO1), and glutathione S-transferase a-1 (GST-a1). HO-1, which catalyzes the degradation of heme to release free iron, carbon

monoxide and biliverdin, has potent antioxidative and cytoprotective properties against various stress stimuli [29], and the importance of HO-1 expression in mediating antioxidant and anti-inflammatory effects has been well characterized in both *in vitro* and *in vivo* models [30-32]. HO-1 expression has been shown to be induced by a variety of stimuli including LPS, cytokines, cigarette smoke, and oxidative stress [33-35]. Resveratrol protected cardiomyocytes against DOX-induced apoptosis by inducing HO-1 in a nude mouse of lymphoma *in vivo* [36]. NQO1 is one of the key antioxidant enzymes that efficiently inhibits ROS production in various cells [37-39]. These up-regulated cellular detoxifying systems can increase resistance to the cell injury caused by DOX.

DOX plays an important role in the treatment of various tumors, but its application carries a risk of serious cardiac toxicity. Our results may therefore provide a treatment option to reduce the risk of serious cardiac complication by administration of tBHQ as an adjuvant. Thus, tBHQ may be useful in the clinical setting to prevent the cardiotoxicity induced by DOX during cancer chemotherapy.

Acknowledgements

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Disclosure of conflict of interest

None.

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