Chapter 7
Beneficial Effect of Taurine Treatment Against Doxorubicin-Induced Cardiotoxicity in Mice

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Abstract Though the administration of taurine is clinically efficacious against heart failure, the mechanism underlying its cardioprotection remains to be established. To provide information on the mechanism, we examined the effects of taurine on doxorubicin (DOX)-induced cardiotoxicity, with an emphasis on ROS generation and cardiac gene inhibition. Oral administration of taurine (3% w/v in tap water) dramatically reduced the mortality rate in both the acute or sub-acute toxic models of DOX toxicity. It was shown that taurine prevented DOX-induced oxidative stress as determined from cardiac glutathione content. Interestingly, Northern blot analysis revealed that DOX altered cardiac gene expression, including that of α-myosin heavy chain, ventricular myosin light chain-2 isoform and brain natriuretic peptide, an effect partially ameliorated by taurine treatment. In conclusion, taurine suppresses ROS generation and regulates gene expression in the DOX treated heart.

Abbreviations *DOX*, doxorubicin; *ROS*, reactive oxygen species; *GSH*, glutathione; α*MHC*, α Myosin heavy chain; *MLC2v*, myosin light chain type 2v; *BNP*, brain natriuretic peptide

7.1 Introduction

Doxorubicin (DOX) is an antineoplastic agent used against a wide variety of malignancies. However, the clinical use of the drug is limited largely because of its cardiotoxicity that leads to the development of a cardiomyopathy and eventually to overt heart failure (Minnotti et al. 2004; Singal and Iliskovic 1998). Although the mechanisms underlying the development of irreversible myocardial damage remain unclear, it has been shown that the generation of reactive oxygen species (ROS) is one of the critical events in the onset of the cardiomyopathy. ROS generation is closely related with the impairment in mitochondrial function and calcium handling. Importantly, the DOX-induced cardiomyopathy is associated with the

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downregulation of cardiac muscle-specific gene expression, such as α-myosin heavy chain (αMHC), myosin light chain ventricular type (MLC2v), brain natriuretic peptide (BNP) and sarcoplasmic reticulum proteins (Arai et al. 1998; Ito et al. 1990), resulting in myofibrillar loss and cardiac dysfunction.

Taurine (2-aminoethylsulfonic acid), a sulfur-containing amino acid, is found in millimolar concentrations in most mammalian tissues, being especially high in the heart. Taurine mediates many physiological functions, such as calcium handling, osmoregulation, membrane stabilization and detoxication (Huxtable 1992; Schaffer et al. 2000a). In cultured cardiomyocytes, taurine promotes cell survival during ischemia (Takatani 2004a, b). Collectively, taurine contributes to the maintenance of cellular homeostasis and is therefore clinically useful in treating certain pathological conditions (Azuma et al. 1982). However, in spite of the pharmacological benefits of taurine, detailed mechanisms of its cardioprotection have not been clarified.

In present study, we tested the effects of taurine on mortality following DOX treatment, focusing on changes in ROS generation and cardiac gene expression. This study provides the biological basis for the treatment of the DOX-induced cardiomyopathy with taurine.

7.2 Methods

7.2.1 Animals and Treatment

The experimental procedures conformed to the guidelines of the Institutional Animal Care and Use Committee of Osaka University. Six-week-old male C57BL6 mice were used in the present study. In the taurine treated group, animals were maintained on tap water containing 3% (w/v) taurine starting 1 week before DOX administration. To produce the acute toxicity model, DOX (Kyowa Hakko, Japan) was administered to mice as a single injection (15 mg/kg i.p.) (Kunisada et al. 2000). In the sub-acute toxicity model, DOX was administered for 6 weeks (5 mg/kg/week i.p.) (Taniyama and Walsh 2002). The survival rate was monitored for either 1 month (acute) or 2 months (sub-acute).

7.2.2 Measurement of Glutathione

The level of total glutathione (GSH+GSSG) was measured by the glutathione reductase (GR)/ 5,5′-dithibis(2-aminobenzoic acid) (DTNB) recycling assay (Anderson 1985). Tissues were homogenized in 0.1 M phosphate buffer, pH 7.5, containing 5% sulfosalicylic acid and were centrifuged at 10000 xg. Aliquots of supernatant were added to phosphate buffer (30°C) containing 0.27 mM NADPH, DNTB and 0.8 U GR. The reaction was monitored at 412 nm for 30 min.

7.2.3 Northern Blotting

Total RNA was isolated from control and DOX treated hearts and Northern blots were obtained as previously described (Ita et al. 2004). cDNA probes for BNP (Nakaoka et al. 2003), and GAPDH were labeled with Megaprime DNA Labeling System (Amersham Bioscience, USA) according to the protocol. The following oligonucleotides (Zhang et al. 2001) (Invitrogen, USA) were labeled with $\gamma -^{32}$P ATP by using T4 polynucleotide kinase (TOYOBO, Japan) according to the protocol. The following probes were used:

α-MHC: 5'-CGA ACG TTT ATG TTT ATT GTG GAT TGG CCA CAG CGA GGG TCT GCT GGA GAG GTT ATT CCT CGT C-3'.
β-MHC: 5'-GAG GGC TTC ACG GGC ACC CTT AGA GCT GGG TAG CAC AAG ATC TAC TCC TCA TTC AGG CC-3'.
MLC2v: 5'-CAC AGC CCT GGG ATG GAG AGT GGG CTG TGG GTC ACC TGA GGC TGT GGT TCA G-3'.
Sarcoplasmic reticulum Ca^{2+}-ATPase 2v (SERCA2a): 5'-TCA GTC ATG CAG AGG GCT GGT AGA TGT GTT GCT AAC AAC GCA CAT GCA CGC ACC CGA ACA-3'.

7.2.4 Statistical Analysis

Each value was expressed as the mean ± SEM. Statistical significance was determined by the Student's t-test. Survival data were analyzed by the Kaplan Meier method. Differences were considered statistically significant when the calculated P value was less than 0.05.

7.3 Results

7.3.1 Taurine Improved the Survival Rate After DOX Treatment

Taurine promotes cardiomyocyte survival in models of DOX toxicity. Therefore, we examined the effect of taurine treatment on the development of the in acute and sub-acute models of DOX cardiotoxic. As seen in Fig 7.1, DOX treated mice maintained on tap water containing 3% taurine observed greater rates of survival than mice maintained on taurine free tap water. In the acute DOX model, mice that were maintained on normal tap water exhibited a mortality rate of 70% 28 days following DOX injection while mice maintained on tap water supplemented with 3% taurine exhibited a mortality rate of only 10%. Similarly, in the sub-acute DOX model, 100% of the mice without taurine treatment were dead 43 days after the initial DOX injection, however, 50% of the mice treated with taurine survived at

least 60 days, at which time the experiment was terminated. Thus, treatment with taurine significantly attenuated DOX-induced mortality.

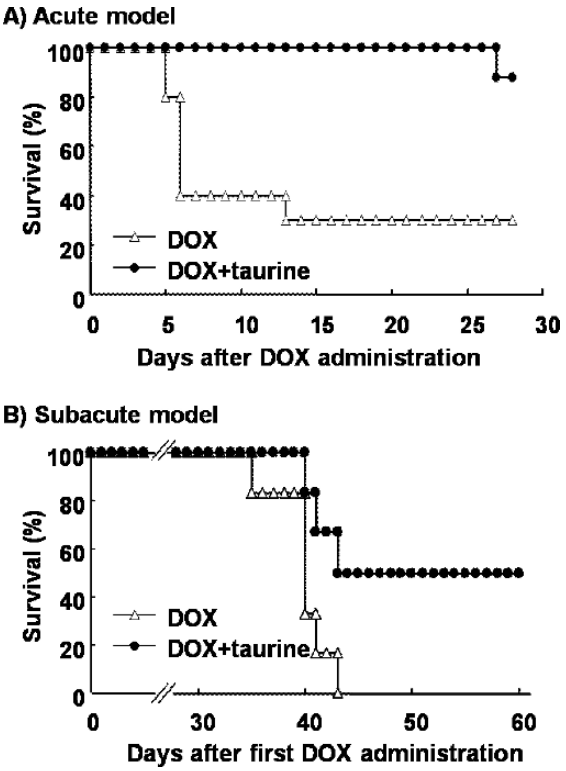

Fig. 7.1 The effects of taurine treatment on survival in acute (A) and sub-acute (B) models of DOX-induced cardiomyopathy. (**A**) Ten mice in each group were subjected to a single injection of 15 mg/kg i.p. of DOX and were monitored for an additional 4 weeks. (**B**) Six mice in each group were subjected to 6 injections of 5 mg/kg i.p. of DOX once a week and were monitored for an additional 8 weeks. *P* values were 0.011 (A) and 0.022 (B)

Cardiac enlargement is a common phenotype of cardiac remodeling seen following pathological stress. In order to examine the effects of taurine on DOX-induced cardiac enlargement, we analyzed the heart weight to body weight (HW/BW) ratio 28 days following DOX injection (15 mg/g BW) (Fig. 7.2). DOX increased the HW/BW ratio by 125%, an effect suppressed by taurine administration.

7.3.2 DOX-Mediated Oxidant Generation was Inhibited by Taurine Treatment

DOX-induced free radical production plays an important role in the progression of the DOX cardiomyopathy. To investigate the effect of taurine on DOX-induced

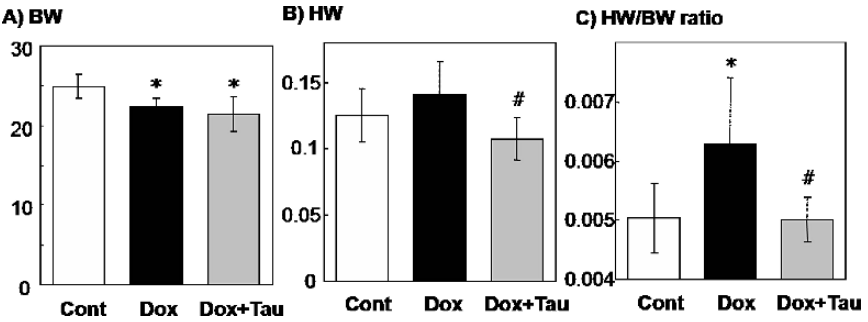

Fig. 7.2 The effect of taurine on DOX-induced cardiac remodeling. In the acute DOX model, body (**A**) and heart (**B**) weight were measured 28 days following initial DOX administration and the heart weight/body weight ratio was calculated (**C**). Data represent means ± S.D. *; $p<0.05$ vs. control group (Cont), #; $p<0.05$ vs. DOX

ROS generation in the heart, glutathione (GSH) was measured, which is known to be inversely associated with ROS production, as GSH is intrinsically reduced by oxidants (Zhou et al. 2001). The GSH content of the heart was reduced by DOX treatment, an effect attenuated by taurine treatment (Fig. 7.3). This result indicates that taurine suppresses DOX-induced ROS generation.

7.3.3 Dox-Induced Alterations in Cardiac Gene Expression were Inhibited by Taurine Treatment

The downregulation of specific genes of the heart is characteristic of the DOX-induced cardiomyopathy (Ito et al. 1990). To examine the effect of taurine treatment on the expression of cardiac genes of mice injected with DOX, Northern blots of

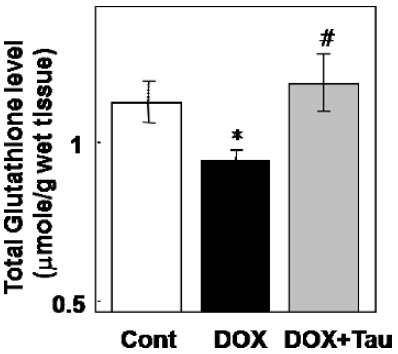

Fig. 7.3 The effect of taurine on the generation of ROS in cardiac tissues after DOX administration. Hearts of mice were removed 2 days after DOX injection and were immediately homogenized. Total glutathione content was then measured. Assays were repeated twice with similar results. Data represent means ± S.D., n=3. *; $p< 0.05$ vs. control group (Cont), #; $p< 0.05$ vs. DOX

αMHC, MLC2v, BNP and SERCA2a were obtained (Fig. 7.4). All of these genes were downregulated 2 days after the administration of DOX, an effect suppressed by taurine treatment. βMHC mRNA was increased in DOX-treated mice, but the change was only partially attenuated by taurine treatment (Data not shown). These results suggest that taurine may ameliorate cardiac remodeling through the suppression of the DOX-induced phenotype.

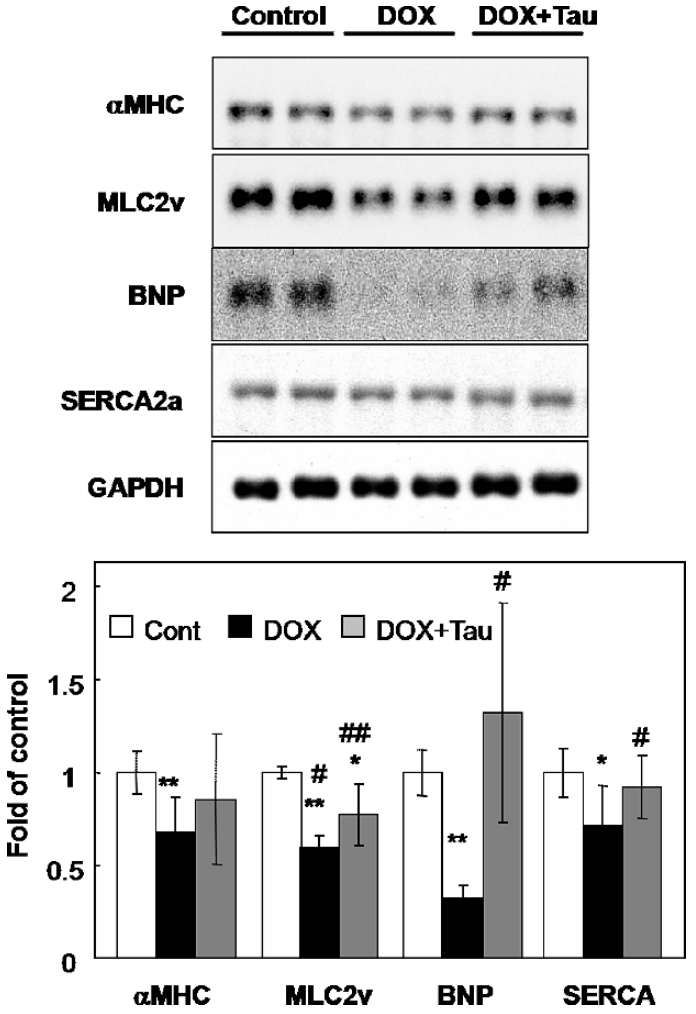

Fig. 7.4 The effects of taurine on cardiac gene expression in DOX-treated mice. Total RNA was prepared from hearts 2 days after DOX injection and then subjected to Northern blot analysis. Representative autoradiograms from 2–3 independent experiments involving 5–12 mice in each group are shown. Data are mean ± S.D. *; $p< 0.05$, **; $p<0.01$ vs. control (Cont), $^{\#}$; $p< 0.05$, $^{\#\#}$; $p< 0.01$ vs. DOX

7.4 Discussion

In the present study, we demonstrated that taurine significantly reduced mortality in both the acute and sub-acute models of DOX cardiotoxicity. Treatment with taurine attenuated the progression of cardiac dysfunction, including the generation of ROS and the alteration in myocardial gene expression. While taurine has been implicated in various functions of the heart, including modulation of ion current, antioxidation and osmoregulation (Huxtable 1992; Schaffer et al. 2000b), the critical cytoprotective role of taurine remains to be clarified. Consistent with the present study, it has been demonstrated that treatment with taurine attenuates the degree of myocardialROS generation caused by various toxins or pathological stimulants (Oudit et al. 2004), an effect unlikely to be related to free radical scavening since taurine chemically reacts with ROS to only a limited extent (Cunningham et al. 1998). Treatment with antioxidant agents, such as probucol (Siveski-Iliskovic et al. 1995) and N-acetylcysteine (Villani et al. 1990), or overexpression of antioxidant proteins, such as Mn-SOD (Yen et al. 1996) and catalase (Kang et al. 1996), attenuate DOX-induced cardiotoxicity. Thus, the antioxidant effect of taurine may contribute to the suppression of DOX-induced cardiac remodeling.

DOX downregulates specific muscle proteins, which contribute to alterations in Z-band structure and to disarray of the thin filaments (Ito et al. 1990). The transcription of these genes is mediated by regulatory factors Nkx2.5 (Lints et al. 1993; Tanaka et al. 1999), MEF2c (Edmondson et al. 1994) and GATA4 (Molkentin et al. 1994, Thuerauf et al. 1994), as well as transcription co-factor p300 (Poizat et al. 2000). Importantly, overexpression of Nkx2.5 or p300 in mice not only blocks DOX-induced inhibition of myocardial gene expression but also increases survival (Kawamura et al. 2004; Toko et al. 2002). Thus, the control of cardiac genes by taurine may be the critical step in the cytoprotective actions of taurine against DOX-induced cardiotoxicity and cardiac remodeling. To test this hypothesis, we measured the levels of the transcriptional factors in our animal model. It was revealed that the levels of neither Nkx2.5 nor MEF2c were influenced by DOX injection *in vivo* (data not shown), although they were reportedly downregulated by DOX in vitro (Poizat et al. 2000). Yet in agreement with a study by Aries et al. (2004), we found that the levels of GATA4 were downregulated by DOX administration. However, taurine treatment did not attenuate the response (data not shown). Thus, although we cannot completely exclude the possibility that alterations in the expression of these transcriptional factors may be partially caused by DOX-induced downregulation of cardiac genes, such as αMHC, MLC2v, BNP and SERCA2a, causality between the downregulation of transcriptional factors and that of cardiac specific genes seems unlikely. However, further studies examining the influence of DOX on myocardial gene regulation, especially in vivo, could be beneficial in understanding the molecular mechanisms underlying the cardioprotective actions of taurine.

Several studies have addressed the relationship between DOX-induced ROS generation and the downregulation of myocardial genes. It has been demonstrated that anti-oxidants, such as N-acetylcysteine and catalase, do not prevent DOX-induced inhibition of gene expression in cultured cardiomyocytes (Torti et al. 1998).

Moreover, while it has been reported that heart-specific overexpression or upregulation of metallothionein in mice protects against DOX-induced cardiac injury through the suppression of ROS generation (Kang et al. 1997; Wang et al. 2001; Oshima et al. 2002), elevated metallothionein did not prevent the downregulation of cardiac genes, including αMHC and MLC2v (our unpublished data). These data suggest that the myocardial genes are downregulated by DOX injection independent of oxidative stress. Collectively, our results indicate that taurine protects cardiac tissue through diverse mechanisms and these pharmacological effects of taurine might explain the remarkable improvement in survival following DOX administration.

7.5 Conclusion

Treatment with taurine confers resistance against DOX-induced cardiotoxicity. The protective effect correlates with the suppression of ROS generation and amelioration of impaired myocardial gene expression. These findings suggest that taurine might be clinically useful in the treatment of the DOX-induced cardiomyopathy. It raises the interesting possibility that overexpression of taurine-linked proteins, such as the taurine transporter, might represent a novel therapeutic approach in reducing the severity of the DOX cardiomyopathy.

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