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TAURINE DEFICIENCY AND DOXORUBICIN: INTERACTION WITH THE CARDIAC SARCOLEMMAL CALCIUM PUMP

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Abstract—An anticancer drug, doxorubicin, and a naturally occurring β-amino acid, taurine, exert opposing actions on myocardial calcium content and lipid peroxidation. Thus, we tested the hypothesis that the two agents may interact to modify cardiac calcium metabolism and indices of lipid peroxidation. Cardiac taurine levels were reduced by half in rats given tap water containing a β-amino transport inhibitor, β-alanine. Taurine deficiency was associated with an increased susceptibility of the heart to doxorubicin-mediated calcium accumulation, a phenomenon commonly associated with doxorubicin cardiotoxicity. Taurine deficiency also predisposed the heart to enhanced formation of malondialdehyde caused by doxorubicin administration. While increases in malondialdehyde levels are often associated with lipid peroxidation, the failure of doxorubicin to cause changes in oxidized glutathione content makes peroxidative mechanisms a less likely explanation for the potentiation of doxorubicin-mediated myocardial calcium accumulation in taurine-deficient rats. A more likely possibility is the interaction between taurine deficiency and doxorubicin to inhibit the sarcolemmal calcium pump. The data also suggest that the interaction between doxorubicin and taurine deficiency does not involve alterations in the pharmacokinetics of doxorubicin or the cardiotoxic metabolite, doxorubicinol. It is concluded that reduction in sarcolemmal calcium pump activity by taurine deficiency may contribute to myocardial calcium accumulation in hearts whose calcium homeostasis has been compromised by doxorubicin.

Taurine is a ubiquitous amino acid present in high concentrations in heart [1]. It has been hypothesized that taurine has an important physiological function in heart because the large intracellular pool is maintained at the expense of ATP in an energy-requiring process [2]. Testing this hypothesis has been difficult because intracellular levels are readily conserved and remain stable [3]. Initially, the role of taurine in cellular function was inferred by administering pharmacological doses of taurine to various experimental preparations. Recently, drugs such as β-alanine have been used to selectively deplete endogenous taurine to assess physiological function. However, much more is known about the pharmacological effects of taurine than is known about its physiological function.

The most important cardiac-related pharmacological effects of taurine include protection against decreases in myocardial function caused by hypoxic injury [4], exposure to cardiotoxic doses of isoproterenol [5], chronic aortic regurgitation [6], and calcium paradox [7, 8]; taurine also protects against cardiac dysfunction that occurs in cardiomyopathic hamsters [9]. Taurine therapy also has been reported to improve cardiac function in patients with congestive heart failure [10]. The mechanism of these multiple cardioprotective effects of taurine appears related to prevention of tissue calcium accumulation and overload [4, 5, 7] presumably by preventing lipid peroxidation [11–13], stabilizing membranes [14, 15] and/or altering calcium transport [16].

Doxorubicin (Adriamycin®) is an antitumor drug that causes cardiotoxicity at therapeutic doses. Although the mechanism of cardiotoxicity is unknown, doxorubicin produces calcium overload and lipid peroxidation in cardiac tissue [17–20]. Because taurine and doxorubicin appear to affect similar aspects of cellular function in an opposing way, we hypothesized that endogenous taurine would modulate the actions of doxorubicin. This hypothesis was tested by comparing the effects of doxorubicin administration on calcium metabolism and redox mechanisms in normal and taurine-deficient rat hearts.

METHODS

Myocardial taurine levels were reduced using the drug-induced model previously described [21]. The model is based on the fact that maintenance of myocardial taurine levels depends on uptake by the myocyte. Thus, the myocardium can be readily depleted by exposure to taurine analogs which interfere with the carrier-mediated taurine transport system [2, 3]. This model was produced in male Wistar rats by feeding them Purina rat chow *ad lib.* and maintaining them for a period of 3–4 weeks on tap water containing 3% β-alanine. This led to a decrease in myocardial taurine levels from 92 ± 7 to 43 ± 3 μmol/g dry wt. The control rats were maintained on tap water throughout the 3- to 4-week period.

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All experiments began with administration of either 5 mg/kg doxorubicin or 250 μL saline into the tail vein of the rat. Animals were killed either 1 or 48 hr postinjection following pentobarbital (50 mg/kg, i.p.) anesthesia and assayed for the desired parameters.

Glutathione levels were assayed according to the method of Adams *et al.* [22]. To prepare hearts for the total glutathione assay, hearts frozen in liquid nitrogen were freeze-dried. A known amount of dried ventricle was homogenized with 3 mL of 1 M perchloric acid containing 2 mM EDTA. After centrifugation the extract was neutralized to pH 7.4 with 2 M KOH. The resulting potassium perchlorate precipitate was removed by centrifugation, and the supernatant fraction was assayed using the glutathione reductase method [22].

Oxidized glutathione was assayed by first homogenizing weighed ventricle with perchloric acid containing 2 mM EDTA and 50 mM *N*-ethylmaleimide (NEM). The homogenate was neutralized to pH 6.2 with 2 M KOH containing 0.3 M *N*-morpholinepropane sulfonic acid. After centrifugation, the homogenate was extracted via a C18 Sep-Pak cartridge (J. T. Baker Research Products) that had been washed previously with methanol and buffer. The column was then washed with 1.0 mL of buffer. An aliquot was removed and assayed by the glutathione reductase method [22]. Reduced glutathione was calculated by subtracting the levels of oxidized glutathione from total glutathione content.

Tissue malondialdehyde (MDA) was determined according to the method described by Ohkawa *et al.* [23]. Hearts rinsed of blood were weighed and homogenized with 3 vol. of 0.9% NaCl. To 0.1 mL of homogenate was added 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% sodium acetate (pH 3.5) and 1.5 mL of 0.8% aqueous solution of thiobarbituric acid. The mixture was made up to 4.0 mL with distilled water and heated at 95° for 60 min. After a period of cooling, 1.0 mL of distilled water and 5.0 mL of *n*-butanol–pyridine mixture (15:1, v/v) were added. The mixture was shaken vigorously and then centrifuged at 4000 rpm for 10 min. The absorbance of the top layer was measured at 532 nm.

Myocardial calcium content was measured according to a modification of the method described by Alto and Dhalla [24]. Hearts were rinsed of blood and then homogenized with 0.9% NaCl. To 2 mL of the homogenate was added 0.2 mL of 5.5 M HCl. The mixture was then held in a boiling water bath for 10 min and centrifuged. The pH of the supernatant fraction was adjusted to 9.7 with KOH. Calcium content was determined spectrophotometrically (Sigma kit).

The plasma concentrations of doxorubicin and the primary alcohol metabolite, doxorubicinol, were measured by HPLC with fluorometric detection. Plasma samples (0.25 to 1.0 mL) were extracted using a modification of the solid phase extraction procedure according to Robert [25]. Briefly, Sep-Pak extraction cartridges were conditioned using 4.0 mL of methanol, 4.0 mL of methanol/water (v/v) and 10 mL of 0.1% ammonium formate buffer (AFB). Plasma samples, diluted with 4 mL of AFB, were passed through the cartridges which then were washed with 3.0 mL of 0.1% ammonium formate buffer followed by 1.0 mL of heptane. Cartridges were aspirated by vacuum for 45 min. Samples, eluted from the Sep-Pak cartridges using 7.0 mL methanol, were dried at 45° using a vacuum evaporator. They were then reconstituted in methanol for analysis by HPLC, and injected on the Waters 4 μm phenyl Radial-Pak column. The HPLC method employed a fluorescence detector with an excitation wavelength of 470 nm and a 550 nm emission wavelength cut-off filter. The mobile phase consisted of 0.1% AFB (pH 4.0) and acetonitrile (v/v) at a flow rate of 3.0 mL/min. The gradient changed at 6 min, progressing to a ratio of 66:34 by 6.5 min and returned to 72:28 at 11.5 min. A standard curve was prepared using human plasma at doxorubicin concentrations between 1.0 and 200 ng/mL. Doxorubicinol concentrations were expressed in doxorubicin equivalents.

Isolated sarcolemmal vesicles were prepared from hearts obtained from non-taurine-deficient and taurine-deficient rats 48 hr after administration of either saline or doxorubicin (5 mg/kg, i.v.) according to the technique of Pitts [26] as previously reported [16]. Enrichment of sarcolemmal preparations was assessed by determining the ratio of vesicle to homogenate activities of Na^+/K^+ ATPase and cytochrome-*c* oxidase as previously described [16]. Isolated sarcolemma from taurine-deficient rats (group 1), doxorubicin-injected rats (group 2) or taurine-deficient rats injected with doxorubicin (group 3) contained the same amount of enrichment as preparations obtained from rats with normal taurine levels not injected with doxorubicin (group 4). In all four groups, enriched sarcolemma did not exhibit oxalate facilitated Ca^{2+} transport, indicating that preparations were devoid of significant amounts of membrane from sarcoplasmic reticulum.

The data were analyzed by analysis of variance using a randomized design. The least significant difference was used to compare the means of treated and control groups. The 0.05 level of probability was used as the criterion of significance.

RESULTS

Doxorubicin cardiotoxicity is associated with calcium overload [17, 18] but the mechanism of calcium overload remains unclear. It is clear, however, that doxorubicin increases calcium influx across sarcolemma [27], causes calcium release from sarcoplasmic reticulum [28] and inhibits calcium uptake by mitochondria [29]. Since taurine stabilizes membranes and prevents calcium overload, it was logical to assume that endogenous taurine might interact with doxorubicin to attenuate cardiac calcium accumulation caused by doxorubicin administration. We tested this idea by determining cardiac calcium concentrations 1 and 48 hr after injection doxorubicin, 5 mg/kg i.v., into male Wistar rats (Table 1) with normal cardiac taurine levels (92 ± 7 μmol/g dry wt) and into male Wistar rats with reduced cardiac taurine levels (43 ± 3 μmol/g dry wt) [21]. Taurine deficiency by itself did not change cardiac calcium levels 48 hr after vehicle (saline) injection

Table 1. Interaction between doxorubicin and taurine deficiency on myocardial calcium content

Group description	Myocardial calcium content (nmol/g wet wt)
Saline-treated, non-taurine-deficient	782 ± 5
Saline-treated, taurine-deficient	754 ± 11
Doxorubicin-treated, non-taurine-deficient (1 hr)	787 ± 3
Doxorubicin-treated, taurine-deficient (1 hr)	790 ± 10
Doxorubicin-treated, non-taurine-deficient (48 hr)	872 ± 9*
Doxorubicin-treated, taurine-deficient (48 hr)	918 ± 9*†

Hearts were removed from taurine-deficient or non-taurine-deficient rats 1 and 48 hr after saline or doxorubicin (5 mg/kg) administration. Calcium content of each sample was determined as described in Methods. The 1- and 48-hr values for the two saline-treated groups were pooled to obtain a single value. Data are means ± SE of 4–6 hearts.

* Significant difference from saline-treated groups ($P < 0.05$).

† Significant difference ($P < 0.05$) between doxorubicin-treated, non-taurine-deficient and doxorubicin-treated, taurine-deficient 48-hr groups.

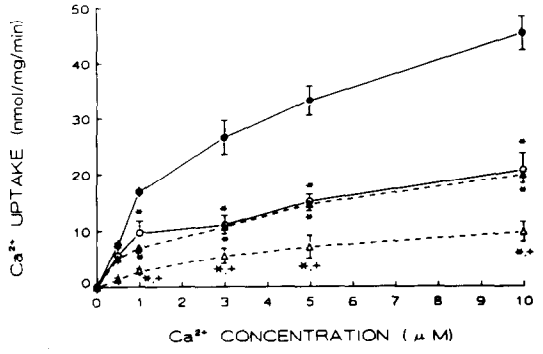

Fig. 1. Effect of doxorubicin and taurine deficiency on sarcolemmal ATP-dependent calcium transport. Cardiac sarcolemma from taurine-deficient (○, △) and non-taurine-deficient (●, ▲) rats administered either 5 mg/kg doxorubicin (▲, △) or saline vehicle (●, ○) were prepared according to the modified method of Pitts [26] as described previously [16]. The rate of ATP-dependent calcium uptake into these isolated membrane vesicles was measured in medium containing free calcium ranging in concentration from 0.05 to 10 μM. Key: (*) significant difference ($P < 0.05$) compared to the non-taurine-deficient, saline-treated group (●——●); (+) difference ($P < 0.05$) between the non-taurine-deficient, doxorubicin-treated (▲---▲) and the taurine-deficient, doxorubicin-treated (△---△) groups. All data are means ± SE of 4–5 different sarcolemmal preparations.

(Table 1). Similarly, doxorubicin did not increase calcium content in normal or taurine-depleted hearts 1 hr after injection. However, 48 hr after injecting doxorubicin into rats with normal cardiac taurine levels, cardiac calcium content significantly increased ($P < 0.05$) from 782 ± 5 to 872 ± 9 nmol calcium/g wet wt (Table 1). Thus, doxorubicin-induced accumulation of cardiac calcium was nearly two times greater in taurine-deficient rats than in rats with normal cardiac taurine levels (90 vs 164 nmol calcium/g wet wt, $P < 0.05$; Table 1).

To provide information on the mechanism underlying calcium accumulation by doxorubicin in taurine-deficient hearts, we evaluated ATP-dependent calcium uptake in isolated sarcolemmal vesicles from rat hearts (Fig. 1). At calcium concentrations from 1 to 10 μM, calcium uptake was reduced in isolated sarcolemmal vesicles obtained from non-taurine-deficient, doxorubicin-treated rats and taurine-deficient rats not exposed to doxorubicin (Fig. 1). When the two treatments were combined (i.e. taurine-deficient rats were injected with doxorubicin), there was an enhanced inhibition of sarcolemmal calcium uptake compared to either treatment alone (Fig. 1). Thus, taurine deficiency interacted with doxorubicin administration to inhibit ATP-dependent Ca^{2+} uptake in isolated cardiac sarcolemmal vesicles.

Doxorubicin can form lipid peroxides and free radicals in *in vitro* systems [30, 31]. Moreover, free radical mediated oxidation reactions have been hypothesized to contribute to calcium overload and cardiotoxicity caused by doxorubicin administration in laboratory animals and patients [32]. Since taurine has been shown to reduce the degree of lipid peroxidation caused by carbon tetrachloride and iron treatment in liver and photoreceptor preparations [11–13], it seemed logical that endogenous taurine might modify the effect of doxorubicin on cardiac MDA and glutathione content, indices of the cellular redox state. In agreement with this concept, it was found that 1 hr after doxorubicin treatment in rats with normal cardiac taurine content, myocardial MDA content was increased 25%; 48 hr after doxorubicin injection MDA content had increased to 56% above saline-treated control values (Table 2). Although taurine deficiency alone did not increase MDA content, it enhanced the doxorubicin-mediated increase in myocardial MDA level ($P < 0.05$, Table 2); in taurine-deficient rats injected with doxorubicin, myocardial MDA levels were 43 and 78% higher than values in non-taurine-deficient, saline-treated rats 1 and 48 hr after injection respectively.

Surprisingly, doxorubicin did not increase cardiac oxidized glutathione (GSSG) levels of normal or taurine-deficient rats either 1 or 48 hr after doxorubicin injection (Table 2). Nevertheless, doxorubicin administration decreased cardiac reduced glutathione content (GSH) 1 hr after injection in both normal and taurine-deficient groups. The doxorubicin-induced decrease in GSH was not altered by

Table 2. Interaction between doxorubicin and taurine deficiency on myocardial glutathione and malondialdehyde content

Group description	Malondialdehyde (nmol/g wet wt)	GSH (μmol/g dry wt)	GSSG (μmol/g dry wt)
Saline-treated, non-taurine-deficient	344 ± 8	6.92 ± 0.41	0.13 ± 0.01
Saline-treated, taurine-deficient	339 ± 13	6.85 ± 0.04	0.14 ± 0.002
Doxorubicin-treated, non-taurine-deficient (1 hr)	430 ± 30*	5.45 ± 0.32*	0.15 ± 0.02
Doxorubicin-treated, taurine-deficient (1 hr)	484 ± 6*	5.50 ± 0.07*	0.16 ± 0.005
Doxorubicin-treated, non-taurine-deficient (48 hr)	536 ± 40*	6.95 ± 0.20	0.16 ± 0.005
Doxorubicin-treated, taurine-deficient (48 hr)	603 ± 8*†	6.58 ± 0.08	0.15 ± 0.006

Hearts from non-taurine-deficient and taurine-deficient rats were removed from the animal 1 or 48 hr after administration of either saline or 5 mg/kg doxorubicin. The 1- and 48-hr values for saline-injected rats were pooled to obtain a single value. Data are means ± SE of 4–5 samples.

* Significant difference from non-taurine-deficient, saline-treated group ($P < 0.05$).

† Significant difference between doxorubicin-treated, non-taurine-deficient and doxorubicin-treated, taurine-deficient groups ($P < 0.05$).

Table 3. Plasma levels of doxorubicin and the primary metabolite, doxorubicinol, at various times after doxorubicin administration

Group description	Plasma doxorubicin (ng/ml) 0.5 hr	1 hr	24 hr	48 hr
Non-taurine-deficient	46 ± 7	38 ± 4	2.06 ± 0.59	1.22 ± 0.75
Taurine-deficient	49 ± 12	43 ± 4	1.98 ± 0.54	3.20 ± 0.54*

Group description	Plasma doxorubicinol (ng/ml) 1 hr	24 hr	48 hr
Non-taurine-deficient	2.3 ± 0.3	ND†	ND
Taurine-deficient	2.2 ± 0.3	ND	ND

Plasma samples were obtained from non-taurine-deficient and taurine-deficient rats or 48 hr after administration of 5 mg/kg doxorubicin. Values are means ± SE of 5 unpaired observations per time period.

* Significant difference from non-taurine-deficient group ($P < 0.05$).

† Not detectable.

taurine deficiency (Table 2) and GSH levels rebounded back to saline-treated control values by 48 hr. Thus, cardiac glutathione content was affected minimally by doxorubicin administration and taurine deficiency did not enhance the doxorubicin effects.

We also considered the possibility that taurine deficiency might alter the pharmacokinetics of doxorubicin or the cardiotoxic metabolite doxorubicinol [33–36], thereby enhancing the doxorubicin effect on cardiac calcium metabolism and MDA formation. This was tested by assessing plasma levels of doxorubicin and doxorubicinol at various times after injecting doxorubicin (5 mg/kg, i.v.) into normal and taurine-deficient rats. Plasma concentrations of doxorubicin in the two groups were not different 0.5, 1 and 24 hr after injecting doxorubicin (Table 3). Forty-eight hours after doxorubicin injection, there was a statistically significant ($P < 0.05$) increase in plasma doxorubicin concentration in the taurine-deficient rats but concentration differences between the two groups were so small that it is unlikely to be an important effect. Plasma levels of doxorubicinol (the primary circulating metabolite of doxorubicin) were not different between the two groups 1 hr after injecting doxorubicin and were not detectable 24 or 48 hr after doxorubicin administration.

DISCUSSION

The hypothesis tested in this study was that taurine deficiency would enhance doxorubicin-mediated calcium accumulation, lipid peroxidation and membrane dysfunction in rat hearts. This hypothesis was based on pharmacological studies which showed that taurine administration can protect against cardiac dysfunction caused by myocardial ischemia [4], catecholamine toxicity [5] and calcium paradox [7, 8]. It seemed reasonable that these beneficial effects of taurine might relate to its ability to prevent cardiac calcium accumulation [4, 5, 7] and lipid peroxidation [11–13] and cause membrane stabilization [14, 15], effects opposite those of doxorubicin.

It is generally accepted that doxorubicin causes an intracellular calcium overload that produces myocyte injury [17, 18]. In agreement with these studies, we found that doxorubicin significantly ($P < 0.05$) increased total cardiac calcium content by 11% 48 hr after injection into non-taurine-deficient rats (Table

1). By comparison, doxorubicin administration increased total cardiac calcium content by 22% 48 hr after injection in taurine-deficient rats (Table 1). Since taurine deficiency alone did not alter myocardial calcium content, doxorubicin administration caused a 2-fold greater accumulation of calcium ($P < 0.05$) in taurine-deficient rats compared to those with normal taurine content. These observations indicate that endogenous taurine attenuates doxorubicin-mediated cardiac calcium accumulation, an effect often associated with doxorubicin cardiotoxicity.

The mechanism of doxorubicin-induced calcium accumulation in heart is still unclear. Concentrations as low as 5×10^{-8} M doxorubicin enhance calcium uptake in cultured rat myocytes, probably by increasing slow calcium channel influx via adenylate cyclase stimulation [27, 37]. Doxorubicin (25 μM) also promotes the release of calcium from sarcoplasmic reticular terminal cisternae, an action that could lead to increased cytoplasmic calcium accumulation [28]. The primary metabolite of doxorubicin (i.e. doxorubicinol) has been shown recently to inhibit calcium uptake in isolated sarcoplasmic reticular vesicles which may also increase sarcoplasmic calcium if this effect occurs in intact myocytes [33–36]. The data from the current study show that doxorubicin administration also inhibited ($P < 0.05$) calcium uptake by sarcolemmal vesicles (Fig. 1). However, combined taurine deficiency with doxorubicin administration caused further inhibition of calcium uptake by sarcolemma vesicles than occurred with either treatment alone (Fig. 1). Since the sarcolemmal Ca^{2+}-ATPase pumps calcium out of the cell, one explanation for enhanced cardiac calcium accumulation by doxorubicin in taurine-deficient rats is potentiation of doxorubicin-mediated decreases in sarcolemmal calcium pump activity.

Among the various explanations of doxorubicin cardiotoxicity, the free radical hypothesis has received the most attention. Clearly, doxorubicin can form lipid peroxides and free radicals *in vitro* [30, 31]. Whether this reaction occurs *in vivo* and mediates doxorubicin-induced cardiotoxicity is less clear. Some investigators find that doxorubicin causes MDA formation, GSH depletion and GSSG formation [20, 38, 39]. Other studies do not support a free radical hypothesis for doxorubicin-induced cardiotoxicity [40, 41]. For example, one study showed that large single doses of doxorubicin failed to produce lipid peroxidation as assessed by MDA [41]. Similarly, multiple doses of doxorubicin did not change GSSG content and actually increased GSH levels by 30%. Porta *et al.* [40] reported that doxorubicin (15 mg/kg) increases plasma creatine kinase activity and produces mitochondrial swelling and myofilament fragmentation in rat hearts without decreasing the activity of catalase or glutathione peroxidase or causing lipid peroxidation in the heart as assessed by diene conjugates or MDA. In the current study, we found that doxorubicin administration significantly increased cardiac MDA formation within 1 hr after injection and taurine deficiency enhanced the doxorubicin-induced increase in MDA (taurine deficiency alone did not promote MDA formation) (Table 2).

While the MDA results are consistent with the notion that the interaction between doxorubicin and taurine deficiency involves lipid peroxidation and oxidative stress, this notion is not supported by three other findings obtained from the current study. First, GSSG was not increased by doxorubicin treatment in hearts with normal or reduced taurine content 1 or 48 hr after doxorubicin injection. Second, doxorubicin caused a small but significant ($P < 0.05$) decrease in GSH 1 hr after injection but the GSH value returned to baseline by 48 hr. Third, taurine deficiency did not potentiate the effects of doxorubicin on GSH or GSSG.

Assessing tissue oxidative stress is a difficult task because species causing oxidative stress, such as oxygen free radicals or lipid peroxides, are short lived and are difficult to measure directly. One technique often used to assess oxidative stress is to measure MDA, a more stable and longer lived degradative product of lipid peroxides. Another way is to measure cellular GSSG levels, which become elevated when a large peroxide stress generates increased GSSG from GSH stores. However, there are limitations in interpreting the results obtained from these assays. For example, cellular GSSG can readily diffuse across plasma membranes and cellular levels may not always reflect cellular formation. Thus, in the present study cardiac GSSG content may not have been a sensitive indicator of oxidative stress. Additionally, MDA can be formed from sources other than lipid peroxides. For example, the prostaglandin endoperoxide PGH_2 is an unstable prostaglandin precursor and can be nonenzymatically converted to a 17 carbon hydroxy acid and MDA [42]. Since the rate-limiting step in prostaglandin synthesis is arachidonic acid availability, which results from activation of phospholipase A_2 by calcium [42], increased MDA formation in the current study may have resulted not from increased lipid peroxidation but from increased PGH_2 production caused by increased calcium accumulation. Thus, it is not altogether surprising that, in the present study, the effects of doxorubicin and taurine deficiency on MDA and glutathione gave disparate results. Rats deficient in taurine and injected with doxorubicin exhibited elevated MDA levels but GSSG levels did not change. As a result, it is difficult to interpret the role of lipid peroxidation in the interaction of taurine deficiency with doxorubicin to cause cardiac calcium accumulation.

Increased cardiac calcium accumulation associated with doxorubicin administration in taurine-deficient rats may result from effects on calcium-transporting mechanisms. Doxorubicin inhibits Na^+/Ca^{2+} exchange [43] and causes calcium release from sarcoplasmic reticulum [28], and the primary metabolite, doxorubicinol, inhibits calcium uptake into sarcoplasmic reticulum [33–36]. Such a compromise in calcium homeostasis caused by doxorubicin may make myocytes susceptible to further reductions in the activity of transporters that control intracellular calcium levels. This idea is consistent with the results presented in the current study. We found that doxorubicin administration caused cardiac calcium accumulation and inhibited the sarcolemmal calcium

pump, which pumps intracellular Ca^{2+} to extracellular sites. Although the pump is not usually considered a major factor in controlling cardiac calcium content, it could be important when other mechanisms of calcium homeostasis are compromised. For example, in the current study taurine deficiency inhibited calcium uptake into sarcolemmal vesicles but did not cause cardiac calcium accumulation. However, when taurine-deficient rats were treated with doxorubicin, there was an additional inhibition of calcium uptake into sarcolemmal vesicles and an enhancement of accumulation of cardiac calcium. These observations are consistent with the idea that modulation of calcium transporters by taurine may modify the effects of doxorubicin on calcium homeostasis.

The interaction between taurine deficiency and doxorubicin on cardiac calcium, MDA and sarcolemmal calcium uptake could result from altered pharmacokinetics of doxorubicin or doxorubicinol, the primary metabolite of doxorubicin whose cardiotoxicity is at least fifty times more potent than doxorubucin [33–36]. However, the plasma concentrations of doxorubicin and doxorubicinol were not different between the taurine-deficient and non-taurine-deficient rats, with the exception that plasma doxorubicin was increased significantly in the taurine-deficient rats 48 hr after injection. This difference at 48 hr is unlikely to be of biological importance because the plasma concentrations were low and the difference would not contribute significantly to area under the curve calculations. Thus, the interactions between taurine deficiency and doxorubicin do not appear to be due to altered pharmacokinetics of doxorubicin or the metabolite doxorubicinol.

In conclusion, these data suggest that the intracellular pool of taurine may play an important function in the heart to modulate, not only the toxicity of doxorubicin, but perhaps other interventions that cause myocardial calcium perturbations.

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