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ORIGINAL ARTICLE

Glutamine regulation of doxorubicin accumulation in hearts versus tumors in experimental rats

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Abstract
Purpose Doxorubicin (DOX), an effective antineoplastic agent is known for its cardiotoxicity attributed mainly to free radical formation. Preliminary data indicated that oral glutamine (GLN) reduced cardiac oxidative damages in experimental rats treated with DOX. This study investigated the effect of GLN on DOX accumulation in tumors and normal tissues, troponin plasma concentration and functional alternations associated with DOX-induced myocardial damage.
Methods Female Fisher344 rats ($n = 40$) with implanted MatBIII mammary tumors were randomized to receive oral GLN (1 g/kg/day) ($n = 20$) or to serve as controls ($n = 20$) and were treated with a single i.p. injection of 12 mg/kg DOX. Ten normal rats ($n = 10$) without treatment served as naive controls. Cardiac physiologic alterations resulting from DOX treatment in GLN-supplemented and control rats were assessed by micro-ultrasound imaging at 3 and 10 days after DOX injection. Ten rats from each GLN-supplemented and control groups were killed at 3 and 10 days after DOX administration. At killing, hearts, livers, spleens, kidneys, tumors and sera were examined for DOX concentration by measuring DOX natural fluorescence. Hearts were examined for Von Willebrand factor (vWF) expression using immunohistochemistry.
Results Glutamine supplementation resulted in a significant reduction of DOX concentration in the normal tissues, without a significant effect on tumor DOX concentration. GLN-supplemented rats had lower plasma cTnI levels and lower cardiac levels of vWF. DOX-induced alterations in the echocardiographic parameters were significantly reduced in the GLN-supplemented rats.
Conclusions These data indicate that GLN supplement is able to reduce DOX-induced cardiac damage and thus to enhance DOX therapeutic effectiveness.

Keywords Glutamine · Doxorubicin · Cardiotoxicity · Plasma cardiac troponin I · Thrombocytopenia · Von Willebrand factor

Introduction

Doxorubicin (DOX) is an anthracycline antibiotic widely used in clinical practice to treat solid tumors and hematologic malignancies in adults and children. Administration of anthracyclines, however, may induce cardiomyopathy and congestive heart failure, which is usually refractory to common medications [1]. For example, adverse cardiac events occurring in breast cancer patients associated with various DOX-containing chemotherapy regimens have been estimated to be between 10 and 21% [2]. The etiology of DOX-induced cardiotoxicity is not completely understood and the suggested mechanisms include free radical formation with oxidative damage of cardiomyocytes; direct

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DNA damage and/or interference with DNA repair [3]. The antitumor activity of DOX is likely to be distinct from the mechanism of its cardiotoxicity. DOX antitumor activity is thought to be due to DNA damage and inhibition of cell replication of highly proliferative tumors [4]. Cardiomyocytes, however, are minimally replicative cells that should be resistant to such antimitotic mechanisms. Free radical generation and oxidative stress significantly contributes to the cardiotoxic effects of DOX [5–7]. Studies have shown that the oxidative stress of the cardiomyocytes occurring rapidly after DOX treatment resulted in harmful modifications to proteins [8], lipids [9] and DNA [10]. Accordingly, the use of antioxidants in combination with chemotherapy prolonged the survival time of patients compared to the expected outcome without the antioxidant supplements [11, 12].

Pharmacokinetic studies have shown that the rapid tissue uptake of DOX within 5 min after administration was followed by a terminal half-life of 20–48 h (http://www.pfizer.com/files/products/uspi_adriamycin.pdf). DOX is metabolized in the liver where the quinone nucleus of DOX undergoes redox cycling. Increased production of superoxide, OH–, conjugated dianes, malonaldehyde and enzymatic activity changes of glutathione peroxidase and catalase following anthracycline treatment has been observed in several biological systems [13].

Glutamine (GLN) is the most abundant amino acid in the human body constituting more than 60% of the total amino acid pool [14]. GLN serves multiple metabolic functions that account for its extremely high turnover rate. The significance of GLN in maintaining the body metabolic homeostasis becomes evident during periods of stress, when it becomes a conditionally essential amino acid [15]. The excessive needs of GLN during catabolic states such as an advanced malignant disease are supplied from the muscle stores and this might lead to a massive depletion of skeletal muscle GLN [16]. GLN starvation results in energy depletion, decreased immune defense, increased apoptosis and reduced levels of GSH [17]. The safety of enteral and parenteral GLN, and its benefits on improving amino acid metabolism, immune function, and outcome in normal volunteers and patients with catabolic diseases has been established in numerous studies [18, 19], including at least 18 clinical trials (reviewed in [20]). As one of the precursors for the synthesis of the major natural antioxidant of the body glutathione (GSH), GLN plays an important role in maintaining the cellular redox state, especially during periods of stress [21, 22]. Our previous studies showed that dietary GLN maintained normal cardiac GSH levels in animals given DOX and prevented cardiac lipid peroxidation [23]. This study aimed to determine the effect of GLN on DOX accumulation in tumors and normal tissues, troponin plasma concentration and functional alternations associated with DOX-induced myocardial damage.

Materials and methods

Animals, cell culture and treatment

A total of 50 ($n = 50$) 3-month-old female Fisher344 rats (NCI, Frederick, MD) weighing 130–150 g were used. All studies were approved by the Animal Care and Use Committee at the Central Arkansas Veterans Healthcare System. The rats were maintained two/cage in standard cages in the animal care facility and were subjected to a 12-h dark/light cycle. One week before tumor cell implantation, the rats were randomized to receive either 5% GLN-enriched diet (TD.07199, Harlan-Teklad, Madison, WI) ($n = 20$) or regular chow ($n = 20$). In order to supplement all rats in the GLN-group with equal amount of GLN, we measured the amount of consumed food daily and the GLN intake was calculated. The rats with a reduced food intake were gavaged daily with fresh 50% GLN suspension calculated to deliver 1 g/kg/day GLN. The controls were gavaged with the same amount of water. Ten untreated rats ($n = 10$) served as naive controls.

To closely model the clinical scenario, we used only rats bearing implanted mammary carcinoma. Rat mammary adenocarcinoma cell line MatBIII (ATCC, Manassas, VA) were grown on plastic culture plates in McCoy's 5A modified medium supplemented with 10% fetal bovine serum (FBS) at 37°C and 5% CO_2. Suspension of 1×10^6 cells in 0.2 ml saline was injected into the mammary fat pad of rats anesthetized with 2% isoflurane/oxygen.

Doxorubicin hydrochloride (Sigma Chemical Co., St. Louis, MO) diluted in saline was administered intraperitoneally (i.p) into the rats after the tumors reached 0.3–0.5 cm with a single injection of 12 mg/kg DOX in 200 µl saline. This dose of DOX is approximately equal to a dose of 60 mg/m^2 (http://www.fda.gov/cder/cancer/animalframe.htm). Rats were monitored daily for tumor growth and body weight (BW). Ten rats ($n = 10$) from each GLN-supplemented and control groups were killed 3 and 10 days after DOX administration. The naive controls were killed with the later group of animals. At killing, tumor volumes and weights were recorded. Tumor volumes were calculated according to the formula: $V = (ab^2)/2$, where a is the longest diameter and b is the shortest diameter of the tumor [24]. Blood was withdrawn by a heart puncture with a syringe containing heparin. Samples from the left ventricle (LV) were fixed in neutral buffered formaldehyde (4% wt/vol) or snap-frozen in liquid nitrogen and stored at −80°C until used. Samples from the tumors, livers, spleens and

kidneys were also snap-frozen in liquid nitrogen and stored at −80°C until used.

Quantification of DOX in tissue samples

The fluorescent properties of DOX were used to quantify DOX concentration in tissue samples. DOX was extracted from tumors, hearts, livers, spleens, kidneys and sera following the method for quantifying total DOX fluorescence of Bachur et al. [25]. Briefly, frozen under CO_2 tissue samples were powdered and extracted over ice with 20 volumes of 0.3 N HC1 in 50% ethanol. After centrifugation at 20,000*g* at 4°C for 20 min, the supernatant was collected. DOX fluorescence was measured in 100 µl with a SpectraMax5 plate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 585 nm using an activation wave length of 470 nm. Plasma DOX concentrations were determined in 50 µl diluted with 100 µl acid alcohol. The fluorescent levels were compared to a series of DOX standards diluted in acid alcohol and expressed as µg/mg protein or µg/ml blood. Protein content of the tissue samples was measured using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA.

Plasma troponin measurement

Plasma concentrations of cardiac troponin I (cTnI) were determined using "High sensitivity rat cardiac troponinI-ELISA" (Life Diagnostics, Inc., West Chester, PA). The assay was performed in eight rats/group. The results were expressed as ng/ml.

Histopathology and immunohistochemistry

Tissue samples from the LV were processed and embedded in paraffin and sectioned at 4 µm. Sections were stained with hematoxylin and eosin for routine histological microscopic analysis. For immunohistochemistry, the slides were stained with anti-Von Willebrand factor (vWF) antibody (Dako, Carpinteria, CA). Briefly, the sections were pretreated with Dako EDTA antigen retrieval (pH 9.0) in a Dako tissue decloaker (Dako, Carpenteria, CA) according to the manufacturer's directions for 20 min. Sections were then incubated in Dako Peroxidase block (Dako Carpenteria, CA) for 10 min at room temperature, and after washing with Tris-buffered saline 0.1% Tween 20 (TBST) were incubated with the primary antibody as recommended by the manufacturer. Following extensive washing the slides were treated with biotinylated secondary antibody for 5 min, rinsed, treated with streptavidin-peroxidase and DAB, and counterstained with hematoxylin before mounting. Primary antibody was omitted as a negative control. Stained slides were scanned to digital file and analyzed using proprietary automated image analysis algorithms (Aperio Scanscope T2, Aperio, Vista, CA).

Echocardiographic assessment of cardiac physiological alterations

Two-dimensional B-mode and anatomical M-mode imaging were performed using ultrasound imaging system Vevo 770 High-Resolution In Vivo Imaging System (VisualSonics, Toronto, ON, Canada) designed for small animals. M-mode images were acquired and used for calculation of LV function using calculation procedures provided by the manufacturer. Ten rats of each GLN-supplemented and control groups treated with 12 mg/kg DOX were examined before DOX administration, 3, and 10 days after DOX administration. The rats were anesthetized with 2% isoflurane/oxygen by a facemask during the whole procedure. The procedure was as follows: the rat was laid supine on the platform with all legs taped to ECG electrodes for heart rate monitoring. All hair was removed from the chest using a chemical hair remover (Nair; Carter-Horner, Mississauga, ON). With the scanhead 716 and on B-mode the short-axis imaging was taken to mainly visualize LV. M-mode was used to obtain anatomically correct LV measurements, including LV posterior wall (LVPW), LV ejection fraction (LVEF), and LV fractional shortening (LVFS). Data analysis was performed offline with the use of a customized version of Vevo 770 Analytic Software.

Statistical analysis

Differences between means were considered significant at $P < 0.05$, using non-paired two-tailed *t* tests of variance (StatView II software) analysis. All data were expressed as mean ± SE.

Results

Quantification of DOX in tissue samples

The fluorescent properties of DOX were used to quantify DOX concentration in tumors and normal tissues. We examined the effect of oral GLN on DOX accumulation at 3 and 10 days after DOX injection. The time-point of 3 days was selected based on data from pharmacokinetic studies showing that the average terminal half-life of DOX and its metabolites elimination was between 12 and 48 h [26, 27]. The results presented in Table 1 showed that 3 days after DOX administration the rats from GLN-supplemented had a significantly lower DOX levels in the normal tissues except for the liver (Fig. 1a). At the same time there was no significant difference between DOX concentration of the

Table 1 Effect of GLN supplementation on DOX accumulation in the tissues of tumor-bearing rats 3 days after DOX administration

	Naive controls	GLN supplemented ($n = 10$)	Controls ($n = 10$)
Tumors		0.073 ± 0.07	0.070 ± 0.09
Hearts	0.0203 ± 0.005	0.221 ± 0.04	0.277 ± 0.07
Livers	0.023 ± 0.008	0.175 ± 0.06	0.144 ± 0.05
Spleens	0.069 ± 0.009	0.411 ± 0.07	0.470 ± 0.009
Kidneys	0.070 ± 0.005	0.359 ± 0.08	0.404 ± 0.004
Serum	0.234 ± 0.04	0.239 ± 0.02	0.288 ± 0.002

Data are presented as mean ± SE. Data for each tissue were compared with the naive controls (except for tumors). $P < 0.05$ for all except for the livers of GLN-supplemented rats versus controls and tumors

tumors of GLN- and control rats (Fig. 1a). DOX concentration was also lower in the plasma of GLN-supplemented rats (Fig. 1b). Low fluorescence was detected 10 days after DOX administration in the livers and tumors but the differences versus the controls were not significant ($P > 0.05$).

Plasma troponin

Cardiac troponins are regulatory proteins of the thin actin filaments of the cardiac muscle. Troponin T and troponin I are highly sensitive and specific markers of myocardial injury [28]. Cardiac troponin I (cTnI) degradation has been noted in the stunned myocardium of rodents after ischemia and reperfusion and is a proposed mechanism for the decreased LV contractility in post-ischemic hearts [29]. In this study, we have used cTnI ELISA that recognized an epitope on rat cTnI in rat plasma. The results showed that 3 days after DOX administration the average cTnI concentration in the plasma of GLN-supplemented rats was 45% less than cTnI concentration in the control rats (mean value in ng/ml ± SE, 0.57 ± 0.035 in GLN group vs. 1.12 ± 0.045 in controls vs. 0.21 ± 0.01 in naive controls, $P < 0.05$ for naive controls vs. both GLN and controls and $P = 0.053$ for GLN vs. control) (Fig. 2a). cTnI levels were still elevated 10 days after DOX administration in both GLN-supplemented and control rats in comparison with the naive controls (mean value in ng/ml ± SE, 0.56 ± 0.03 in GLN group vs. 0.75 ± 0.05 in controls vs. 0.26 ± 0.01 in naive controls, $P < 0.05$ for all) but remained lower in the GLN group (Fig. 2b).

Histopathology and immunohistochemistry

The results from the histopathological examination showed a presence of necrotic lesions in both DOX-treated groups on average of 15% of the rats without a significant difference between the groups. However, the presence of edema and hemorrhage was found in all of the animals in the control DOX-treated group versus only 50% of the GLN-supplemented rats treated with DOX (not shown).

Elevated plasma or tissue levels of vWF have been established in thromboembolic cardiovascular events [30, 31]. We determined the protein expression of vWF in the LV of tumor-bearing rats 3 days after DOX administration using immunohistochemistry. The results showed lower average vWF intensity of the staining in the LV of

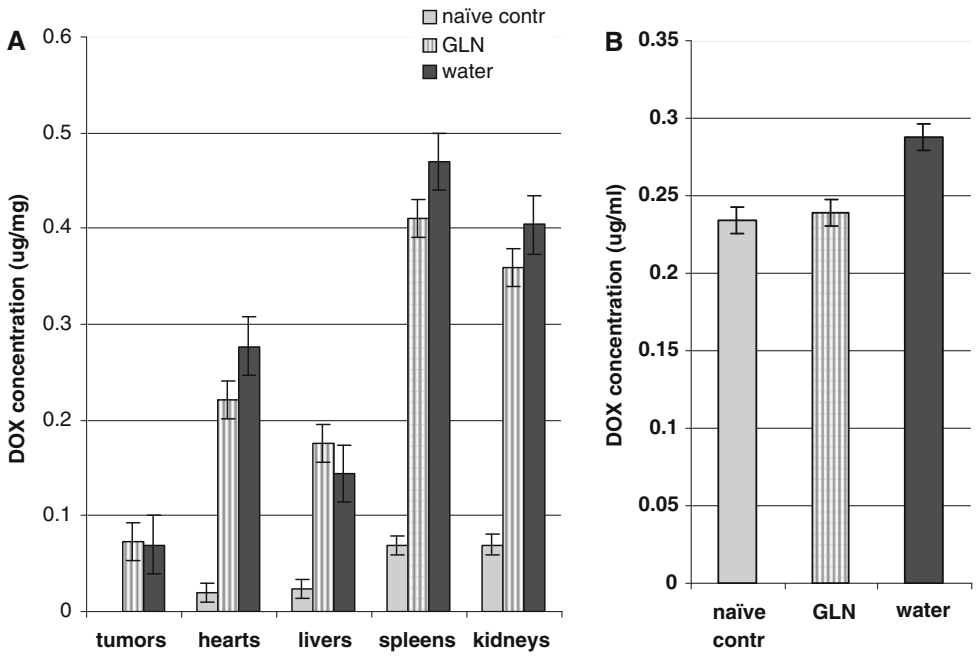

Fig. 1 Effect of oral GLN on the average DOX concentrations of tumors, normal tissues (**a**) and plasma (**b**) 3 days after a single DOX administration of 12 mg/kg. DOX concentration was calculated per mg protein or ml (blood) based on its natural fluorescence

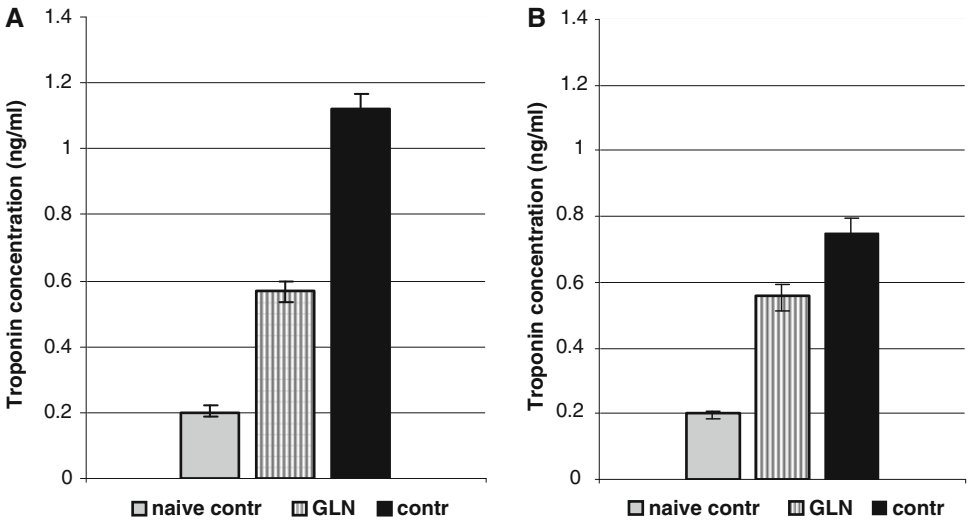

Fig. 2 Average plasma cTnI concentration 3 days (**a**) ($P < 0.05$ for naive control vs. both GLN-supplemented and controls; $P = 0.053$ for GLN vs. controls) and 10 days (**b**) after DOX administration ($P < 0.05$ for all) ($n = 8$ in GLN group and $n = 7$ in control group at 10 days time-point)

GLN-supplemented rats (average score 3.58) versus that of the controls (average score 38.6) (representative images in Fig. 3).

Echocardiographic assessment of cardiac physiological alterations

Heart rate was similar among the groups and was not affected significantly by tumor presence or by DOX treatment (260–25 b.p.m.). At 3 days after DOX administration, the average LVPW end-diastolic thickness of the control rats increased from 1.6 to 2.57 mm, while in the GLN group the average LVPWd increased from 1.6 to 1.9 mm (Table 2). At 10 days after DOX administration, the average LVPWd of the control rats was 1.82 mm, while in the GLN-supplemented rats LVPWd was similar to the baseline. Similar effect of GLN on LVEF was detected at 10 days after DOX administration. At 3 days after DOX administration the average reduction of LVEF in GLN-supplemented group was 4% in comparison with the baseline (before DOX injection), while in the controls the reduction was 12%. At 10 days after DOX administration, the average LVEF of the controls was lower by 10% in comparison with the baseline, while in the GLN-supplemented rats it was only 2%.

Discussion

The present study aimed to examine the effect of GLN on DOX-induced cardiotoxicity in a tumor-bearing host. To closely monitor the clinical scenario, we used only rats with implanted breast tumors. DOX-induced cardiotoxicity of rats recapitulated the physiological and histological findings in patients, making this model particularly suitable for experimental studies (discussed in [32]). The results showed that GLN supplement differentially affected DOX accumulation in tumors and normal tissues by reducing DOX levels in normal tissues without affecting it into the tumors. The plasma concentration of cTnI and cardiac protein expression of vWF were significantly lower in the GLN-supplemented rats. Our results also consistently

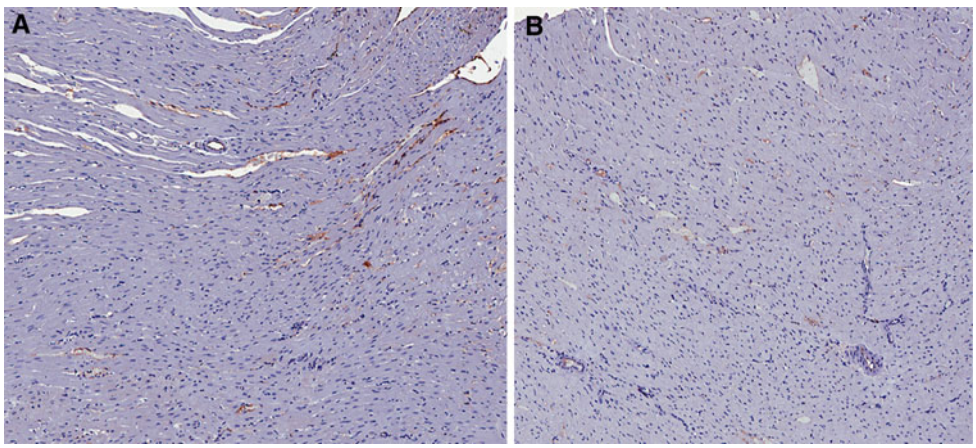

Fig. 3 Representative pictures of high (**a**) and low (**b**) intensity of vWF staining. The intensity of the staining was determined using proprietary automated image analysis algorithms (Aperio Scanscope T2, Aperio, Vista, CA). No staining was observed in the negative control (not shown)

Table 2 Effect of GLN on left ventricular parameters obtained from M-mode echocardiograms of rats treated with a single dose of 12 mg/kg DOX

	GLN supplementation			Controls		
	Before DOX ($n = 10$)	3 Days after DOX ($n = 10$)	10 Days after DOX ($n = 8$)	Before DOX ($n = 10$)	3 Days after DOX ($n = 10$)	10 Days after DOX ($n = 7$)
LVPWd (mm)	1.618 ± 0.19	2.0 ± 0.2	1.601 ± 0.11	1.696 ± 0.11	2.576 ± 0.77	1.82 ± 0.14
LVEF (%)	85.5 ± 12.1	80.56 ± 13.8	83.65 ± 18.9	85.3 ± 13.8	75.2 ± 20.3	76.06 ± 16.8
LVFS (%)	55.5 ± 10.1	50.3 ± 12.1	55.2 ± 13.5	56.9 ± 11.6	46.3 ± 9.2	48.7 ± 12.6

Values are mean ± SE. $P < 0.05$, LVPWd before DOX versus 3 and 10 days after DOX administration for both GLN-supplemented and control rats; $P < 0.05$, LVEF before DOX versus 3 and 10 days after DOX for both GLN-supplemented and control rats

showed that DOX-induced alterations of the cardiac electrophysiological parameters, including LVPW, EF and FS were significantly reduced by GLN supplementation. These findings were associated with reduced tumor volumes in GLN group in comparison with the controls.

Dose-dependent cardiotoxicity of DOX remains a major limitation in the standard and high dose chemotherapy, strongly impacting the quality of life and overall survival of cancer patients [33]. DOX cardiotoxicity in patients has been subdivided into acute and chronic effects, depending on their occurrence following administration of the drug. The most frequently encountered and investigated form of cardiac toxicity is a cumulative, dose-related myocardial dysfunction occurring 1–6 months after chemotherapy [34, 35]. However, in human heart samples, contraction and ring formation of the nuclei suggestive of apoptosis have been documented as early as 24 h after DOX injection [36].

Several methods to detect subclinical myocardial damage during and after the chemotherapy have been used in the clinical practice. The commonly used approaches include echocardiographic assessment of LVEF and measurement of plasma concentration of troponins [33]. The rat is frequently used as an experimental animal in cardiovascular research, although the values of the echocardiographic parameters of normal rats obtained with different rat strains, age and gender [37–39]. This study assessed the effect of GLN supplement on the physiological characteristics of the hearts of 3-month-old female Fisher344 rats with implanted breast tumors and treated with a single dose of DOX. The effect of DOX on echocardiographic parameters we have recorded is similar to those reported by others [40] in rats. A decline of LVEF by more than 10% was suggested as a criterion for suspending the treatment [41]. In the present study, the echocardiographic examination showed 10% reduction of LVEF in tumor-bearing rats treated with DOX. This effect of DOX was significantly reduced by dietary GLN supplement resulting in only 2% reduction of LVEF.

The clinical application of cardiac troponin as a cardiotoxicity biomarker was analyzed in several clinical studies involving more than 1,500 patients with cancer [33]. The results showed that in approximately one-third of patients treated with potentially cardiotoxic chemotherapy, the increase in troponin concentrations in the blood underlines the occurrence of irreversible myocardial cell injury. In the present study, the plasma levels of cTnI in GLN-supplemented rats was more than 40% less at 3 days after DOX administration and 25% less at 10 days after DOX administration in comparison with the rats that did not receive GLN.

VWF, a plasma glycoprotein synthesized by endothelial cells, binds to subendothelial collagen and platelets to induce platelet aggregation. It mediates platelet adhesion to the subendothelium at sites of vascular injury [42]. Various lines of evidence indicate that VWF is not only a marker but also actually an important effector in the pathogenesis of myocardial infarction [32]. Increased expression of vWF was detected in the endocardium of patients with a variety of underlying heart diseases [33, 43] as well as in animal studies [44]. In our present study, the level of vWF in the hearts of rats treated with DOX was much higher than in the GLN-supplemented rats. These data support the conclusion that vascular damage is reduced by GLN supplementation after DOX treatment, and suggest that DOX cardiotoxicity may be mediated, in part, through the prothrombotic effects of vWF.

Although DOX-induced cardiotoxicity has been suggested to be mediated through different mechanisms, the generation of free radicals is the main widely accepted mechanism [3]. The oxidative stress of cardiomyocytes appeared to play an important role in the early injury of cardiomyocytes by DOX [45]. The heart is particularly susceptible to DOX toxicity due to its low antioxidant enzyme activities, including catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) [3]. As such, the heart is more vulnerable to DOX-generated reactive oxygen species (ROS) insults than other organs in the body [46, 47]. For example, the level of DOX-induced oxidative stress was found to be up to ten times greater in the heart than in the liver, kidney and spleen [48]. A number of reports using DOX acute toxicity have shown a marked cardiac protection by antioxidant supplements. Antioxidants such as GSH

[49], vitamin C [50], coenzyme Q [51], *N*-acetylcysteine [52] were able to reduce DOX cardiotoxicity through prevention of the early oxidative effects of DOX. Dexrazoxane, a derivative of EDTA that acts as an intracellular chelating agent has been used to protect against anthracycline-associated cardiotoxicity in patients with breast cancer [53]. The proposed mechanism of dexrazoxane-mediation cardioprotection is through the chelation of intracellular iron, which may decrease doxorubicin-induced free radical generation. However, side effects include pain on injection and augmented myelosuppression [54]. Studies on rats [55] showed that dexrazoxane increased hepatic protein synthesis, which may represent its putative cytotoxic effects, as indicated by raised serum activities of liver enzymes.

Experimental data suggest that GLN may protect the cardiac metabolism during periods of stress. For example, Khogali et al. [56, 57] found that in the absence of GLN, ischemia–reperfusion caused an immediate fall in myocardial glutamate, decrease in myocardial ATP and accumulation of myocardial lactate, while the presence of GLN prevented these deleterious changes in myocardial metabolites. Post-ischemic treatment with GLN completely restored the myocardial metabolites to normal and significantly enhanced the myocardial ATP/ADP and GSH/GSSG ratio.

Our previous studies found that oral GLN stimulated GSH production of the heart, blood, normal mammary tissue and gut, while significantly reducing tumor GSH in implantable sarcoma and 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast cancer models [58–60]. This is important, because GSH is a key regulator of the cellular redox state and as such it also determines the response of tumors (and protection of the normal cells) to chemotherapy and radiation [61, 62]. The paradoxical effect of GLN on GSH metabolism in tumor and host tissues may be a result of the relatively more acidic intracellular environment of tumors compared to normal structures, thus inactivating the pH-sensitive enzyme 5-oxoprolinase, which catalyzes the formation of gamma-glutamyl-glutamine dipeptide, an immediate precursor of GSH in gamma-glutamyl cycle to glutamate [60]. This hypothesis is supported by data showing that tumors containing high levels of γ-glutamyl transferase and glutaminase are more resistant to cytotoxic chemotherapy (reviewed in [63]). Our data showed that dietary GLN significantly down-regulated several enzymes of the γ-glutamyl cycle, including 5-oxoprolinase and glutaminase in tumor but upregulated them in normal tissues [64]. The differential effect of GLN on GSH recycling of normal tissues and tumors was associated with a reduction of tumor growth in breast cancer model [59]. We have also found that dietary GLN increased blood and cardiac GLN and GSH in rats treated with DOX [23], cyclophosphamide [65] or methotrexate (MTX) [66] and this inversely correlated with cardiac tissue lipid peroxidation. In a phase I trial, nine patients diagnosed with inflammatory breast cancer received GLN (0.5 g/kg/day) during MTX neoadjuvant therapy, followed by a DOX-based regimen. No patient showed any sign of chemotherapy-related toxicity [67]. A review of the literature to date showed no reported side effects of GLN supplementation. Moreover, a recent review of the data over the past 15–20 years by Wischmeyer [68] showed that GLN supplementation improved infectious morbidity and mortality, reduced hyperglycemia, reduced inflammation and improved liver dysfunction in critically ill patients.

The present study showed that GLN protected the heart of a tumor-bearing host from DOX toxicity by reducing DOX accumulation in the heart without affecting it in the tumors. These data indicate that GLN may be a useful adjunct for the treatment of cancer patients with DOX.

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