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BIOCHEMICAL DETERMINANTS OF ADRIAMYCIN® TOXICITY IN MOUSE LIVER, HEART AND INTESTINE

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Abstract—Biochemical characteristics relevant to the differential susceptibilities of liver, heart, and intestine to acute Adriamycin® toxicity were examined in female CD-1 mice with and without intravenous Adriamycin (dose range 23–30 mg/kg). The liver which, unlike heart and intestine, is relatively resistant to Adriamycin toxicity, had high levels of glutathione and glutathione peroxidase, and exhibited a sharp decline in non-protein thiol concentrations within 1–3 hr with rebound by 6 hr after Adriamycin. Covalent binding to Adriamycin or its metabolites could not account quantitatively for the loss of non-protein thiols, implicating an oxidative mechanism. No lipid peroxidation was observed in the liver, apparently due to effective utilization of antioxidant defenses. Adriamycin caused significant increases in cardiac lipid peroxides, indicative of oxidative tissue damage, which would be expected to exacerbate cardiotoxicity. However, non-protein thiol concentrations did not decrease in heart or in intestine in response to Adriamycin. Both heart and intestine had extremely low levels of glutathione peroxidase activity, which may limit glutathione utilization for protection against oxidative toxicity. The activity of DT diaphorase, which may have an activating role in Adriamycin metabolism, was high in heart and intestine and was induced 4-fold in liver in response to Adriamycin.

The widely used anticancer drug Adriamycin® produces toxic effects in numerous organs [1–11]. In addition to the transient myelosuppression characteristic of many anticancer drugs, a clinical use of Adriamycin frequently results in potentially lethal acute gastrointestinal and cardiac toxicities and its chronic administration leads to cumulative cardiomyopathy, whereas the liver is relatively resistant to damage by either acute or chronic Adriamycin [1, 10]. Biochemical effects observed in experimental animals have yielded considerable information about the mechanism of toxicity [11–15]. Oxidative damage to membrane lipids and other cellular components is believed to be a major factor in the toxicities of Adriamycin and other anthracyclines [12–14]. Adriamycin and its iron chelate undergo redox cycling, resulting in the generation of free radical metabolites and active oxygen [12–14]. Lipid peroxidation and depletion of tissue non-protein sulfhydryl compounds in response to i.p. Adriamycin also support an oxidative mechanism of toxicity [16–19]. However, the concept of a single biochemical mechanism to account for the toxic effects of Adriamycin on several organs appears untenable since the recent report of Ito *et al.* [15] that Adriamycin selectively inhibits expression of cardiac muscle genes *in vivo*. Adriamycin administration has also been reported to lead to inactivation of glutathione (GSH‖) peroxidase (EC 1.11.1.9) *in vivo* [20, 21]. Since this enzyme protects against Adriamycin-induced oxidative damage [22], its inactivation would be expected to exacerbate the toxic effects of Adriamycin.

In the present investigation, potential biochemical determinants of toxicity and their responses to the acute i.v. administration of Adriamycin have been examined in mouse liver, heart, and intestine to further define the biochemical basis of differing organ susceptibilities in relation to the underlying mechanisms of Adriamycin toxicity.

MATERIALS AND METHODS

Chemicals. Adriamycin hydrochloride, GSH, 2-thiobarbituric acid, NADPH, 5,5′-dithio-bis(2-nitrobenzoic acid), 2,6-dichloroindophenol, cumene hydroperoxide, 1,1,3,3-tetraethoxypropane, 3,5-di-*tert*-butyl-4-hydroxytoluene, dicumarol, yeast glutathione reductase, and crystalline bovine serum albumin were purchased from the Sigma Chemical Co. Sephadex G-10 was from Pharmacia. Adriamycin was dissolved at a concentration of 3.0 mg/mL in either sterile water or 0.9% (w/v) NaCl (saline), and its concentration was determined either by weight or spectrophotometrically [23].

Animals. CD-1 mice, from Charles River Breeding Laboratories, were housed in stainless steel cages with hardwood bedding in an environmentally

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‖ Abbreviation: GSH, glutathione.

controlled room with a 12-hr light/dark cycle. They received Purina laboratory chow and tap water *ad lib*. Adriamycin was administered i.v. via the tail vein either in sterile saline or in sterile water (10 μL/g body wt). Mice were killed by cervical dislocation, and organs were excised and processed immediately as described below. Organs were collected from Adriamycin-treated mice and from untreated or vehicle-treated mice at each time point (below) after injection to avoid errors due to diurnal variations.

Measurement of enzyme activities. GSH peroxidase and DT diaphorase (EC 1.6.99.2) activities were measured at 25° in cytosol fractions of livers, hearts, and the mucosa of the proximal half of the small intestine. Immediately after excision, livers and hearts were perfused with cold 0.15 M KCl in 2 mM EDTA (Na_2), pH 7.0. Tissues for enzyme determinations were frozen in liquid N_2 and stored at −80°. Homogenates were prepared at 0° in 0.25 M sucrose (3 mL/g tissue) in a glass/Teflon homogenizer, and cytosol fractions were obtained as described previously [24]. GSH peroxidase activities were measured with H_2O_2 and cumene hydroperoxide as substrates, using a modification [25] of the method of Paglia and Valentine [26]. DT diaphorase activity was measured as the dicumarol-sensitive enzymic reduction of 2,6-dichloro-indophenol by NADH as described previously [27].

Evaluation of lipid peroxidation. Freshly excised hearts and livers were rinsed quickly in cold KCl/EDTA solution. Each heart was placed in a tube with 1 mL of cold 20 mM potassium phosphate buffer (pH 7.4) containing butylated hydroxytoluene (0.5 mg/100 mL). Hearts and livers were frozen in liquid nitrogen and stored at −80°. For further processing, the organs were thawed on ice, blotted dry, weighed, minced, and homogenized (10–15 firm strokes in a ground glass homogenizer) at 0° in 2 mL of the phosphate buffer containing butylated hydroxytoluene. Further treatment of homogenates was based primarily on methods described by Lenzhofer *et al.* [16]. Each homogenate was transferred with 7.5 mL of deionized water into a 25-mL distillation flask. After the addition of 0.5 mL of 2 M HCl, the flasks were heated in a 120° bath in a distillation apparatus and the first 5 mL of distillate was collected. Malonaldehyde was measured spectrophotometrically after reaction with 2-thiobarbituric acid under the conditions described by Lenzhofer *et al.* [16]. An aliquot (1.0 mL) of the distillate was added to 1 mL of a solution containing 0.02 M 2-thiobarbituric acid in 90% (v/v) acetic acid and heated at 95° for 35 min. The samples were cooled and their absorbances were measured at 538 nm against blanks prepared with deionized water in place of distillate. Assays were performed in duplicate. Quantitation was by use of standard curves prepared with malonaldehyde synthesized from 1,1,3,3-tetraethoxypropane [28]. The identity and concentration of the synthesized malonaldehyde were verified spectrophotometrically and by gel filtration under the conditions described by Lenzhofer *et al.* [16].

Measurement of non-protein sulfhydryl compounds. Concentrations of acid-soluble thiol compounds were determined in freshly excised tissues. Samples consisted of one heart (weighed, perfused with cold 0.9% NaCl, and minced), 100 mg of liver, or the mucosa from the proximal 6 inches of the small intestine. Each tissue sample was promptly placed in a pre-chilled glass/Teflon homogenizer and homogenized at 0° in N_2-flushed 0.02 M EDTA(Na_2) (9 mL/g tissue). A portion of each homogenate was reserved for protein determinations. To the remaining homogenate, 1/9 volume of cold 50% trichloroacetic acid (w/w, in H_2O) was added. At this stage the samples were kept on ice for 1 hr or less before centrifugation at 10,000 *g* for 10 min at 0–4°. The supernatants were assayed as described below. Blood collected from the retroorbital sinus in heparinized capillary tubes was centrifuged at 1000 *g* for 10 min. The packed erythrocytes were then lysed by the addition of 5 volumes of cold 0.02 M EDTA. After the removal of an aliquot of the lysate for measurement of hemoglobin, cold 50% trichloroacetic acid (167 μL/mL) was added to the remaining hemolysate. After centrifugation the supernatants were assayed for sulfhydryl concentrations by the method of Sedlack and Lindsay [29]. The reactions were run in 1-mL and 3-mL polystyrene cuvettes. Cuvettes containing 0.8 mL of N_2-flushed 0.4 M Tris–HCl in 20 mM EDTA(Na_2), pH 8.9, received either 200 μL of supernatant from erythrocyte or heart samples or 2.0 mL of H_2O plus 200 μL of supernatant from liver or intestine mucosa samples. To each cuvette was added 20 μL of 0.01 M 5,5′-dithio-bis(2-nitrobenzoic acid), and the absorbances at 412 nm were read within 5–30 min. Thiols were quantitated by the use of a standard curve prepared with a solution of GSH in cold, N_2-flushed 20 mM EDTA(Na_2).

Measurement of protein concentrations. Protein concentrations were measured by the methods described for soluble proteins and for tissue homogenates by Lowry *et al.* [30]. Hemoglobin was measured according to Tentori and Salvati [31].

RESULTS

GSH peroxidase and DT diaphorase. The activities of these enzymes in cytosol fractions from perfused livers and hearts and from small intestine mucosa from untreated 9-week-old female CD-1 mice are shown in Table 1. GSH peroxidase activity toward H_2O_2 was 27-fold higher in the livers than in the hearts of these mice, in contrast to the finding that male CDF_1 mice exhibit similar specific activities of this enzyme in cardiac muscle and liver [32]. The small intestine, which like the heart is a target organ for Adriamycin toxicity, also had very low GSH peroxidase activity (Table 1). In heart and in intestinal mucosa similar activities were observed with the two substrates, H_2O_2 and cumene hydroperoxide, suggesting the presence of the selenoenzyme, GSH peroxidase I, and the virtual absence of GSH peroxidase II activity which is due to GSH transferases that utilize cumene hydroperoxide but not H_2O_2 [33]. In liver cytosol the ratio of activities toward these two substrates was consistent with high activities of both types of GSH peroxidase. The distribution of GSH peroxidases in liver, heart, and intestine of female

Table 1. GSH peroxidase and DT diaphorase activities in organ cytosols from untreated female CD-1 mice

		Specific activity (nmol/min/mg protein)		
		GSH peroxidase		
Organ	N	H_2O_2	Cumene hydroperoxide	DT diaphorase
Liver	6	510 ± 13*	1130 ± 23	135 ± 6
	12	609 ± 20	1155 ± 20	130 ± 15
Heart	6	19 ± 1	16 ± 1	ND†
	12	22 ± 1	19 ± 1	545 ± 19
Intestine‡	18	12 ± 1	13 ± 1	788 ± 53

* Mean ± SEM.
† Not determined.
‡ Mucosa from upper half of small intestine.

CD-1 mice resembles that found in rats [34]. DT diaphorase activity, which is almost 6-fold higher in the intestinal mucosa than in the livers of female CD-1 mice [35], was present in cardiac tissue at levels 4-fold greater than in liver (Table 1).

Effects of Adriamycin on GSH peroxidase and DT diaphorase activities. Enzyme activities were measured in mouse heart, liver, and intestinal mucosa daily for 4 days after i.v. administration of Adriamycin at 30 mg/kg. Toxicity was evidenced by a 9% decrease in body weight by 96 hr after Adriamycin, from 23.8 ± 0.4 g (mean ± SEM; N = 12) to 21.1 ± 0.8 g in mice treated with Adriamycin (N = 6; $P < 0.01$). In other experiments this dosage of Adriamycin led to continued weight loss and lethality (data not shown). The largest effects on enzyme activities were observed in the liver where a decline in GSH peroxidase activity toward both substrates, observed 24 hr after Adriamycin injection, was followed by a 4-fold induction of DT diaphorase (Table 2). Maximal levels of hepatic DT diaphorase activity were attained 72 hr after Adriamycin administration, coincident with an increase in liver weight from 5.4 ± 0.1% of body weight (N = 12) to 6.4 ± 0.3% of body weight (N = 6; $P < 0.001$). By 96 hr, when DT diaphorase activity had started to decline, liver weight had returned to normal, being 5.5 ± 0.1% of body weight (N = 6). Although Adriamycin caused substantial decreases in cardiac GSH peroxidase in other animal models [20, 32], we observed no significant change in either GSH peroxidase or DT diaphorase of cardiac tissue in response to Adriamycin. The intestinal mucosa exhibited little if any change in GSH peroxidase or DT diaphorase activity, although intestinal DT diaphorase of female CD-1 mice is inducible by other xenobiotics [27, 35].

Non-protein sulfhydryl compounds. Acid-soluble thiols were measured in preparations from livers, hearts, proximal small intestine mucosae, and erythrocytes from Adriamycin-treated and vehicle-treated 7- to 8-week-old mice. The results are shown in Table 3. All mice in these experiments were killed between 10:00 a.m. and 2:00 p.m. on consecutive days. Much lower concentrations of non-protein thiols were present in heart than in liver, or intestinal mucosa. Measurements 1, 3, and 6 hr after administration of Adriamycin (23 mg/kg, i.v.) revealed that non-protein thiol concentrations were unaffected in heart, intestine, and erythrocytes relative to values from simultaneously vehicle-treated control mice. In liver, a significant decrease in thiol concentrations was observed at the 1-hr and 3-hr time points, with rebound by 6 hr after Adriamycin.

Lipid peroxidation. Tissue distillates, in which malonaldehyde is produced by the acid-catalyzed breakdown of peroxidized lipids, were used to avoid interference from other 2-thiobarbituric acid-reactive substances present in crude extracts of tissues. When examined by gel filtration on Sephadex G-10, the malonaldehyde synthesized from 1,1,3,3-tetraethoxypropane and the 2-thiobarbituric acid-reactive material in distillates of heart and liver homogenates appeared as a single peak in the low molecular weight region of the chromatogram (data not shown), as also observed by Lenzhofer *et al.* [16]. Examination of the effects of acute i.v. Adriamycin on lipid peroxidation in hearts and livers of female CD-1 mice yielded the results shown in Table 4. In the first series of experiments, cardiac lipid peroxides were unchanged 24 hr after Adriamycin at 30 mg/kg (Expt. 1A) but were elevated significantly (+83% per g of tissue; +67% per heart) relative to levels in untreated controls at 48 hr post-Adriamycin (Expt. 1B). The time course of lipid peroxidation in response to i.v. adriamycin was examined more closely in Expt. 2. A single 28 mg/kg dose of Adriamycin elicited a 65% elevation in cardiac concentrations of lipid peroxides observed 24 hr later. At 48 hr post-Adriamycin, lipid peroxide levels appeared higher than in controls but statistical significance was not achieved due to mouse-to-mouse variations. The increase in cardiac lipid peroxides was transient, being followed by decline to levels found in vehicle-treated control mice. Thus, both sets of experiments showed that Adriamycin increased cardiac lipid peroxides although the time course of this response appeared more rapid in Expt. 2. It is not clear whether the somewhat more rapid time course of this response in Expt. 2 is related to differences in the age of the mice or to some unidentified experimental variable. Livers from Adriamycin-treated mice showed no increase in lipid peroxides at any of the time points examined (Table 4).

DISCUSSION

The results of this investigation reveal ways in which biochemical differences in liver, heart, and intestine may provide a rationale for the differing responses of these organs to Adriamycin. The liver utilizes an antioxidant defense mechanism to protect against the toxic effects of Adriamycin. Hepatic levels of GSH and GSH peroxidases I and II were relatively high, non-protein thiol concentrations declined in response to Adriamycin, and no increase in lipid peroxide levels was observed. These results are in accord with the concept that redox cycling of

Table 2. Effects of acute i.v. Adriamycin (30 mg/kg) on cytosolic activities of GSH peroxidase and DT diaphorase

Organ	Expt.	Hr after Adriamycin	Specific activity (% of mean for controls)		
			GSH peroxidase		
			H_2O_2	Cumene hydroperoxide	DT diaphorase
Liver	A	24	83 ± 2*†	83 ± 2†	140 ± 6†
	B	48	113 ± 2‡	104 ± 1	228 ± 25§
		72	93 ± 3	92 ± 1‖	405 ± 53†
		96	102 ± 4	99 ± 4	328 ± 77
Heart	A	24	90 ± 4	92 ± 4	ND¶
	B	48	91 ± 4	95 ± 5	110 ± 1
		72	87 ± 3	96 ± 3	113 ± 5
		96	89 ± 4	88 ± 3	114 ± 8
Intestine**	B	24	89 ± 9	88 ± 9	116 ± 17
		48	97 ± 6	99 ± 3	98 ± 4
		72	87 ± 5	81 ± 5‡	100 ± 9

* Values are means ± SEM, N = 5–6.
† $P < 0.001$ vs controls. The means ± SEM for controls are in Table 1.
‡ $P < 0.05$ vs controls.
§ $P < 0.01$ vs controls.
‖ $P < 0.02$ vs controls.
¶ Not determined.
** Mucosa of the upper half of the small intestine.

Table 3. Non-protein thiol concentrations in mouse tissues at 1, 3, and 6 hr after i.v. Adriamycin

Tissue	Adriamycin dose (mg/kg)	Non-protein thiols		
		Time after Adriamycin administration		
		1 hr	3 hr	6 hr
		(nmol thiols/mg tissue protein)		
Liver	0	46.7 ± 2.6*	42.6 ± 3.0	34.8 ± 2.8
	23	30.2 ± 1.2†	29.0 ± 1.9‡	31.0 ± 3.6
Heart	0	6.5 ± 0.2	6.9 ± 0.6	5.6 ± 0.3
	23	7.7 ± 0.7	6.5 ± 0.5	5.8 ± 0.1
Small intestine§	0	39.3 ± 1.3	31.7 ± 1.9	29.1 ± 1.5
	23	35.9 ± 2.6	33.1 ± 2.8	28.9 ± 1.8
		(nmol thiols/mg hemoglobin)		
Erythrocytes	0	6.1 ± 0.5	5.8, 5.7	5.3 ± 0.2
	23	6.8 ± 0.4	6.9, 7.7	5.3 ± 0.2

* Values are means ± SEM, N = 4, except for erythrocytes 3 hr after Adriamycin where only two values were obtained.
† $P < 0.01$ vs controls.
‡ $P < 0.02$ vs controls.
§ Mucosa from the proximal 6 inches of the small intestine was used.

Adriamycin did occur in the liver but peroxidative damage to hepatocytes was obviated by GSH. The decrease observed in hepatic concentrations of non-protein thiols may be regarded as protective since it did not progress to levels associated with necrosis [36]. The decrease in thiols may also reflect their covalent reaction with Adriamycin or its metabolites. Although enzymic conjugation of Adriamycin with GSH has not been demonstrated, covalent binding of GSH to Adriamycin or, more likely, to its free radical metabolites has been observed [37, 38]. In the present investigation the decline in non-protein thiols observed in mouse liver was in at least 4-fold molar excess of the Adriamycin injected. Thus, the major portion of the decline in hepatic GSH appeared due to its use in the detoxification of

Table 4. Effects of acute i.v. Adriamycin on lipid peroxides in hearts and livers of female CD-1 mice

Expt. No.	Adriamycin (mg/kg)	Hr after Adriamycin	N	MDA* (nmol/heart)	MDA (nmol/g cardiac tissue)	MDA (nmol/liver)	MDA (nmol/g liver protein)
1A	0		6	3.8 ± 0.6†	24 ± 4		
	30	24	6	3.7 ± 0.4	26 ± 4		
1B	0		6	3.0 ± 0.3	24 ± 2	13.5 ± 0.7	
	30	48	6	5.0 ± 0.6‡	44 ± 6‡	13.2 ± 1.5	
	30	72	6	4.5 ± 1.0	41 ± 9	14.5 ± 1.6	
2	0		10	3.9 ± 0.4	37 ± 4		44 ± 6
	28	24	5	5.9 ± 0.6§	61 ± 10§		36 ± 6
	28	48	5	4.8 ± 0.5	42 ± 3		35 ± 3
	28	72	4	3.6 ± 0.3	34 ± 2		32 ± 4
	28	96	5	3.6 ± 0.1	40 ± 2		37 ± 5

Mice were 9 weeks old in Expt. 1A, 19 weeks old in Expt. 1B, and 13 weeks old in Expt. 2.
* Abbreviation: MDA, malonaldehyde.
† Mean ± SEM.
‡ $P < 0.01$ vs controls.
§ $P < 0.02$ vs controls.

reactive oxygen intermediates formed by redox cycling of Adriamycin or its metabolites.

In contrast to the liver, the heart and the intestine had in common extremely low activities of GSH peroxidase. This antioxidant enzyme protects against Adriamycin toxicity, as recently demonstrated in Adriamycin-resistant tumor cells by Sinha *et al.* [22]. Non-protein thiol concentrations were also very low in the heart and, although relatively high non-protein thiol levels existed in the intestine, no decrease in thiol concentrations was observed in either of these organs in response to Adriamycin. Thus, the toxicity of Adriamycin to heart and intestine correlates with a very limited capability in these organs to utilize non-protein thiols to protect against oxidative toxicity resulting from redox cycling of Adriamycin, due to the low levels of GSH in the heart and the very low GSH peroxidase activities in both of these organs.

While the increase in lipid peroxides that we observed in the hearts of Adriamycin-treated mice provided evidence of oxidative toxicity, its limited extent and transient nature are compatible with the coexistence of another predominant mechanism of toxicity. Such a mechanism was revealed in the recent report of Ito *et al.* [15] that Adriamycin selectively inhibits the expression of genes encoding cardiac muscle proteins. The combination of oxidative damage and loss of the biosynthetic capability to repair damaged cells may act synergistically to cause the myofibrillar loss characteristic of the cardiac toxicity of Adriamycin.

Heart and intestine also had in common relatively high activities of DT diaphorase. The role of this enzyme in Adriamycin metabolism is unclear. Sinha *et al.* [12, 37] have proposed that DT diaphorase catalyzes reductive activation of Adriamycin, leading to the formation of a quinone methide capable of alkylating DNA. However, Wallin [39] found that Adriamycin was not a substrate for this enzyme. Whether DT diaphorase acts on Adriamycin aglycones is under investigation. Activation by DT diaphorase could further explain the cardiac and intestinal toxicity of Adriamycin. Also, the 4-fold induction of hepatic DT diaphorase by Adriamycin may affect toxicity, particularly in a multidose protocol or in combination with other drugs whose metabolism may be affected.

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REFERENCES

1. Billingham ME, Mason JW, Bristow MR and Daniels JR, Anthracycline cardiomyopathy monitored by morphological changes. *Cancer Treat Rep* **62**: 865–872, 1978.
2. Phillips FS, Gilladoga A, Marquardt H, Sternberg SS and Vidal PM, Some observations on the toxicity of adriamycin (NSC-123127). *Cancer Chemother Rep* **6**: 177–181, 1975.
3. Doroshow JH, Locker GY and Myers CE, Experimental animal models of Adriamycin cardiotoxicity. *Cancer Treat Rep* **63**: 855–860, 1979.
4. Dethlefsen LA and Riley RM, The effects of Adriamycin on murine duodenal crypt cell proliferation. *Int J Radiat Oncol Biol Phys* **5**: 501–506, 1979.
5. Van Vleet JF, Greenwood LA and Ferrans VJ, Pathologic features of adriamycin toxicosis in young pigs: Nonskeletal lesions. *Am J Vet Res* **40**: 1537–1552, 1979.
6. Unverferth DV, Magorien RD, Leier CV and Balcerzak SP, Doxorubicin cardiotoxicity. *Cancer Treat Rev* **9**: 149–164, 1982.
7. Burholt DR, Hagemann RF, Schenken LL and Lesher S, Influence of adriamycin and adriamycin–radiation combination on jejunal proliferation in the mouse. *Cancer Res* **37**: 22–27, 1977.
8. Van Hoesel QGCM, Steerenberg PA, Vos JG, Hillen FC and Dormans JAMA, Antitumor effect, cardiotoxicity, and nephrotoxicity of doxorubicin in the IgM solid immunocytoma-bearing LOU/M/WSL rat. *J Natl Cancer Inst* **72**: 1141–1147, 1984.
9. Bertazzoli C, Bellini O, Magrini U and Tosana MG, Quantitative experimental evaluation of Adriamycin

cardiotoxicity in the mouse. *Cancer Treat Rep* **63**: 1877–1883, 1979.
10. Yates J, Glidewell O, Wiernik P, Cooper MR, Steinberg D, Dosik H, Levy R, Hoagland C, Henry P, Gottlieb A, Cornell C, Berenberg J, Hutchison JL, Raich P, Nissen N, Ellison RR, Frelick R, James GW, Falkson G, Silver RT, Haurani F, Green M, Henderson E, Leone L and Holland JF, Cytosine arabinoside with daunorubicin or adriamycin for therapy of acute myelocytic leukemia: A CALGB study. *Blood* **60**: 454–462, 1982.
11. Singal PK, Deally CMR and Weinberg LE, Subcellular effects of adriamycin in the heart: A concise review. *J Mol Cell Cardiol* **19**: 817–828, 1987.
12. Sinha BK and Politi PM, Anthracyclines. *Cancer Chemother Biol Resp Modifiers Annu* **11**: 45–57, 1990.
13. Olson RD and Mushlin PS, Doxorubicin cardiotoxicity: Analysis of prevailing hypotheses. *FASEB J* **4**: 3076–3086, 1990.
14. Myers C, Anthracyclines. *Cancer Chemother Biol Resp Modifiers Annu* **10**: 33–39, 1988.
15. Ito H, Miller SC, Billingham ME, Akimoto H, Torti SV, Wade R, Gahlmann R, Lyons G, Kedes L and Torti FM, Doxorubicin selectively inhibits muscle gene expression in cardiac muscle cells *in vivo* and *in vitro*. *Proc Natl Acad Sci USA* **87**: 4275–4279, 1990.
16. Lenzhofer R, Magometschnigg D, Dudczak R, Cerni C, Bolebruch C and Moser K, Indication of reduced doxorubicin-induced cardiac toxicity by additional treatment with antioxidative substances. *Experientia* **39**: 62–64, 1983.
17. Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K and Young RC, Adriamycin: The role of lipid peroxidation in cardiac toxicity and tumor response. *Science* **197**: 165–167, 1977.
18. Doroshow JH, Locker GY, Baldinger J and Myers CE, The effect of doxorubicin on hepatic and cardiac glutathione. *Res Commun Chem Pathol Pharmacol* **26**: 285–295, 1979.
19. Olson RD, MacDonald JS, van Boxtel CJ, Boerth RC, Harbison RD, Slonim AE, Freeman RW and Oates JA, Regulatory role of glutathione and soluble sulfhydryl groups in the toxicity of Adriamycin. *J Pharmacol Exp Ther* **215**: 450–454, 1980.
20. Revis NW and Marusic N, Glutathione peroxidase activity and selenium concentration in the hearts of doxorubicin-treated rabbits. *J Mol Cell Cardiol* **10**: 945–951, 1978.
21. Julicher RHM, Sterrenberg L, Haenen GRMM, Bast A and Noordhoek J, The effect of chronic adriamycin treatment on heart, kidney, and liver tissue of male and female rat. *Arch Toxicol* **61**: 275–281, 1988.
22. Sinha BK, Mimnaugh EG, Rajagopalan S and Myers CE, Adriamycin activation and oxygen free radical formation in human breast tumor cells: Protective role of glutathione peroxidase in Adriamycin resistance. *Cancer Res* **49**: 3844–3848, 1989.
23. Vigevani A and Williamson MJ, Doxorubicin. *Anal Profiles Drug Substances* **9**: 245–274, 1980.
24. Benson AM, Cha Y-N, Bueding E, Heine HS and Talalay P, Elevation of extrahepatic glutathione *S*-transferase and epoxide hydratase activities by 2(3)-*tert*-butyl-4-hydroxyanisole. *Cancer Res* **39**: 2971–2977, 1979.
25. Tappel AL, Glutathione peroxidase and hydroperoxides. *Methods Enzymol* **52**: 506–513, 1978.
26. Paglia DE and Valentine WN, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* **70**: 158–169, 1967.
27. Benson AM, Hunkeler MJ and Talalay P, Increase of NAD(P)H:quinone reductase by dietary antioxidants: Possible role in protection against carcinogenesis and toxicity. *Proc Natl Acad Sci USA* **77**: 5216–5220, 1980.
28. Kwon TW and Watts BM, Determination of malondialdehyde by ultraviolet spectrophotometry. *J Food Sci* **28**: 627–630, 1963.
29. Sedlak J and Lindsay R H, Estimation of total, protein bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Anal Biochem* **25**: 192–205, 1968.
30. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
31. Tentori L and Salvati AM, Hemoglobinometry in human blood. *Methods Enzymol* **76**: 707–715, 1981.
32. Doroshow JH, Locker GY and Myers CE, Enzymatic defenses of the mouse heart against reactive oxygen metabolites. Alterations produced by doxorubicin. *J Clin Invest* **65**: 128–135, 1980.
33. Prohaska JR and Ganther HE, Glutathione peroxidase activity of glutathione *S*-transferases purified from rat liver. *Biochem Biophys Res Commun* **76**: 437–445, 1977.
34. Lawrence RA and Burk RF, Species, tissue and subcellular distribution of non Se-dependent glutathione peroxidase activity. *J Nutr* **108**: 211–215, 1978.
35. Bensom AM, Barretto PB and Stanley JS, Induction of DT-diaphorase by anticarcinogenic sulfur compounds in mice. *J Natl Cancer Inst* **76**: 467–473, 1986.
36. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR and Brodie BB, Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. *J Pharmacol Exp Ther* **187**: 211–217, 1973.
37. Sinha BK and Gregory JL, Role of one-electron and two-electron reduction products of adriamycin and daunomycin in deoxynucleic acid binding. *Biochem Pharmacol* **30**: 2626–2629, 1981.
38. Julicher RHM, van der Laarse A, Sterrenberg L, Bloys van Treslong CHF, Bast A and Noordhoek J, The involvement of an oxidative mechanism in the adriamycin induced toxicity in neonatal rat heart cell cultures. *Res Commun Chem Pathol Pharmacol* **47**: 35–47, 1973.
39. Wallin R, Adriamycin and DT-diaphorase. *Cancer Lett* **30**: 97–101, 1986.