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Doxorubicin-induced acute changes in cytotoxic aldehydes, antioxidant status and cardiac function in the rat

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Abstract

Doxorubicin (DOX)-induced cardiotoxicity is thought to be caused by free radical-mediated mechanisms. An in vivo rat model was developed to investigate the DOX-induced cascade of early biochemical changes focusing on the central role of the aldehydic lipid peroxidation products. Antioxidant status was evaluated by glutathione measurements. Creatine Kinase (CK) activity was measured as an index of cardiac injury. Development of functional abnormalities were documented by echocardiography. The results showed that aldehydes in rat plasma and heart tissues increased significantly following DOX treatment. The changes occurred early, peaked around 2 h after DOX administration, and the levels declined or returned to baseline value within 8–24 h. Toxic aldehyde levels including malondialdehyde, hexanal and 4-hydroxy-non-2-enal also increased. Acyloin levels, metabolic products of aldehydes, increased early and then decreased in plasma, and there was a significant decrease in heart tissues after DOX treatment. GSH levels decreased early, then increased by 24 h, while GSSG levels decreased initially, then increased after DOX treatment, suggesting early depletion of GSH and a later rebound phenomenon. CK levels were elevated after treatment. The functional abnormalities were documented by stress echocardiography in some rats although the changes were not consistent at such an early stage following treatment. Our data confirmed the involvement of free radicals, and suggested that the cytotoxic aldehydes play a central role in initiating the steps that lead to functional impairment of the myocardium following DOX administration. Scavengers and the metabolic removal of some of the aldehydes also play a role in protecting the myocardium against injury.

Keywords: Doxorubicin; Aldehyde; Lipid peroxidation; Free radical; Antioxidant; Echocardiography

Abbreviations: DOX, doxorubicin; CK, creatine kinase; NICIGC-MS, negative ion chemical ionization gas chromatography-mass spectrometry; HPLC, high pressure liquid chromatography; GSH, reduced glutathione; GSSG, oxidized glutathione; HNE, 4-hydroxy-non-2-enal; MDA, malondialdehyde; V_{cfc}, velocity of circumferential fiber shortening; PDH-AS, pyruvate dehydrogenase-acyloin synthase; SOD, superoxide dismutase; GP, glutathione peroxidase.

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1. Introduction

The anthracycline antibiotic, doxorubicin (DOX, also known as adriamycin) is a potent broad spectrum chemotherapeutic agent that is effective against human malignancies such as leukemias, lymphomas and many solid tumors, and is widely used in both pediatric and adult patients [1–4]. However, the clinical use of doxorubicin is limited by its severe, cumulative, dose-dependent cardiotoxicity [4,5]. DOX has both acute and chronic, long-term toxic effects on the heart. The acute effects are characterized by a variety of acute atrial and ventricular dysrhythmias, a pericarditis-myocarditis syndrome, and acute hypertensive reactions occurring shortly after the administration of the drug, which are usually transient and clinically manageable [6]. The long-term effects include the insidious onset of cardiomyopathy that often leads to congestive heart failure. These life threatening effects often develop only after several weeks or months of treatment, and sometimes even years after therapy has been completed. The incidence of cardiotoxicity increases at cumulative doses in excess of 500 mg/m^2 and is age related. Younger children are at higher risk and DOX treatment in children may lead to a lifelong reduction in myocardial mass that, with further growth may result in decreased cardiac reserve [7,8].

The precise mechanism of DOX-induced cardiotoxicity is not entirely clear [9,10]. Although acute alterations in platelet-activating factor, prostaglandins, histamine, and intracellular calcium have been implicated, drug-stimulated reactive oxygen metabolism that causes peroxidation of membrane lipids is thought to be most likely factor responsible for the development of cardiotoxicity [11–13].

The quinone structure of the anthracycline C ring has a highly reactive potential, and a 1-electron reduction results in the formation of a semiquinone free radical in the presence of certain flavin enzymes and mitochondrial complex I–III [14,15]. Under the aerobic conditions existing in the myocardial cell, this semiquinone free radical intermediate can readily donate its unpaired electron to molecular oxygen to produce superoxide radical, which is a potent initiator of other oxygen radicals as well as activated oxygen species. The repetitive reduction and oxidation (known as redox cycling) of DOX generates semiquinone radicals, singlet oxygen, superoxide, and hydrogen peroxide. In the presence of a suitable transition metal catalyst (DOX-iron complex), hydroxyl radicals form through a Fenton-type reaction [16]. Free radicals, especially the highly reactive hydroxyl radical, readily react with a variety of molecules and cause peroxidation of membrane lipids, resulting in membrane degradation, protein aggregation and, ultimately, cell death [17,18].

Free radicals have very short half lives and are very difficult to measure. The major stable products of lipid peroxidation, saturated and unsaturated aldehydes, are considered to be some of the few measurable parameters indicative of lipid peroxidation in vivo [19]. In the present study, we have developed a short-term, in vivo rat model in which DOX was given by a bolus injection, to investigate the DOX-induced cascade of early biochemical changes focusing on the central role of toxic aldehydes that initiate the process leading ultimately to cardiomyopathy. Some of the acyloins (3-hydroxyalkan-2-ones), pyruvate dehydrogenase (PDH) catalyzed condensation products between pyruvate and saturated aldehydes, were also determined at the same time. Antioxidant status was evaluated by measurement of blood glutathione levels. Creatine Kinase (CK) levels were measured as an index of early myocardial injury. The development of functional abnormalities following DOX treatment were documented by echocardiography.

2. Materials and methods

2.1. Animals and drug treatment

Male Wistar rats (Charles River, Montreal, Canada, 350–400 g) were used. Animals were housed in stainless steel cages and maintained under a controlled environment (temperature 24°C, relative humidity 50–55%, light cycle 06:00 h to 18:00 h), and were allowed free access to commercial rat chow (PROLAB R-M-H 1000, AGWAY, St. Mary's, OH) and tap water ad libitum. A total of 36 rats were randomly divided into six groups. Twelve rats served as control. To determine whether diurnal variation exists in the levels of any of the measured parameters, 4 rats were injected with 0.9% NaCl vehicle solution intraperitoneally each time, at 9 a.m., 1 p.m.

and 5 p.m., respectively. The rats were sacrificed by exsanguination 1 h later under sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, Ontario, Canada) injection anesthesia. Blood samples were taken from femoral vein, and the heart was rapidly removed and frozen in liquid nitrogen for later analysis. For the five experimental animal groups (4 rats in each group), rats were treated with a bolus injection of 10 mg/kg of DOX (Adria Laboratories, Columbus, OH) in 0.9% NaCl solution intraperitoneally, and were sacrificed at 1 h, 2 h, 4 h, 8 h and 24 h after DOX injection. The experimental protocol and all procedures were approved by the review board of the Animal Facility of the Hospital for Sick Children Research Institute.

2.2. Measurement of aldehydes and acyloins

Aldehydes and acyloins in plasma and heart tissues were measured by a newly developed method in our laboratory using capillary column gas chromatography negative-ion-chemical ionization mass spectrometry (NICIGC-MS) [20]. Ethanal was excluded from this study because of possible contamination from solvents. The acyloins we have monitored included 3-hydroxyheptan-2-one, 3-hydroxyoctan-2-one, 3-hydroxynonan-2-one, 3-hydroxydecan-2-one and 3-hydroxydodecan-2-one. The characteristic ions for selected ion recording (SIR) were 126, 140, 244, 258 and 287, respectively. Since the standard acyloins were not commercially available, their concentrations were estimated according to their relevant aldehydes. The coefficient of variability (CV) for within-run and between-run assay precision for measuring aldehydes was assessed using 10 replicate samples of 5 separate experiments, and was found to be between 5–10%.

2.3. Assay of glutathione

Reduced and oxidized glutathione (GSH and GSSG) in whole blood were separated by HPLC and quantitated using an electrochemical detector [21]. 0.2 ml of blood was added to 1.3 ml mobile phase for GSH, and 0.2 ml of blood was added to 1.0 ml mobile phase and 0.3 ml N-ethylmaleimide for GSSG, respectively. Samples were mixed well and centrifuged at 10000 rpm for 5 min. The supernatants were stored at −80°C, and were analyzed within 24 h.

2.4. Creatine kinase (CK) activity

Plasma CK activity was measured by Kodak Ektachem 700 Analyzer (Kodak Canada, Toronto) within 24 h.

2.5. Echocardiography

Systolic blood pressure, and 2-dimensional targeted *m*-mode echocardiography in all 12 control rats and 2 of each experimental group rats were measured using a Programmed Electro-Sphygmomanometer PE-300 (Narco Bio-Systems, Houston, TX), and an ATL Ultramark 9 HDI ultrasound system (Advanced Technology Laboratories, Bothel, WA) equipped with a 7 or 4 MHz broadband phased array transducer, respectively. The relationship between heart rate corrected velocity of circumferential fiber shortening (V_{cfc}) and meridional wall stress was used as a measure of contractility [7].

2.6. Statistical analysis

ANOVA (single-factor) and *F*-test of two-sample for variances was used to check the possible diurnal variation of aldehydes, acyloin and glutathione measurements within the control group. Since there was no significant diurnal variation during the experiment period observed, all control data were combined as one group. Student *t*-test of unpaired two-sample for means were calculated for aldehyde and acyloin concentrations, GSH and GSSG levels, and CK activities from control and experimental groups. $P < 0.05$ (two-tail) was considered significant for all analyses.

To assess contractility, V_{cfc} and meridional wall stress were analyzed by linear regression analysis, and a normal relationship defined from control animals. Values significantly below the normal regression line may indicate impaired contractility, points above suggest an enhancement. The change in the experimentally determined V_{cfc} following DOX was considered an indicator of the effect of treatment.

3. Results

Fig. 1 shows total aldehyde concentrations in rat plasma (A) and heart (B) at various times after 10

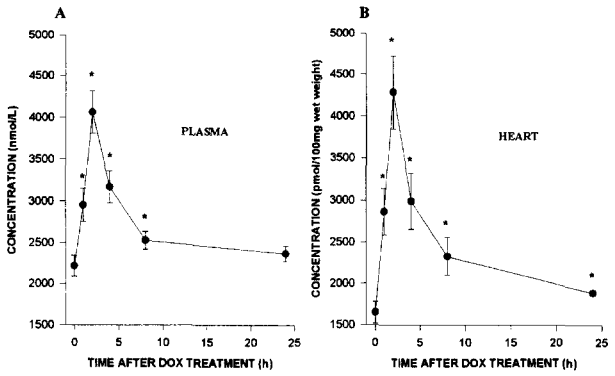

Fig. 1. Total aldehydes in rat plasma and heart. Total aldehyde concentrations in rat plasma and heart (A, plasma; B, heart). Data presented as mean ± SD. * $P < 0.01$.

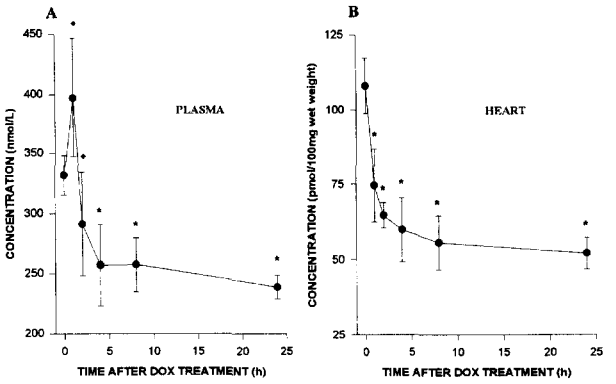

Fig. 2. Total acyloins in rat plasma and heart. Total acyloin concentrations in rat plasma and heart (A, plasma; B, heart). Data presented as mean ± SD. * $P < 0.01$, ♦ $P < 0.05$.

mg/kg of bolus injection of DOX intraperitoneally. Total aldehydes in rat plasma and heart tissue increased significantly, peaked at 2 hours following in vivo DOX treatment. The aldehyde levels declined or returned to control values 8–24 h after DOX administration. Fig. 2 shows the changes of acyloins in rat plasma (A) and heart (B) after DOX treatment. Total acyloin levels in rat plasma increased at 1 h after

Table 1
Aldehydes and acyloins concentration in rat plasma following doxorubicin treatment

Aldehydes and Acyloins	Rat-control ($n = 12$)	Rat-1 h ($n = 4$)	Rat-2 h ($n = 4$)	Rat-4 h ($n = 4$)	Rat-8 h ($n = 4$)	Rat-24 h ($n = 4$)
Propanal	117.0 ± 20.9	211.5 ± 16.5 *	318.5 ± 13.5 *	252.4 ± 22.1 *	162.5 ± 21.4 *	139.8 ± 11.7
Butanal	160.4 ± 42.5	204.6 ± 16.2	286.4 ± 68.8 *	217.4 ± 37.5 ♦	164.5 ± 16.7	159.2 ± 19.0
Pentanal	85.3 ± 13.2	108.0 ± 13.1 *	154.2 ± 48.7 *	129.6 ± 42.0 *	77.2 ± 13.5	82.3 ± 18.2
Furfural	23.2 ± 4.6	20.1 ± 1.4	29.5 ± 9.8	39.5 ± 12.9 *	34.4 ± 5.7 *	27.8 ± 9.3
Hexanal	287.5 ± 35.6	399.6 ± 66.4 *	505.3 ± 41.2 *	346.7 ± 59.5 *	320.4 ± 80.5	277.2 ± 49.2
trans-2-Hexenal	16.7 ± 1.4	18.2 ± 5.0	18.7 ± 4.8	19.4 ± 4.6	16.2 ± 4.1	17.5 ± 3.9
Heptanal	182.7 ± 35.6	184.3 ± 18.9	228.4 ± 85.7	195.3 ± 65.0	184.9 ± 40.6	164.9 ± 29.1
trans-2-Heptenal	104.4 ± 19.2	210.1 ± 78.8 *	357.8 ± 89.6 *	209.9 ± 26.9 *	115.4 ± 37.1	161.2 ± 102.8
trans,trans-2,4-Heptadienal	16.8 ± 3.9	21.0 ± 4.1	22.0 ± 9.0	17.3 ± 5.1	17.1 ± 6.8	15.7 ± 2.5
Octanal	219.0 ± 34.9	341.3 ± 18.5 *	373.5 ± 84.3 *	260.2 ± 36.3	234.0 ± 45.9	206.8 ± 40.7
trans-2-Octenal	23.0 ± 5.7	28.5 ± 6.4	35.2 ± 8.3 *	25.3 ± 7.0	21.0 ± 7.3	21.1 ± 7.4
Nonanal	57.0 ± 18.7	85.1 ± 5.9 ♦	133.2 ± 14.9 *	107.8 ± 11.0 *	80.9 ± 17.8 ♦	69.9 ± 19.1
trans-2-Nonenal	123.3 ± 16.2	156.4 ± 46.3 ♦	208.2 ± 33.9 *	157.5 ± 47.1 ♦	158.2 ± 30.1 *	140.4 ± 31.2
trans-2,cis-6-Nonadienal	2.7 ± 0.8	3.8 ± 1.2 *	9.4 ± 3.8 *	4.9 ± 0.9 ♦	3.9 ± 1.0	3.2 ± 1.1
trans,trans-2,4-Nonadienal	26.8 ± 1.8	27.9 ± 4.3	31.2 ± 4.1 *	77.3 ± 10.6	25.8 ± 4.2	24.0 ± 6.8
4-Hydroxy-non-2-enal	86.0 ± 14.2	142.7 ± 38.9 *	189.7 ± 72.6 *	135.1 ± 53.6 *	86.9 ± 27.6	94.3 ± 9.4
Decanal	165.6 ± 59.1	198.3 ± 34.2	278.1 ± 74.9 *	197.1 ± 89.6	159.9 ± 60.2	162.2 ± 51.3
trans-4,cis-4-Decenal	56.8 ± 9.4	92.9 ± 14.6 *	141.8 ± 47.4 *	111.1 ± 21.6 *	89.2 ± 20.9 *	81.5 ± 32.7 ♦
Dodecanal	163.7 ± 32.0	185.3 ± 77.2	289.4 ± 38.7 *	250.1 ± 37.8 *	212.4 ± 55.1 ♦	167.7 ± 37.6
Malondialdehyde	299.0 ± 53.7	312.6 ± 67.4	452.5 ± 85.8 *	410.5 ± 86.5 *	363.8 ± 69.6	354.1 ± 50.9
3-Hydroxyheptan-2-one	105.1 ± 15.9	129.8 ± 15.3 *	75.3 ± 11.8 *	70.9 ± 15.3 *	82.5 ± 14.2 ♦	108.7 ± 14.7
3-Hydroxyoctan-2-one	18.7 ± 2.0	22.7 ± 7.0	18.9 ± 3.4	19.2 ± 1.6	21.1 ± 7.0	19.4 ± 2.3
3-Hydroxynonan-2-one	11.4 ± 1.9	17.6 ± 5.8 *	8.9 ± 1.7 *	7.5 ± 2.1 *	4.8 ± 1.6 *	4.1 ± 2.7 *
3-Hydroxydecan-2-one	106.9 ± 18.5	132.1 ± 46.7	100.5 ± 40.6	92.0 ± 13.9	73.3 ± 6.0 *	61.0 ± 5.0 *
3-Hydroxydodecan-2-one	89.5 ± 15.4	94.5 ± 32.7	87.6 ± 30.8	67.4 ± 18.8 ♦	75.7 ± 32.9	45.6 ± 12.2 *

Data presented as mean ± SD (nmol/l), * $P < 0.01$, ♦ $P < 0.05$ compared with control.

DOX treatment, and then significantly decreased. The ratio of aldehydes to acyloins, which reflects the proportion of saturated aldehydes detoxified by Pyruvate Dehydrogenase (PDH) to acyloins, increased following DOX treatment. The plasma ratio doubled at 2 h, then declined towards control values. There was a significant, sustained decrease of acyloins in heart tissue following DOX treatment. In heart the ratio increased more than four-fold by 2 h suggesting that the increase in toxic aldehydes was accompanied by inhibition of the detoxification mechanisms, possibly because of decreased PDH activity.

Tables 1 and 2 showed the individual aldehyde and acyloin concentrations in rat plasma and heart tissue following DOX treatment. There was a remarkable increase in most aldehydes with a peak around 2 h. Significant changes of some well-known toxic aldehydes included hexanal, 4-hydroxy-non-2-enal (HNE) and malondialdehyde (MDA). There was a early increase followed by decreases in some of the acyloins in rat plasma, while most of the acyloins in rat heart decreased significantly.

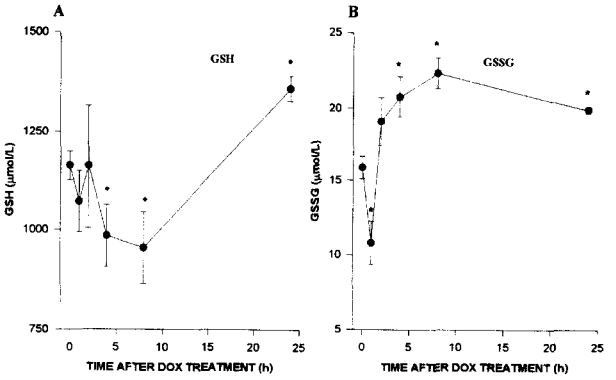

Fig. 3. GSH and GSSG levels in rat blood following DOX treatment. GSH and GSSG levels in rat blood following DOX treatment (A, GSH; B, GSSG). Data presented as mean ± SEM. * $P < 0.01$, ♦ $P < 0.05$.

Table 2
Aldehydes and acyloins concentration in rat heart following doxorubicin treatment

Aldehydes and Acyloins	Rat-control ($n = 12$)	Rat-1 h ($n = 4$)	Rat-2 h ($n = 4$)	Rat 4 h ($n = 4$)	Rat-8 h ($n = 4$)	Rat-24 h ($n = 4$)
Propanal	60.5 ± 8.9	150.6 ± 30.8 *	260.8 ± 49.2 *	192.0 ± 24.5 *	118.6 ± 29.6 *	74.1 ± 14.6 ♦
Butanal	15.1 ± 4.3	42.0 ± 6.8 *	88.9 ± 24.7 *	55.2 ± 16.4 *	35.8 ± 3.4 *	22.3 ± 9.7
Pentanal	10.3 ± 4.3	25.3 ± 4.9 *	73.8 ± 21.0 *	61.5 ± 23.6 *	22.6 ± 9.7 *	10.6 ± 2.0
Furfural	2.8 ± 0.8	13.0 ± 1.2 *	21.9 ± 1.9 *	14.1 ± 2.9 *	8.2 ± 4.5 *	5.2 ± 1.5 *
Hexanal	138.0 ± 26.0	277.5 ± 24.6 *	417.4 ± 100.5 *	271.0 ± 41.2 *	187.9 ± 51.3 ♦	159.6 ± 30.9
trans-2-Hexenal	13.0 ± 1.1	35.4 ± 10.8 *	45.1 ± 7.2 *	30.0 ± 5.9 *	15.9 ± 5.2	13.5 ± 2.7
Heptanal	157.6 ± 26.8	251.0 ± 64.6 *	362.8 ± 69.0 *	221.4 ± 16.3 *	175.6 ± 14.4	159.1 ± 6.6
trans-2-Heptenal	99.4 ± 35.8	219.9 ± 81.7 *	324.2 ± 61.3 *	214.1 ± 76.4 *	150.3 ± 42.5 ♦	112.9 ± 16.5
trans,trans-2,4-Heptadienal	21.4 ± 4.8	21.5 ± 2.2	28.7 ± 10.6	27.5 ± 10.8	19.4 ± 4.1	19.5 ± 2.8
Octanal	191.6 ± 27.9	268.1 ± 19.9 *	348.4 ± 88.9 *	263.3 ± 26.3 *	213.8 ± 14.4	187.9 ± 34.1
trans-2-Octenal	12.5 ± 3.1	19.9 ± 3.2 *	22.3 ± 5.4 *	18.4 ± 1.3 *	14.4 ± 3.9	17.1 ± 2.9
Nonanal	241.1 ± 71.6	248.8 ± 38.4	362.7 ± 136.5 ♦	207.6 ± 46.1	232.7 ± 107.5	219.7 ± 64.1
trans-2-Nonenal	95.0 ± 20.4	205.8 ± 84.2 *	224.2 ± 60.3 *	182.6 ± 39.0 *	142.8 ± 54.3 ♦	104.5 ± 21.3
trans-2,cis-6-Nonadienal	2.4 ± 0.7	5.1 ± 2.7 *	9.6 ± 2.2 *	4.5 ± 2.6 ♦	3.7 ± 2.4	2.5 ± 0.6
trans,trans-2,4-Nonadienal	14.8 ± 4.8	16.3 ± 3.2	19.9 ± 5.6	14.4 ± 4.8	16.5 ± 1.4	13.6 ± 3.2
4-Hydroxy-non-2-enal	61.7 ± 20.6	126.9 ± 13.3 *	194.7 ± 40.8 *	101.5 ± 37.6 ♦	85.7 ± 28.7	76.2 ± 9.2
Decanal	57.1 ± 15.5	127.9 ± 36.7 *	179.7 ± 39.2 *	101.1 ± 55.3 ♦	95.9 ± 41.4 ♦	85.7 ± 24.2 ♦
trans-4,cis-4-Decenal	107.5 ± 16.5	162.4 ± 33.0 *	187.9 ± 23.8 *	139.7 ± 28.3 ♦	115.2 ± 29.5	93.7 ± 15.5
Dodecanal	100.2 ± 13.4	209.6 ± 53.5 *	238.2 ± 53.8 *	163.9 ± 47.5 *	112.8 ± 40.3	100.4 ± 19.8
Malondialdehyde	245.5 ± 50.0	439.3 ± 94.2 *	872.6 ± 159.9 *	704.8 ± 195.8 *	564.1 ± 162.3 *	405.4 ± 36.2 *
3-Hydroxyheptan-2-one	32.2 ± 9.8	20.2 ± 7.3 ♦	21.0 ± 6.2	24.4 ± 6.9	27.3 ± 7.3	24.1 ± 4.2
3-Hydroxyoctan-2-one	10.6 ± 1.4	9.0 ± 0.9	7.1 ± 2.5 *	4.4 ± 2.2 *	3.8 ± 1.6 *	4.3 ± 1.5 *
3-Hydroxynonan-2-one	12.7 ± 3.0	8.8 ± 4.6	7.2 ± 5.2 *	4.0 ± 2.3 *	4.0 ± 2.6 *	5.1 ± 2.0 *
3-Hydroxydecan-2-one	35.8 ± 8.9	25.3 ± 5.9 ♦	20.8 ± 8.6 ♦	20.1 ± 6.6 *	13.0 ± 3.6 *	13.5 ± 5.0 *
3-Hydroxydodecan-2-one	16.7 ± 3.9	11.2 ± 2.6 ♦	8.5 ± 2.2 *	6.9 ± 1.4 *	7.4 ± 1.7 *	5.0 ± 1.4 *

Data presented as mean ± SD (pmol/100 mg wet wt.). * $P < 0.01$, ♦ $P < 0.05$ compared with control.

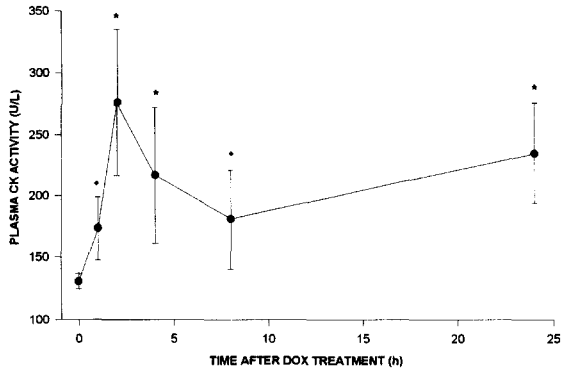

Fig. 4. CK activity in rat plasma following DOX treatment. Data presented as mean ± SEM. * $P < 0.01$, ◆ $P < 0.05$.

Fig. 3 showed the GSH (A) and GSSG (B) levels in rat blood following DOX treatment. GSH levels decreased early (4 and 8 h), and increased by 24 h, while GSSG levels were reduced at 1 h, and significantly elevated 4–24 h after DOX treatment.

CK activity in rat plasma following DOX treatment was shown in Fig. 4. CK levels were significantly elevated in all time point following DOX treatment.

Fig. 5 showed the wall stress/V_{cfc} relationship in normal and DOX-treated rats. The relationship between wall stress and the velocity of circumferential fiber shortening is an index of contractility. This was determined to be a normal inverse linear one in our control rat population. Line A in Fig. 9 is from the control rats, and was obtained by least square linear regression analysis. The equation for this line was $Y = 2.4788 - 0.0404X$, and the correlation coefficient, r^2 was 0.6480 ($P < 0.01$). DOX treatment altered contractility in these rats. The regression line for the treated animals (line B) shifted upward, and although the relationship was still an inverse linear one, the scatter was much greater. The equation for the line for the post-DOX animals was $Y = 2.2921 - 0.0183X$, and r^2 was 0.3017 ($P > 0.05$). After DOX, contractility was enhanced in some of animals, while in others the shift from normal to abnormal suggested a functional impairment, the changes however were not consistent.

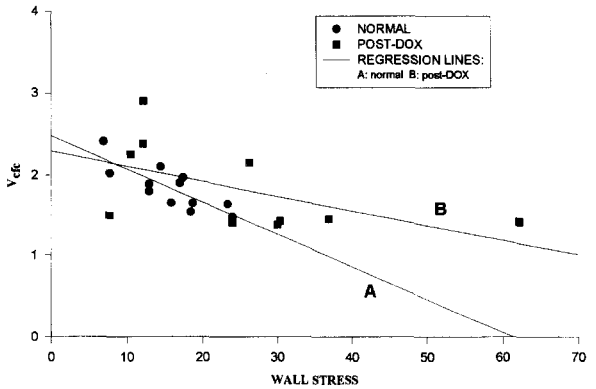

Fig. 5. Wall stress/V_{cfc} relationship in normal and DOX treated rats. Linear regression between wall stress and V_{cfc} for control animals is represented by line A ($Y = 2.4788 - 0.040X$, $r^2 = 0.6480$, $P < 0.01$). Line B ($Y = 2.2921 - 0.0183X$, $r^2 = 0.3017$, $P > 0.05$) is from post-DOX treated animals.

4. Discussion

Free radicals are generally short lived and thus inflict damage only in the local environment where they are produced. Recent findings indicate that lipid peroxidation also results in the production of a great variety of stable, diffusible saturated and unsaturated aldehydes including MDA, 4-hydroxy-alkenals, alkanals, 2-alkenals, and 2,4-alkadienals. The cytotoxic aldehydes are extremely active, they can diffuse within or even escape from the cell and attack targets far from the site of the original free radical initiated event, and therefore act as 'second cytotoxic messengers' [19,22,23]. Among them, HNE is an α,β-unsaturated aldehyde that can be formed by peroxidation of ω-6 unsaturated fatty acids such as linoleic and arachidonic acids [24]. HNE originates almost exclusively from phospholipid-bound arachidonic acid and may be a highly reliable index of free-radical induced lipid peroxidation. HNE exhibits a variety of cytopathological effects such as enzyme inhibition, inhibition of DNA and RNA synthesis, inhibition of protein synthesis, and induction of heat shock proteins. It also shows genotoxic and mutagenic effects as well as inhibitory effects on cell proliferation [22,25,26].

Aldehydes are potent cytotoxic agents that react with different biomolecules, thus changing the biochemical status of the cell, resulting finally in cell damage [27]. Our data clearly demonstrate that the aldehydes in rat plasma and heart tissue increased significantly following in vivo DOX treatment. The changes occurred early, peaked around 2 h after DOX

administration, and the levels declined or returned to normal values within 8–24 h. These changes included some of the well-known toxic aldehydes such as hexanal, HNE and MDA.

Recently studies have shown that pyruvate dehydrogenase (PDH) catalyzes a little known conjugation reaction between pyruvate and saturated aldehydes to produce acyloins (3-hydroxyalkan-2-ones), which can then be reduced endogenously to the corresponding 2,3-alkanediols. Since acyloins and 2,3-alkanediols are stable and freely diffusible, PDH could catalyze an alternative and ubiquitous detoxification mechanism for saturated aldehydes produced by lipid peroxidation [28,29]. We found that the acyloin levels increased and then decreased in plasma, and there was a significant decrease of acyloins in heart tissues after DOX treatment. Although the metabolic detoxification of toxic aldehydes is not well understood, our data suggest that there may be an inhibition by DOX of PDH, or of the PDH-Acyloin Synthase (PDH-AS) activity. An impairment of PDH and/or PDH-AS activity may play a role in DOX-induced cardiotoxicity.

The body has elaborate defenses to prevent injury caused by free radical-induced lipid peroxidation. The enzyme systems functioning in this capacity include catalase, superoxide dismutase (SOD), glutathione peroxidase (GP), and a number of free radical scavengers such as glutathione (GSH), Vitamins E and A, etc. [24,27,30–32]. GSH levels decreased early, then increased by 24 h, while GSSG levels decreased, then increased after DOX treatment, suggesting a early depletion of GSH and later overcompensation. GSH, a tripeptide synthesized by glutathione synthetase, is thought to be one of the most effective primary antioxidants against oxidizing agents. The electron-rich sulfhydryl group in GSH can neutralize free radicals or conjugate with aldehydes and intercept their reactions with cell constituents. GSH synthetase requires 1–2 h to return the GSH level to its original or even higher concentrations after detecting a reduction in the cellular GSH concentration [27,33]. A bolus injection of a large dose of DOX will induce an overwhelming oxidative stress and may cause the depletion of some antioxidants.

Echocardiography is one of the most important techniques for early detection of anthracycline-induced cardiomyopathy [8,34]. In our study, we were able to evaluate the relationship between the wall stress and the velocity of circumferential fiber shortening in normal rats. CK levels were elevated after DOX administration, indicating myocardial injury. Our results indicated that in some of the animals following treatment there was a shift from normal to abnormal (increased or decreased contractility) as a consequence of DOX exposure, this however was not a consistent finding. Acute, transient effects of DOX have been reported to included tachycardia, arrhythmias, and increased beta adrenergic tone. The increased contractility in some animals may be a reflection of these changes. Since the cardiac functional abnormalities following DOX treatment have been documented to occur much later than the initial biochemical changes presented here, a few hours or days might be too short a period for the development of an overt cardiomyopathic process.

DOX is eliminated from the body within 2 days after administration, and the increase in hydroxyl radicals and cytotoxic aldehydes is an early transient event. In contrast, in human subjects who have been treated with DOX, cardiomyopathy may take months or years to develop fully. The development of DOX-induced cardiomyopathy must therefore be due to a complex sequence of events in which the presence of DOX is required only at the beginning to initiate the process. Our hypothesis is that the cytotoxic aldehydes produced as a consequence of DOX-induced lipid peroxidation are the main culprits of the short-term abnormalities, and initiate the signals leading to the chronic, long-term injury, particularly with repeated exposure as in humans. The reactions initiated by the cytotoxic aldehydes result in impaired energy production, membrane damage, protein and DNA damage, and the induction of inflammatory reactions leading to increased synthesis of adhesion molecules and cytokines, infiltration of neutrophils and macrophages, and necrotic or apoptotic death of heart cells.

The present study confirms the involvement of free radicals, and suggests that cytotoxic aldehydes play a key role in initiating the steps that lead to functional impairment of the myocardium following DOX administration. Scavengers and the metabolic removal of some of these aldehydes also play a role in protecting the myocardium against injury. Further

investigations concerning the cascade of events following DOX treatment that lead ultimately to cardiomyopathy, and a study of the effectiveness of specific cardioprotective agents to interfere with the initial biochemical changes are underway in our laboratory.

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