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Effects of chronic administration of doxorubicin on plasma levels of prostaglandins, thromboxane B_2, and fatty acids in rats

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Summary. The effects of multiple doses of doxorubicin (DXR) on hematocrit and plasma levels of prostaglandins (PG), thromboxane B_2 (TxB_2), total lipid, esterified and free fatty acids, and proteins were investigated in male rats. The rats received DXR (2mg/kg) or vehicle weekly by the subcutaneous route for 2, 4, 8, and 13 weeks and were killed 1 week after their last dose. Another group of rats treated for 13 weeks was sacrificed at 19 weeks, 6 weeks after the last dose. No changes in hematocrit or plasma PG, TxB_2, or total levels of fatty acids were noted between control and DXR-treated rats at either 3, 5, or 9 weeks. The hematocrit was slightly depressed from control levels at 14 and 19 weeks. Plasma PGE, $PGF_{2\alpha}$, and TxB_2 were elevated over control levels at 14 and 19 weeks. Plasma 6-keto-$PGF_{1\alpha}$ was increased over the control level only at 19 weeks. Total plasma lipid and esterified fatty acids were highly elevated over control levels at 14 and 19 weeks. Plasma free arachidonic acid was elevated over control levels at 14 and 19 weeks, while levels of other free fatty acids were unchanged. Plasma protein levels were slightly depressed from control levels at 3, 9, and 14 weeks. Elevations of plasma free arachidonic acid, PG, TxB_2, esterified fatty acids, and total lipid might be involved in cardiotoxicity and nephrotoxicity found with chronic administration of DXR.

Introduction

Doxorubicin (DXR) is an effective chemotherapeutic agent employed for the treatment of a variety of human neoplasms [3]. Unfortunately the use of DXR is associated with the development of congestive heart failure [5]. DXR-induced cardiac damage, which is characterized by an insidious progressive degeneration of heart muscle, has been found to be dependent on the total cumulative dose administered [26]. All patients given DXR ultimately develop some degree of cardiac damage, which in advanced stages causes congestive heart failure [11]. This side effect poses the problem of discontinuing effective cancer treatment due to the risks of severe irreversible damage to the heart [5].

The physiological and biochemical manifestations of this drug-induced cardiotoxicity are often delayed in their nature. The mechanism of DXR-induced cardiotoxicity is not clear, and definition of the biochemical effects that lead to cardiac damage is difficult [36]. One mechanism of cardiotoxicity that has received a great deal of attention is the ability of DXR to generate free radicals as a consequence of redox cycling [16].

Many studies have linked the toxicity of DXR to the ability of this drug to form a semiquinone free radical intermediate [19]. This reactive intermediate is able to interact with molecular oxygen to produce superoxide anion through a reduction-autoxidation cycle [19]. Hydroxyl radicals, peroxy radicals, and hydrogen peroxide, derived from the decomposition of superoxide anion, are known to initiate free-radical-mediated chain reactions which result in conversion of membrane-unsaturated fatty acids to lipid peroxides [6]. Free radical reactions and lipid peroxidation may play a major role in the cardiotoxicity of DXR.

Numerous studies have suggested that lipid peroxides derived from the autoxidation of membrane-unsaturated fatty acids stimulate the biosynthesis of prostaglandins (PG) and thromboxane A_2 (TxA_2) [25, 33]. It has been postulated that the enzyme cyclooxygenase contains a saturable activator site for lipid peroxides [25]. The activity of this enzyme is dependent on a continous low level of peroxide [25]. These findings have important implications in cell function, since any process which generates peroxides may activate cyclooxygenase [25]. The quinone structure of DXR is unstable and may be converted to the free radical semiquinone in cardiac sarcosomes; the semiquinone is a probable cofactor for PG synthesis [4].

Ohuchi and Levine [31] have shown that DXR stimulates PG synthesis in dog kidney cells. Bristow et al. [4] found that levels of immunoreactive PGE and PGF in coronary sinus blood of open-chest dogs began to rise 30 min after initiation of a continuous infusion of DXR and increased slowly thereafter for the remainder of the 3-h study. Sanma and Nakamura [35] noted the appearance of leukotriene C, an arachidonate metabolite, in hearts of guinea pigs treated with DXR (5 mg/kg/day, i.v.) for 2 consecutive days.

The present study was designed to determine whether alterations in PG and TxA_2 occur with chronic administration of DXR. Plasma levels of arachidonic acid, PG, and TxB_2 were examined in rats treated chronically with DXR. Rats treated with DXR have been found to be severely hyperlipidemic, so plasma levels of palmitic acid, stearic

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acid, oleic acid, linoleic acid, and total lipids were also measured. Hematocrit and plasma protein values were also monitored to assess the animals' general health.

Materials and methods

Male Sprague-Dawley rats approximately 2 months old and weighing 150–200 g were purchased from Bentim and Kingom, Inc. (Oakland, Calif, USA). The rats were housed two to three per cage in approved facilities and had full access to laboratory chow and tap water ad libitum.

Doxorubicin hydrochloride was a generous gift from Farmitalia Carlo Erba of Milan, Italy. Additonal supplies of DXR were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). The radioimmunoassay (RIA) kits for the assay of $PGF_{2\alpha}$ and PGE ($PGE_1 + PGE_2$) were purchased from Clinical Assays (Cambridge, Mass, USA). [^{3}H]-$PGF_{2\alpha}$ (specific activity 165 Ci/mmol), [^{3}H]-PGE_2 (specific activity 165 Ci/mmol), [^{3}H]-TxB_2 (specific activity 100 Ci/mmol), and [^{3}H]-6-keto-$PGF_{1\alpha}$ (specific activity 120 Ci/mmol) were purchased from New England Nuclear Corporation (Boston, Mass, USA). Unlabeled TxB_2 and 6-keto-$PGF_{1\alpha}$ and antibodies specific to TxB_2 and 6-keto-$PGF_{1\alpha}$ were purchased from Upjohn Diagnostic Laboratories (Kalamazoo, Mich, USA and Seragen, Inc. (Boston, Mass, USA), respectively. Palmitic, stearic, oleic, linoleic, and heptadecanoic acid standards were purchased from Nu-Chek (Elysia, Minn, USA). Heptadecanoic acid methyl ester and arachidonic acid standards were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). GP 10% SP-2330 on 100/120 Chromosorb W was purchased from Supelco, Inc. (Bellefonte, Pa, USA). Other reagents were of analytical grade and obtained from standard commercial sources.

Treatment of animals. Rats were treated with DXR according to a modification of the procedure devised by Mettler et al. [28]. They received a subcutaneous dose of 2 mg/kg DXR once per week for 2, 4, 8, and 13 weeks. DXR was freshly dissolved in phosphate-buffered saline (PBS, 0.05 *M* KH^2P0^4, 0.9% NaCl, pH 7.4). Corresponding control rats received an equal volume of PBS. Body weights were recorded weekly. Rats were killed 1 week after the last dose at 3, 5, 9, and 14 weeks. One group of rats that received 13 consecutive weekly doses of DXR plus a corresponding control group were killed at 19 weeks, 6 weeks after the last dose.

Preparation of blood for analysis of fatty acid, PG and TxB_2 levels. At the time of killing rats were anesthetized with pentobarbital (60 mg/kg, i.p.) and blood was drawn from the inferior vena cava in a heparinized syringe. Blood from three rats of the same group was pooled into a tube kept on ice containing indomethacin (1 mg/10 ml blood). This amount of indomethacin has been shown to prevent artifactual arachidonate metabolism [14]. The blood was centrifuged at 3000 rpm for 20 min in a refrigerated centrifuge at 4 °C. The plasma was then aspirated without disturbing the buffy coat and aliquoted. Plasma (2.5 ml) for analysis of PG and TxB_2 was acidified with 6 *N* HCl (0.1 ml/ml plasma), immediately spiked with 2000 cpm of [^{3}H]-PGE_2, [^{3}H]-$PGF_{2\alpha}$, or [^{3}H]-TxB_2, and then frozen at −80 °C until extraction. Plasma spiked with [^{3}H]-TxB_2 was used for determination of both TxB_2 and 6-keto-$PGF_{1\alpha}$, since these were found to have almost identical recovery and no cross-reactivity with each other's antibody [7]. Plasma for analysis of fatty acids (2–3 ml) and total lipids (1 ml) was aliquoted into test tubes and sealed under nitrogen.

Extraction of PG and TxB_2 from plasma. Acidified plasma was thawed at room temperature. The samples for PG and TxB_2 analysis were extracted 3 times in chloroform (40 ml total volume), the chloroform was evaporated under a stream of nitrogen, and the dried samples were then frozen at −20 °C.

The dried samples for plasma PG and TxB_2 analysis were subjected to silicic acid column chromatography according to the technique described by Giri and Krishna [13], with slight modification. Briefly, each sample, was first reconstituted in 1 ml of a toluene:ethylacetate mixture (60:40). This was loaded on a silicic acid column (0.5 g silicic acid slurry). The tube was consecutively rinsed 3 times with 1 ml of the same mixture and loaded on the column. After the mixture had run through the column the PG or TxB_2 was eluted by adding 8 ml of absolute methanol. The methanol was collected and evaporated to dryness under nitrogen. The dried samples were stored at −20 °C until assayed.

Measurement of plasma TxB_2 and 6-keto-$PGF_{1\alpha}$ by RIA. The TxB_2 (stable metabolite of TxA_2) and 6-keto-$PGF_{1\alpha}$ (stable metabolite of PGI_2) concentrations of plasma were measured by RIA as described in our earlier papers [7, 14]. Briefly, dried samples for determination of plasma concentrations of TxB_2 or 6-keto-$PGF_{1\alpha}$ were reconstituted in 500 µl of 0.1 *M* PBS gelatin (pH 7.4). Predetermined aliquots of 50 µl in duplicate of the reconstituted solution were used for RIAs of plasma TxB_2 and 6-keto-$PF_{1\alpha}$. Aliquots of 200 µl of the reconstituted solution were used to determine the percentage of TxB_2 recovered from the plasma. The recovery value of TxB_2 of each sample was also used for the recovery value of 6-keto-$PGF_{1\alpha}$ of the same plasma sample, since the recoveries of these substances have been found to be similar. Recoveries of TxB_2 ranged from 30% to 70% and were used to correct for loss of TxB_2 or 6-keto-$PGF_{1\alpha}$ during extraction and column chromatography to estimate the amount in plasma samples. Cross-reactivities of TxB_2 and 6-keto-$PGF_{1\alpha}$ antiserum were the same as those reported in our earlier papers [7, 14]. The sensitivity limits of the RIAs for TxB_2 and 6-keto-$PGF_{1\alpha}$ were both 10.0 pg/ml plasma.

Measurement of plasma PGE and $PGF_{2\alpha}$ by RIA. The PGE and $PGF_{2\alpha}$ concentrations of plasma were measured by RIA, as described in our earlier papers [7, 14]. Briefly, dried samples for plasma PGE were reconstituted in 1 ml Tris-HCl buffer (pH 7.4) containing 0.1% gelatin and then converted to PGB according to the RIA kit instructions. In this study, PGE refers to PGE_1 and PGE_2 converted to PGB_1 and PGB_2 respectively. The final volume of the reconstituted plasma PGE samples was 1.2 ml. Predetermined aliquots of 25 µl in duplicate for samples were used for measurement of PGB concentrations in each sample, employing the RIA kit for PGE. Aliquots from the reconstituted solutions of 1.2 ml were used to determine the percentage of PGE recovered. PGE concentrations in plasma were corrected for loss during extraction and column

chromatography using the respective recovery values. Recovery for PGE ranged from 20% to 45%. The cross-reactivity for PGB antiserum was the same as that reported in our earlier papers [7, 14]. The sensitivity limit for PGE was 25 pg/ml plasma.

Dried samples for $PGF_{2\alpha}$ were reconstituted in 500 µl Tris-HCl buffer (pH 7.4) containing 0.1% gelatin. Predetermined aliquots of 50 µl in triplicate for plasma samples were used for measurement of $PGF_{2\alpha}$ concentrations in each sample, employing the RIA kit for $PGF_{2\alpha}$. Aliquots from the reconstituted solutions of 200 µl from samples were used to determine the percentage of $PGF_{2\alpha}$ recovered. $PGF_{2\alpha}$ concentrations in plasma determined by RIA were corrected for loss using the respective recovery values. Recovery for $PGF_{2\alpha}$ ranged from 40% to 80% in samples. The cross-reactivity of the $PGF_{2\alpha}$ antiserum was the same as that reported in our earlier papers [7, 14]. The sensitivity limit for $PGF_{2\alpha}$ was 12.5 pg/ml plasma.

Validation of plasma $PGF_{2\alpha}$ and TxB_2 measurements by RIA. Rats killed at 14 and 19 weeks after DXR treatment were found to have severe hyperlipidemia. Some investigators have reported that lipids, particularly free fatty acids, interfere with RIA measurements of PG by displacing the antibody from its antigen leading to artifical values [17]. The validity of the RIA for measurement of TxB_2 and $PGF_{2\alpha}$ was checked by spiking plasma with a known amount of cold PG plus the corresponding radiolabeled PG to monitor recovery, then proceeding with the extraction and column chromatography as described above. Finally, measurement was perforemd by RIA. An aliquot of 0.1 ml plasma was spiked with 500 pg $PGF_{2\alpha}$, while an equal amount of TxB_2 was added to 1 ml of plasma. After RIA and determination of recovery due to losses from the extraction and column procedures, the recovery of the exogenous 500 pg $PGF_{2\alpha}$ or TxB_2 was calculated after correcting for the endogenous levels.

Determination of total lipids in plasma and in the sample residue for PG and in analysis after extraction and column chromatography. Determination of total lipids in plasma from rats sacrificed at 14 and 19 weeks was based upon the gravimetric method described by Naito and David [29]. A Mettler microbalance Model M3 (Mettler Instruments Corp., Hightstown, NJ, USA) was used for this work.

The total lipid in samples for PG measurement after extraction and column chromatography was also determined. Plasma (5.0 ml) was subjected to extraction and column chromatography followed by gravimetric determination of total lipid.

Measurement of esterified and free fatty acids in plasma. Esterified and free palmitic, stearic, oleic, linoleic, and arachidonic acids in plasma were measured using a modification of the procedure described by Hagenfeldt [18] with heptadecanoic acid or its methyl ester as the internal standard. Methylation was performed using the method of Fales et al. [10]. Esterified and free fatty acids were determined for rats killed at 14 and 19 weeks.

Individual fatty acid methyl esters were separated by gas chromatography. The packing material, GP 10% SP-2330 on 100/120 Chromosorb W, was packed in a 6-ft-long by 1/8-in. internal diameter glass column. A Perkin Elmer Sigma 2000 gas chromatograph with a flame ionization detector (FID) was used for this work. Injector, oven, and detector temperatures were 250°, 200°, and 250 °C, respectively. Helium was used as carrier gas and the flow rate was set at 30 ml/min. Oxygen and hydrogen flow rates for the FID were both set at 20 ml/min. Data was collected and integrated with a Perkin Elmer 3600 computer using a Chrom 2 software system. Fatty acid methyl esters were identified by comparison of retention times with authentic fatty acid standards that had been methylated. Peak areas were measured and compared with the peak area of the internal standard. Fatty acid values are expressed as micrograms per milliliter of plasma.

Hematocrit and plasma proteins. Hematocrit and plasma proteins were determined in rats treated with either DXR or PBS. Hematocrit and plasma proteins were determined by the method of Jasper and Jain [22]. Due to the presence of a severe hyperlipidemia at 14 and 19 weeks, protein content in the plasma was determined by the method of Lowry [27].

Statistical methods. The data are expressed as the mean ± standard error of the mean (SEM). Statistical differences between control and DXR-treated rats were assessed using Student's *t* test. Probability values of ≤ 0.05 were considered significant.

Results

Rats killed at 14 and 19 weeks had very prominent gross pathological signs of DXR-induced congestive heart failure. Rats at both 14 and 19 weeks exhibited severe plasma lipemia. Histological analysis (not shown) revealed that sections of liver and lung did not show treatment-related changes, while lesions were consistently present in heart and kidney of DXR-treated rats. Lesions in the heart were most severe in the DXR-treated group at 19 weeks, relatively mild in the DXR-treated group at 14 weeks, and absent in both corresponding PBS-treated groups. Myocyte changes were most severe in the left ventricle, occurred in scattered individual fibers, and included loss of striations with clumping of the sarcoplasm into granules, phagocytosis of degenerate fibers by macrophages, and proliferation of myocyte nuclei. Renal lesions, which included glomerular, interstitial, and tubular changes, were apparent at 14 and 19 weeks and were similar to those described by Van Hoesel et al. [40]. The weight gain of DXR-treated rats paralleled that of the corresponding controls for the first 3 weeks of the study and then began to increase at a much slower rate than that of the controls for the remaining period of the study. A slight increase in weight gain of drug-treated animals occurred from 14 to 19 weeks after discontinuation of DXR treatment.

Figures 1–4 show changes in plasma levels of PGE, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and TxB_2 of DXR-treated rats respectively. Plasma PGE was elevated to 642 and 206 ng/ml from control (PBS) levels of 60 and 6 ng/ml at 14 and 19 weeks respectively. Plasma $PGF_{2\alpha}$ was elevated to 16 and 21 ng/ml respectively, from a control level of 1.2 ng/ml at both 14 and 19 weeks. Plasma 6-keto-$PGF_{1\alpha}$ was elevated to 2000 pg/ml from a control level of 760 pg/ml at 19 weeks. Plasma TxB_2 was elevated to 2600 and 5000 pg/ml from control levels of 1550 and 1040 pg/ml at 14 and 19 weeks, respectively. Pace-Asciak and Micallef [32] de-

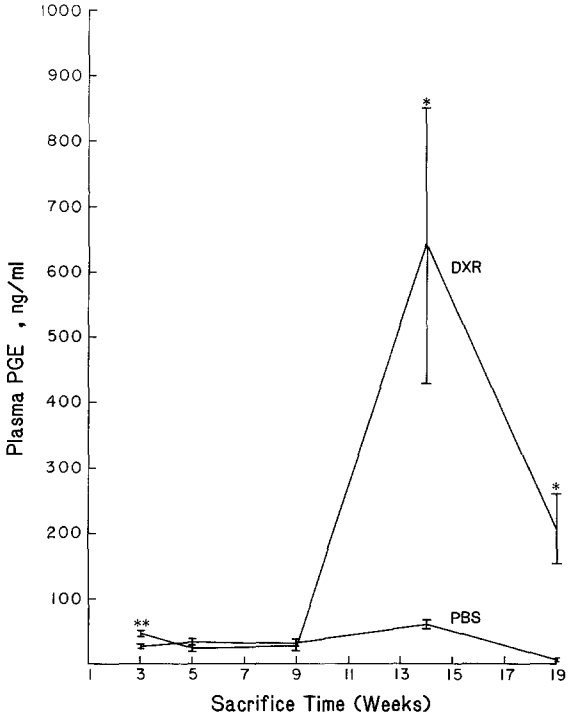

Fig. 1. Effect of multiple doses of doxorubicin on plasma PGE. Rats received a subcutaneous dose of phosphate-buffered saline (PBS) or 2 mg/kg doxorubicin (DXR) weekly for 2, 4, 8, and 13 weeks and killed 1 week after the last dose, at 3, 5, 9, and 14 weeks. One group of rats that received 13 consecutive weekly doses of DXR and a corresponding control group were killed at 19 weeks, 6 weeks after the last dose. Each point represents the mean ± SEM of five different pooled samples (each from three rats). $*p<0.05$

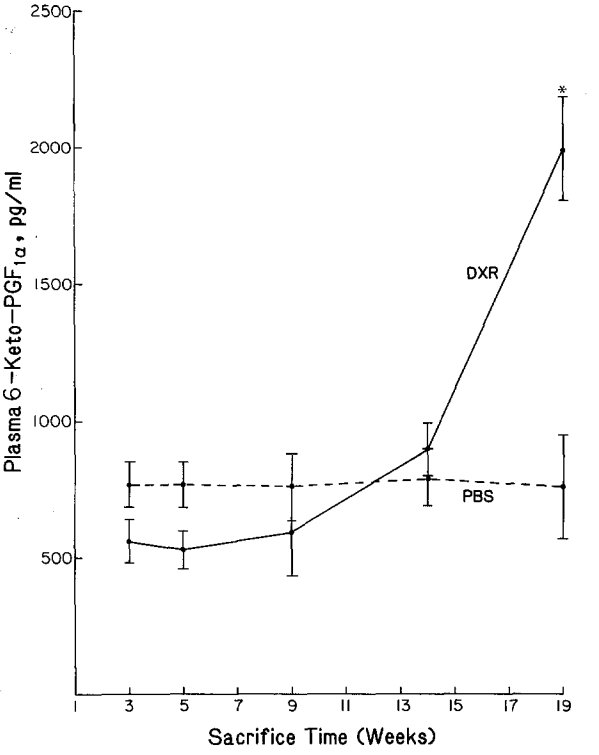

Fig. 3. Effect of multiple doses of doxorubicin on plasma 6-keto-$PGF_{1\alpha}$. Rats received a subcutaneous dose of phosphate-buffered saline (PBS) or 2 mg/kg doxorubicin (DXR) weekly for 2, 4, 8, and 13 weeks. Rats were killed 1 week after the last dose, at 3, 5, 9, and 14 weeks. One group of rats that received 13 consecutive weekly doses of DXR and a corresponding control group were killed at 19 weeks, 6 weeks after the last dose. Each point represents the mean ± SEM of five different pooled samples (each from three rats). $*p<0.05$

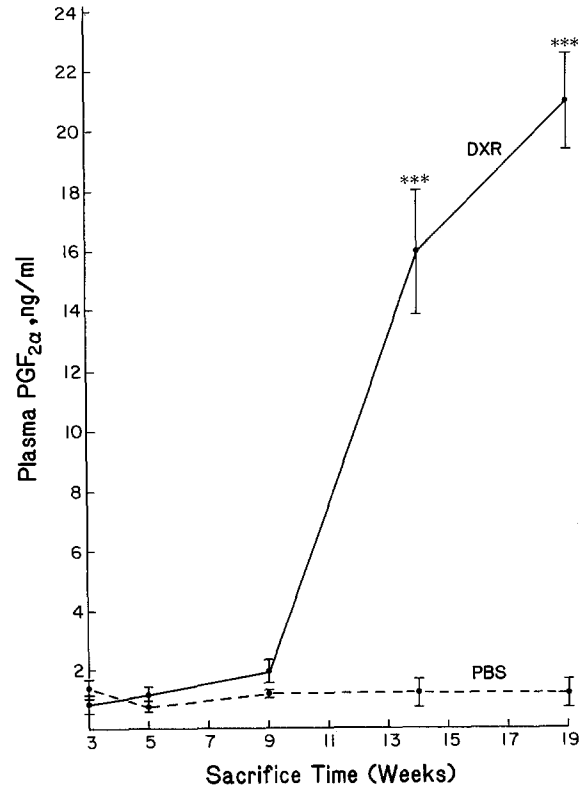

Fig. 2. Effect of multiple doses of doxorubicin on plasma $PGF_{2\alpha}$. Rats received a subcutaneous dose of phosphate-buffered saline (PBS) or 2 mg/kg doxorubicin (DXR) weekly for 2, 4, 8, and 13 weeks. Rats were killed 1 week after the last dose, at 3, 5, 9, and 14 weeks. One group of rats that received 13 concecutive weekly doses of DXR and a corresponding control group were killed at 19 weeks, 6 weeks after the last dose. Each point represents the mean ± SEM of five different pooled samples (each from three rats) $***p<0.001$

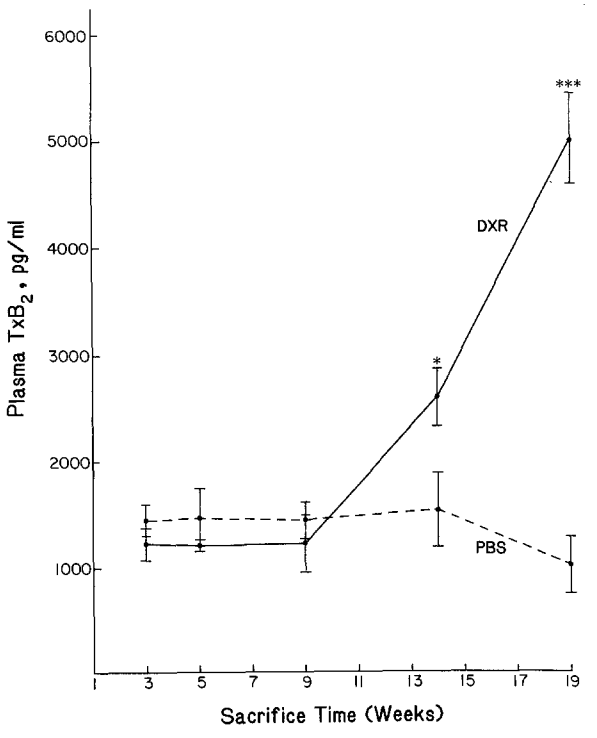

Fig. 4. Effect of muptiple doses of doxorubicin on plasma TxB_2. Rats received a subcutaneous dose of phosphate-buffered saline (PBS) or 2 mg/kg doxorubicin (DXR) weekly for 2, 4, 8, and 13 weeks. Rats were killed 1 week after the last dose, at 3, 5, 9, and 14 weeks. One group of rats that received 13 consecutive weekly doses of DXR and a corresponding control group were sacrificed at 19 weeks, 6 weeks after the last dose. Each point represents the mean ± SEM of five different pooled samples (each from three rats). $*p<0.05$; $***p<0.001$

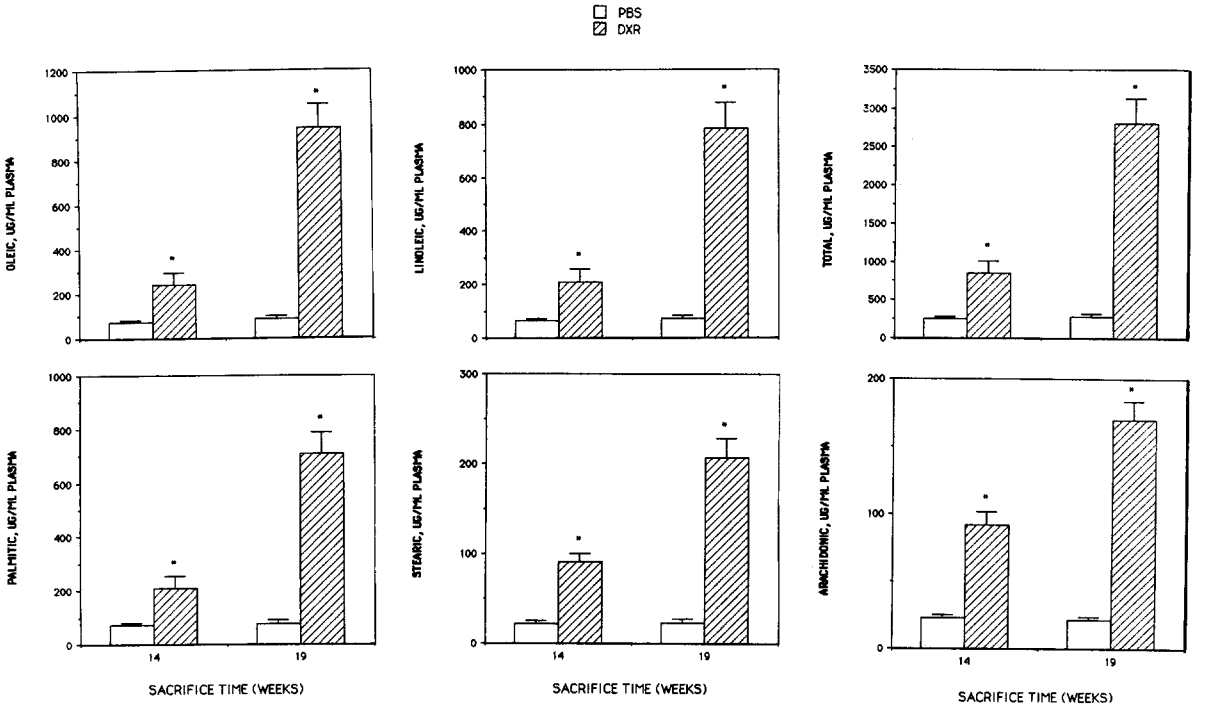

Fig. 5. Effect of multiple doses of doxorubicin on plasma esterified fatty acids. Rats received a subcutaneous dose of phosphate-buffered saline (PBS) or 2 mg/kg doxorubicin (DXR) weekly for 13 weeks. Rats were killed at 14 and 19 weeks, 1 and 6 weeks respectively after the last dose. Each point represents the mean ± SEM of five animals. $*p < 0.0001$

Table 1. Plasma free fatty acids following 13 consecutive weekly doses of doxorubicin (DXR) at 2 mg/kg/week or an equal volume of phosphate-buffered saline (PBS)

Killing time (weeks)	Plasma free fatty acids (μg/ml; $\bar{x}$ ± SEM of five animals)									
	Palmitic (16:0)		Stearic (18:0)		Oleic (18:1)		Linoleic (18:2)		Arachidonic (20:4)	
	PBS	DXR	PBS	DXR	PBS	DXR	PBS	DXR	PBS	DXR
14	40.7±9.2	38.9±2.1	37.0±4.5	46.8±4.3	36.3±12.2	22.5±1.4	28.4±10.7	21.7±1.7	6.4±0.6	17.0±1.9[a]
19	37.2±6.7	39.9±3.9	36.1±6.9	43.3±5.3	19.8± 4.9	20.9±3.5	18.0± 3.6	15.8±1.0	6.3±0.4	16.6±4.2[a]

Rats were killed at 14 and 19 weeks, 1 and 6 weeks respectively after the last dose
[a] $p < 0.0001$

Table 2. Hematocrit and plasma protein levels following multiple doses of doxorubicin

Killing time (weeks)	Percentage hematocrit (PCV; $\bar{x}$ ± SEM of five animals)		Plasma proteins (g/100 ml; $\bar{x}$ ± SEM of five animals)	
	PBS	DXR	PBS	DXR
3	39.4±0.4	38.7±0.8	5.23±0.07	5.00±0.05[a]
5	41.0±0.5	39.9±0.5	5.22±0.04	5.16±0.07
9	39.8±0.5	39.0±0.1	5.56±0.08	5.27±0.06[a]
14	32.6±1.0	40.4±0.8[c]	6.27±0.15	5.72±0.08[b]
19	28.6±1.1	38.4±0.2[c]	6.37±0.23	6.24±0.20

Rats received a subcutaneous dose of 2 mg/kg doxorubicin (DXR) weekly for 2, 4, 8, and 13 weeks. Corresponding controls received an equal volume of phosphate-buffered saline (PBS). Rats were killed 1 week after the last dose, at 3, 5, 9, and 14 weeks. One group of rats that received 13 consecutive weekly doses of DXR plus a corresponding control group were killed at 19 weeks, 6 weeks after the last dose
[a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.001$

monstrated the presence of $PGF_{2\alpha}$, PGE_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ in plasma from rats by a gas chromatographic-mass spectrometric method, and levels of $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ were comparable to those found in control rats in the present study.

Validations of RIAs for the measurement of plasma TxB_2 and $PGF_{2\alpha}$, by spiking plasma with 500 pg of either authentic TxB_2 or $PGF_{2\alpha}$ and then determining the recovery, were performed to insure that lipemia present in DXR-treated rats at 14 and 19 weeks did not interfere with the assay. The recovery of exogenously added TxB_2 and $PGF_{2\alpha}$ to plasma from control and DXR-treated rats in four separate determinations averaged 107.5±13.3 (SEM) and 107.5±6.6%, respectively. This suggests that lipemia does not interfere with the RIA procedures employed for the measurement of TxB_2 and PG.

Total plasma lipid in DXR-treated rats was elevated to 21.8±5.8 and 26.9±4.7 mg/ml plasma over control levels of 1.7±0.2 and 1.1±0.4 mg/ml plasma at 14 and 19 weeks, respectively. Measurement of total plasma lipid in samples for plasma PG and TxB_2 analysis showed that the

procedures of chloroform extraction and silicic acid column chromatography had removed greater than 90% of the total lipid originally present in the samples.

Levels of plasma esterified fatty acids are shown in Fig. 5. Levels of esterified palmitic, stearic, oleic, linoleic, and arachidonic acids in DXR-treated rats were all highly elevated over control levels at both 14 and 19 weeks. The total level of plasma esterified fatty acids in DXR-treated rats was significantly increased to 335% and 1013% of controls at 14 and 19 weeks respectively. The total level of esterified fatty acids constituted 3.9 and 14.5% of the total plasma lipid in DXR and PBS-treated rats, respectively, at 14 weeks, while at 19 weeks the corresponding values were 10.5 and 24.8%.

Levels of plasma free fatty acids are shown in Table 1. In contrast to levels of esterified fatty acids, levels of palmitic, stearic, oleic, and linoleic free fatty acids in DXR-treated rats were unchanged from control levels at both 14 and 19 weeks. Fatty acid levels (free + esterified) in plasma were unchanged between DXR and PBS-treated rats at 3, 5, and 9 weeks (data not shown).

Hematocrit and plasma protein values for DXR- and PBS-treated rats are shown in Table 2. Hematocrit values of DXR-treated rats were significantly depressed to 93% of control levels at both 14 and 19 weeks. Plasma protein values in DXR-treated rats were significantly depressed to 95%, 95%, and 91% of control values at 3, 9, and 14 weeks respectively.

Discussion

Biochemical indices of injury became apparent only in rats sacrificed at 14 and 19 weeks. These rats received a cumulative dose of DXR equivalent to 26 mg/kg or 252 mg/m^2. This dose is considerably lower than the 550 mg/m^2 dose of DXR set as a ceiling in human cancer patients due to the increasing risks of cardiac damage [5]. Severe hyperlipidemia was present in DXR-treated rats at 14 and 19 weeks. Hematocrit levels were depressed in DXR-treated rats at 14 and 19 weeks, which may be suggestive of bone marrow toxicity. Histopathological analysis revealed that the levels of damage, such as that found in the heart and kidney, is much greater in rats examined at 19 weeks than at 14 weeks. Kidney damage by this drug, independent of its cardiotoxic effects, has been frequently reported in rats [1, 2, 40].

Plasma levels of PGE, $PGF_{2\alpha}$, and TxB_2 were elevated at both 14 and 19 weeks, while 6-keto-$PGF_{1\alpha}$ was elevated at only 19 weeks. Removal of a major portion of the lipid (>90%) by silicic acid column chromatography prior to RIA and the nearly 100% recovery of the exogenously added $PGF_{2\alpha}$ or TxB_2 to lipemic plasma from DXR-treated and plasma from PBS-treated rats argue against the presence of interfering materials in the lipemic plasma. In addition, the plasma PG and TxB_2 values found in the present study are in good agreement with the values reported by other investigators using either gas chromatography-mass spectrometry or RIA methods [23, 32].

The level of free arachidonic acid, precursor of PG and TxB_2 [20], was highly elevated in the plasma at 14 and 19 weeks. This elevation of plasma free arachidonic acid also displayed specificity, since levels of other free fatty acids were unchanged. The free (unesterified) fatty acids are generally only present in trace amounts in cells and tissues [20]. Plasma esterified linoleic and arachidonic acids, indirect precursors of PG and TxB of the one and two series respectively, were highly elevated at 14 and 19 weeks. The elevation of PG and TxB of the two series reflects either an increase in synthesis and/or a decrease in degradation of these substances in the tissues. The increases in plasma arachidonic acid, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, and TxB_2 from 14 to 19 weeks coincide with the appearance of cardiac and renal damage. No attempt was made to measure the PG of the one series originating from linoleic acid.

It has been demonstrated in the present study and also by others that rats treated chronically with DXR exhibit lipemia (characterized by increases in total lipid, triglycerides, esterified fatty acids, and cholesterol) [24, 28], and elevated levels of lipid peroxides [37]. DXR has been found to stimulate peroxidation of membrane phospholipids in vitro [8, 39] by formation of either a semiquinone radical or, possibly, an iron-ADP-DXR complex. DXR-induced free radical damage to isolated heart mitochondrial membranes leads to rapid progressive inactivation of respiratory chain function [8]. It is possible that the serum lipid peroxides found in these chronically treated rats were formed by DXR-initiated free radical reactions in tissues. Free radical generation has been shown to stimulate biosynthesis of PG and TxA_2 by promoting the peroxidation of unsaturated fatty acids [25, 33]. Lipid peroxides stimulate cyclooxygenase activity by a direct interaction with this enzyme [25]. It has been shown that DXR has a very high affinity for cardiolipin, which is found in very high concentrations in the heart mitochondrial membrane [8, 39]. It has also been shown that heart mitochondrial membrane is a rich source of linoleic acid, a precursor of PG of the one series [39]. DXR, by initiating free radical reactions in association with cardiolipin, could stimulate PG biosynthesis.

Numerous studies have demonstrated increased myocardial release of PG in response to injury and ischemia [9, 21]. Newman et al. [30] have observed increased cardiac release of 6-keto-$PGF_{1\alpha}$ from dogs with cardiac hypertrophy and heart failure. PG and TxA_2 released into the coronary and systemic circulation bind to specific tissue receptors and, through unknown mechanisms, mediate increased levels of cyclic nucleotides, as well as alterations in cellular calcium pools. These changes are ultimately translated into changes in cell physiology [21]. Also, PG have been shown to have profound effects on inotropy, chronotrophy, coronary blood flow, systemic vascular resistance, and arterial pressure [21]. As suggested by Bristow et al. [4], PG and TxA_2 could amplify the cardiotoxic effects of DXR. A number of studies have reported changes in heart levels of cyclic nucleotides and calcium after DXR treatment [15, 34]. It is conceivable that alterations in the levels of PG and TxA_2 caused by chronic administration of DXR could alter calcium and cyclic mucleotide levels, leading to cardiac damage.

Numerous studies have reported that the long-term effects of DXR sometimes include not only cardiomyopathy, but also nephropathy [1, 2, 40]. This nephropathy causes hyperlipidemia, albuminuria, and hypoalbuminemia, finally resulting in a nephrotic syndrome [1, 2]. In the present study, plasma protein levels were depressed in DXR-treated animals at 3, 9, and 14 weeks, which may be indicative of some protein loss in the urine due to kidney damage. A loss of protein accompanying kidney damage

may be responsible for increased apoprotein synthesis, or a loss of factors activating lipoprotein accompanying kidney damage may be responsible for either increased apoprotein synthesis or loss of factors activating lipoprotein lipase, leading to accumulation of lipid [1, 2, 38]. The increased levels of total plasma lipid in DXR-treated rats at 14 and 19 weeks demonstrate marked hyperlipidemia. In human and experimental nephrosis, there is increased production of PG by the kidney possibly in response to stimulation by substances such as norepinephrine, angiotensin II, and vasopressin [12]. These PG of renal origin, such as TxB_2 and PGE_2, may enter the circulation, leading to increased plasma concentrations of these substances in a nephrotic syndrome [12]. Renal damage caused by DXR may lead to the release of PG into the systemic circulation. PGE_2 has natriuretic and diuretic effects which can play an important role in edema found in a nephrotic syndrome or congestive heart failure [12].

The data collected in the present study indicate that severe alterations in the levels of plasma arachidonic acid and its metabolites occur following chronic administration of DXR. These changes coincide with the appearance of cardiac and renal lesions between 14 and 19 weeks. Arachidonic acid and its metabolites have very potent biological activities and could potentially mediate pathological changes leading to cardiac or renal damage. Further study is needed to define a cause or effect relationship: whether, and how, PG may mediate this drug-induced damage, or whether they are produced in response to injury caused by DXR. An understanding of the role of PG in this problem may offer a greater insight into the mechanism of this cardiac damage and provide ways to alleviate or minimize adverse cardiac effects of DXR while increasing its chemotherapeutic value against cancer.

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