0022-3565/85/2351-0234$02.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 235, No. 1

Printed in U.S.A.

Cardiovascular Effects of Doxorubicin-Induced Toxicity in the Intact Lou/M Wsl Rat and in Isolated Heart Preparations

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Accepted for publication June 28, 1985

ABSTRACT

Hemodynamic effects were followed for 25 days in conscious nontumor bearing Lou/M Wsl rats during i.v. administration of doxorubicin (DXR) (1 mg/kg) on 5 consecutive days and then weekly. At day 24 cardiac output was significantly reduced in the DXR-treated group (cumulative dose of 7 mg/kg) in comparison with a saline-treated group, suggesting a reduction in myocardial performance. Urethane anesthesia at day 25 depressed cardiac output in control rats whereas this variable was not influenced in DXR-treated rats. Furthermore, blood pressure was significantly higher within DXR-treated rats, suggesting the presence of compensatory mechanisms. Separate experiments 25 days after the first DXR administration (cumulative dose of 7 mg/kg) demonstrated that the inotropic response toward dobutamine or norepinephrine as well as the vasoconstrictor response toward norepinephrine were impaired profoundly, suggesting compensatory mechanisms were functioning within the DXR-treated rats around day 25. In the isolated and perfused rat heart no changes in myocardial contraction under either basal or inotropic stimulatory conditions were observed 24 days after DXR treatment, indicating extracardiac phenomena have to contribute to a reduction in cardiac output and the occurrence of counter regulation mechanisms as observed in the *in vivo* experiments. However, after a cumulative dose of 11 mg/kg (at day 52), contraction function appeared to be disturbed upon contractility demand by dobutamine in the isolated heart. This observation supports the histological evidence of cardiomyopathy occurring at that time. On the other hand, continuous exposure of the heart to compensatory mechanisms as derived from the *in vivo* experiments might have contributed to the overall cardiac injury at day 52. Furthermore, it is suggested that induction of compensatory mechanisms might be caused by the nephrotoxicity due to DXR which in the present study was already established around day 24 by the marked increase in urinary proteins.

DXR is an antitumor agent whose long-term administration in humans is limited by the development of a dose-related cardiomyopathy (Young *et al.*, 1981). The occurrence of clinically manifest heart failure is a long-term complication of DXR therapy (Praga *et al.*, 1979; Young *et al.*, 1981). Although the morphological changes within the myocardium due to chronic or late DXR cardiotoxicity were demonstrated in humans and experimental animals (Jaenke, 1974; Olsen and Capen, 1977; Young *et al.*, 1981; Bristow *et al.*, 1981; Mettler *et al.*, 1977; van Hoesel *et al.*, 1984a,b), scarce information is available about myocardial performance and hemodynamic effects of this drug during intoxication.

In previous experiments (van Hoesel *et al.*, 1984a,b) we have studied DXR-induced cardiomyopathy in a Lou/M Wsl rat bearing a transplantable solid immunocytoma. This model might be utilized in dual experimentation to determine oncolytic and cardiotoxic activity of DXR (de Jong *et al.*, 1983). Animals treated with i.v. injections of DXR (1 mg/kg) on 5 consecutive days, followed by one weekly injection for 7 weeks (cumulative dose of 11 mg/kg), showed histologic evidence of cardiotoxicity scored as grade III (van Hoesel) *et al.*, 1984b). However, this model appears to be sensitive to nephrotoxic effects of DXR (van Hoesel *et al.*, 1984a,b) as was also described for other strains of rats (Sternberg, 1970; Hu *et al.*, 1983; Poggi *et al.*, 1979; Bristow *et al.*, 1981; Bertani *et al.*, 1982) and rabbits (Young, 1975; Fajardo *et al.*, 1980).

The present study was designed to assess functional consequences of DXR-induced (cardio)toxicity. Inasmuch as renal damage and subsequent extracardiac factors due to DXR can strongly influence the function of the cardiac pump irrespective of myocardial contractility, DXR-induced cardiac damage was assessed by complete hemodynamic studies within intact nontumor-bearing animals and compared with experiments on isolated hearts.

Received for publication January 2, 1985.

Methods

Animals. Breeding pairs of Lou/M Wsl rats were kindly provided by Dr. H. Bazin (Catholic University of Louvain, Brussels, Belgium)

ABBREVIATIONS: DXR, doxorubicin; max, maximal; TPR, total peripheral resistance; MAP, mean arterial pressure.

(Bazin *et al.*, 1972). Animals were bred at our Institute. Nontumor-bearing male rats, weighing 250 to 280 g were used.

Experimental design. DXR (Adriablastine, Farmitalia Carlo Erba Benelux, Brussels, Belgium) was prepared fresh from commercially available 10-mg vials by reconstituting the lyophilized powder with sterile water to a concentration of 2.0 mg/ml.

Injections *via* the tail vein were performed on 5 consecutive days and then weekly. In all experiments the treated group received DXR (1 mg/kg) every injection whereas the notreatment group was injected with physiological saline (0.9%). The first day of injection was assigned day 0. The following types of experiments were performed.

Chronic aortic flow measurements in conscious Lou/M rats. Pentobarbitone (60 mg/kg i.p.) provided surgical anesthesia for the procedure of flowprobe implantation. Artificial ventilation was carried out during the surgical period with a Harvard model 680 small animal respirator.

Ascending aortic flow (=cardiac output minus coronary flow) was measured by electromagnetic flowmetry according to the method of Smith and Hutchins (1979) and de Wildt *et al.* (1983). An electromagnetic flowprobe (Skalar Medical, Delft, The Netherlands) with an internal diameter of 2.1 mm was placed around the ascending aorta. The connection end of the probe cable was exteriorized and sutured on the head of the rat. After appropriate chest closure the animals were allowed to recover for at least 4 days.

Aortic flow was measured continuously with an electromagnetic flowmeter (Transflow 601, Skalar Medical, Delft, The Netherlands). Both pulsatile and mean aortic flow signals were then displayed on a Hewlett Packard recorder (HP 7758). Zeroflow reference was taken to be the diastolic level of the instantaneous aortic blood flow signal. The max dF/dt was measured by differentiating the pulsatile flow signal by means of an analog device (HP 8814 A derivative computer).

Heart rate was derived from the pulse wave of the aortic flow. Recordings were made at the same time of day between 9:00 A.M. and 1:00 P.M. 4 to 7 days after implantation of the sensor. During 1 hr the aorta flow and derived variables were registered continuously until a steady state was reached for the different variables.

DXR or saline (0.9%) injections after the protocol as mentioned above were given just after the flow measurement.

Hemodynamic measurements in urethane anesthetized Lou/M rats. Rats from the above mentioned chronic flowmetry study were anesthetized with urethane (1 g/kg i.p.) 25 days after starting treatment.

Artificial ventilation was carried out throughout the experiment using a Harvard model 680 respirator. Frequency of the pump was set to 60 strokes/min and the tidal volume was 3.5 ml (FiO_2 being 0.32). Blood pressure was recorded continuously from a cannulated femoral artery *via* a pressure transducer (HP type 1280 C) connected to a Hewlett-Packard recorder. Aortic flow, dF/dt max and heart rate were estimated as mentioned above. TPR was calculated from MAP and cardiac output. After an adequate stabilization period of 30 min the hemodynamic variables were registered.

Hemodynamic reactivity toward catecholamines in urethane anesthetized Lou/M rats. After a 25-day treatment according to the above mentioned protocol rats were anesthetized with urethane (1 g/kg i.p.). Artificial ventilation was carried out throughout the experiment (60 strokes/min, 3.5 ml, FiO_2 being 0.32). After thoracotomy a flowprobe (inside diameter, 2.1 mm) was placed around the aorta. After appropriate chest closure aortic flow and dF/dt max could be measured as mentioned above (de Wildt and Sangster, 1983). Blood pressure, heart rate and TPR were measured as stated above.

After a stabilization period of 30 min the various hemodynamic variables were noted and drugs were subsequently infused in a volume of 200 μl over a period of 1 min *via* a cannulated femoral vein. For each drug a dose-response relationship was established.

The inotropic drug dobutamine (5, 15 and 30 μg/kg) (generously provided by Eli Lilly and Company, Indianapolis, IN) and the α-sympathomimetic drug norepinephrine (1, 3, 7 and 10 μg/kg) (Sigma Chemical Co., St. Louis, MO) were administered at approximately 15-min intervals between each dose. Between the drugs there was an interval period of 30 min.

For each dose of every drug the cardiovascular effects were considered with respect to preadministration levels measured during control recordings.

Isolated hearts. After treatment with either saline or DXR according to the above-mentioned protocol the hearts were quickly excised from heparinized and ether-anesthetized rats at day 24 and 52. Hearts were perfused retrogradely at 37 ± 0.5°C and pH = 7.4 with perfusion medium equilibrated with 95% O_2 + 5% CO_2 according to the Langendorff procedure. The composition of the perfusate (mOsmol/l) was NaCl, 256.4; KCl, 9.4; $CaCl_2$, 3.9; $NaHCO_3$, 40.4; NaH_2PO_4, 1.0; $MgCl_2$, 3.0; and glucose, 12.1. Isolated hearts were stimulated at 300 pulses/min (6 V, 3 msec) with a Grass stimulator (S44) connected to a stimulation isolation unit (SIU 5) after a total atrioventricular block was made by cutting the bundle of His. The vertical apicobasal shortening of the heart can be regarded as a parameter of contractile function (Meyler *et al.*, 1958; Stam and de Jong 1977). The apicobasal shortening was converted to an electrical signal by means of a displacement transducer (Schaevitz, type 125 HPD). Applied preload amounted to 2 g. Velocity of shortening (dL/dt max) was measured continuously by differentiating the displacement signal by means of an analog device (HP 8814 A derivative computer). Displacement and velocity of shortening were recorded continuously on a Hewlett Packard Recorder (7754 A).

Coronary flow rate was measured continuously using an extracorporal flowprobe (inside diameter, 1.0 mm; preset 50 ml/min) placed just above the aortic cannula. Flow was measured with a flowmeter (Transflow 601, Skalar Medical).

After a 30-min control perfusion at a pressure of 60 mm Hg a dose-response curve was made for the *beta*-1 adrenoceptor agonist dobutamine. Therefore, dobutamine was added cumulatively (0.1 ml) to the perfusion medium just above the aortic cannula.

In an additional group perfusion pressure was varied stepwise from 60 up to 100 mm Hg as measured in the coronary flowtract using a pressure transducer (HP, type 1280 C) after a 30-min control perfusion at a pressure of 60 mm Hg.

Proteinuria, edema and hematocrit. Proteinuria was assessed with Albustix (Ames, Division of Miles, Weesp, The Netherlands) at day 24 and 52. The occurrence of ascites and hydrothorax was assessed qualitatively post mortem. Furthermore, hematocrit values were determined at day 24 and 52.

Statistical analysis. Values were given as $\bar{x}$ ± S.E.M. and statistical analysis was performed using the paired or unpaired Student's t test and analysis of variance as appropriate. $P = .05$ was considered the upper limit of significance.

Results

Chronic aortic flow measurements in conscious Lou/M rats during 25 days. From preliminary studies it was observed that after the applied dose scheme for DXR aorta flow and dF/dt max lowered progressively from day 19 to 24 ($n = 4$). Both variables were not significantly influenced within the saline-treated group ($n = 3$).

In the present experiment for both groups eight animals were used. Within the DXR group, however, a total of three animals died spontaneously at day 3 and 10, respectively (cause of death unknown, probably a multifactorial problem as a consequence of operation procedure, handling, drug administration, etc.) and day 25 (cause of death probably respiratory insufficiency as a consequence of pleural effusion). In the control group three animals died at day 13 (infection), day 21 (aortic rupture) and day 24 (cause of death unknown).

In figure 1 the results are demonstrated after administration of a cumulative dose of 7 mg/kg. It is shown that aortic flow

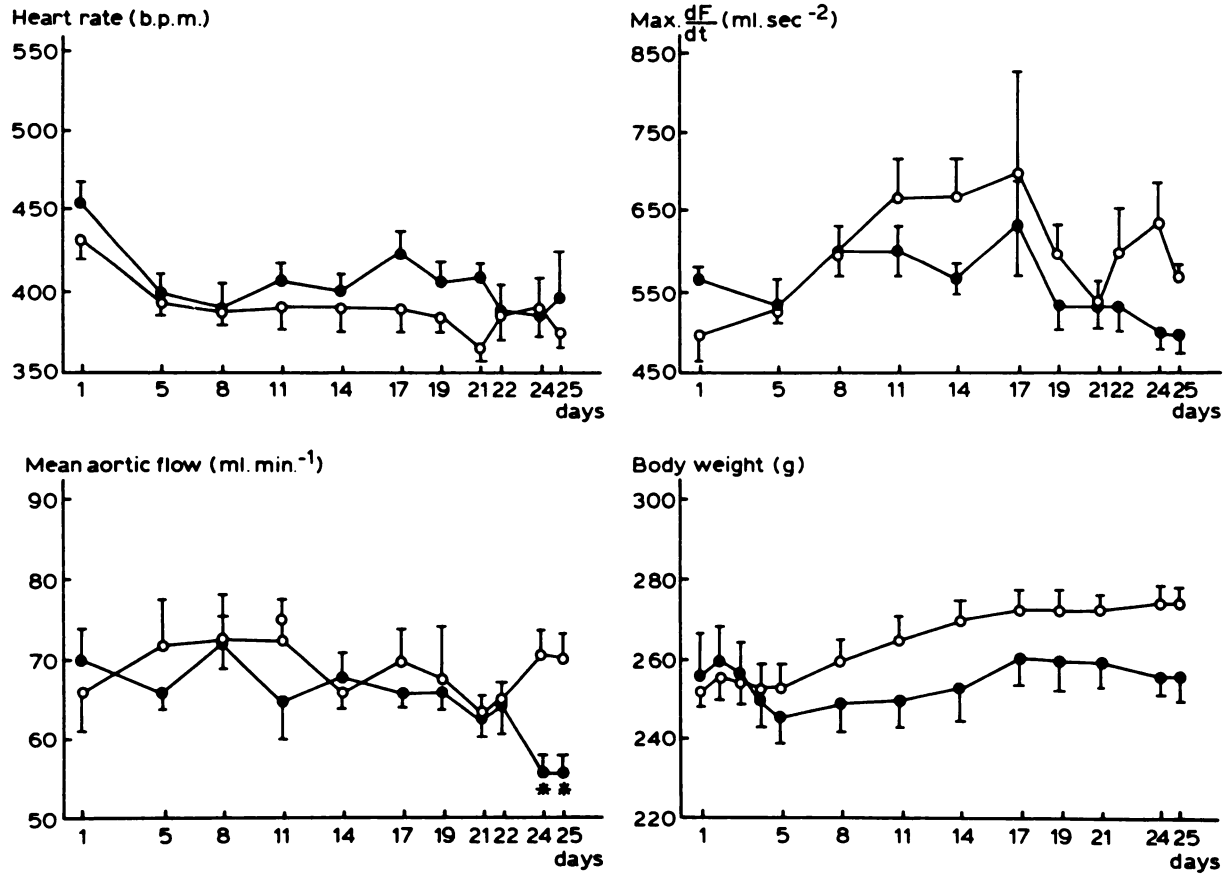

Fig. 1. Effect of DXR (1 mg/kg i.v. on 5 consecutive days, then weekly one injection) upon various cardiovascular variables and body weight of conscious Lou/M Wsl rats. O, control: 0.9% saline-treated group; ● DXR-treated group. Results are expressed as $\bar{x} \pm$ S.E.M. ($n = 5$). *Significantly different from control ($P < .05$). b.p.m., beats per minute.

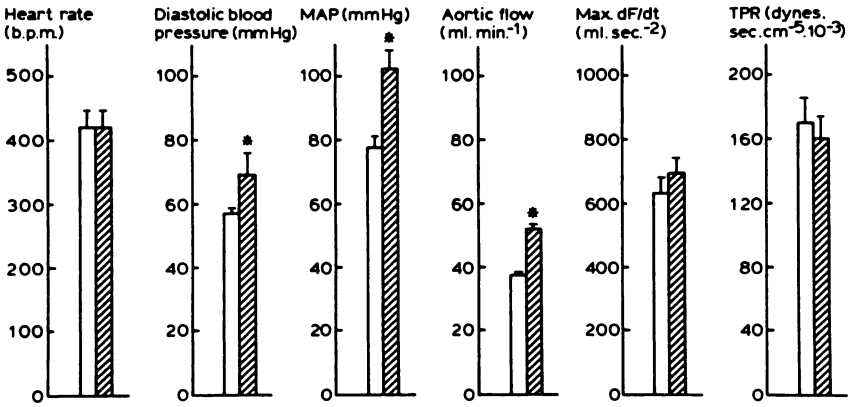

Fig. 2. Basal values of various hemodynamic values in urethane-anesthetized Lou/M Wsl rats, instrumented for chronic flow measurement, 25 days after the start of administration of 0.9% saline (open bars) or DXR (1 mg/kg) (hatched bars) (i.v. administered on 5 consecutive days, then weekly one injection; cumulative dose of 7 mg/kg). Results are expressed as $\bar{x} \pm$ S.E.M. ($n = 5$). *Significantly different from control ($P < .05$). b.p.m., beats per minute.

was significantly reduced at day 24 and 25 with respect to both the control saline-treated group and the initial value at day 0. Aortic flow diminished from 70 ± 4 ml/min ($n = 5$) at day 0 to 56 ± 2 at day 24 whereas in the control group the aortic flow did not change [from 68 ± 5 ($n = 5$) at day 0 up to 71 ± 3 at day 24].

At day 24 and 25 *dF/dt* max appeared to be slightly but not significantly reduced in the DXR-treated group with respect to control. Heart rate did fall approximately 10% during the first 5 days after administration of either DXR or saline (0.9%). During the further course of the study heart rate remained about the same level for both groups.

Hemodynamic measurements in the urethane anesthetized rat at day 25. As both the preliminary and the present study in conscious animals demonstrated a progressive lowering of cardiac output starting from at least day 24, it was decided to perform a more extensive hemodynamic study at day 25 under anesthesia.

From figure 2 it can be seen that the diastolic blood pressure and MAP were significantly higher in the DXR-treated animals than in the saline group under anesthetic and ventilated conditions. Aortic flow in the DXR-treated group was slightly but not significantly reduced with respect to that observed in the awake animals at day 25 (see fig. 1). However, aortic flow was reduced profoundly in the saline-treated group as compared with the values observed in the conscious animals (see fig. 1). Therefore, aortic flow in the DXR-treated rats was significantly higher than in the saline-treated group under these conditions.

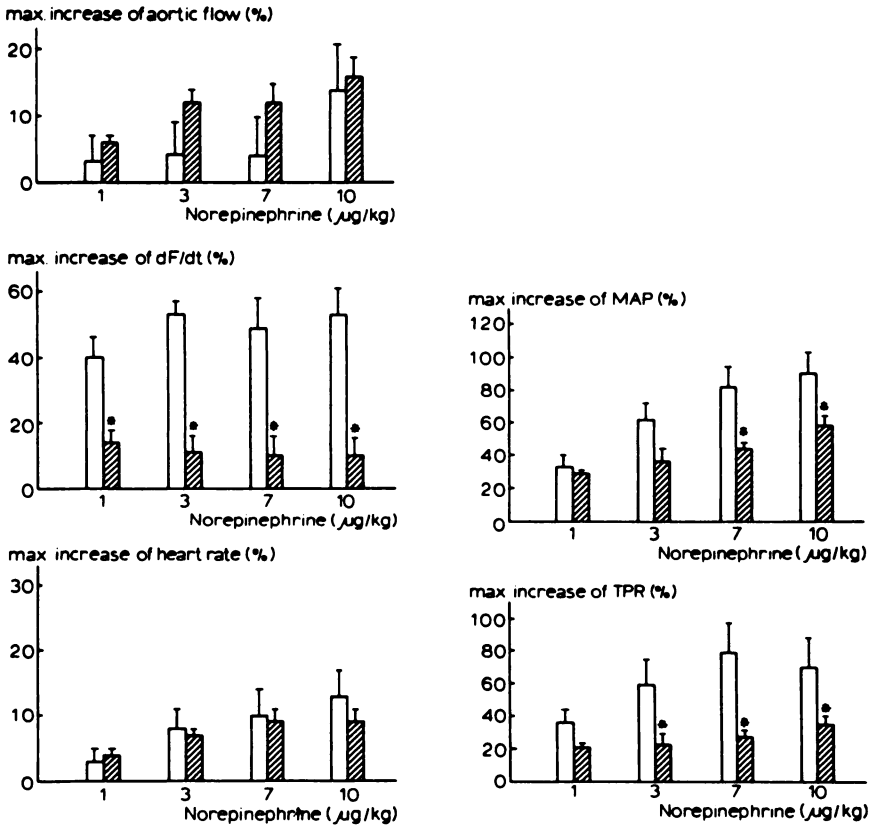

Fig. 3. Dose-response relationship of i.v. infused doses of norepinephrine (1–10 μg/kg) with respect to different hemodynamic variables in urethane-anesthetized Lou/M rats instrumented for acute flow measurements, 25 days after the start of administration of 0.9% saline (open bars) or DXR (1 mg/kg) (hatched bars) (i.v. administered on 5 consecutive days, then weekly one injection; cumulative dose of 7 mg/kg). Results are expressed as $\bar{x}$ ± S.E.M. (n = 6). *Significantly different from control ($P < .005$). The initial values of aortic flow, max dF/dt, MAP, heart rate and TPR amounted to 53 ± 7 ml·min^{-1}, 570 ± 50 ml·sec^{-2}, 56 ± 8 mm Hg, 395 ± 25 beats/min and 86 ± 8 dynes/sec/cm^{-5}·10^{-3}, respectively, for the control group and 70 ± 4 ml·min^{-1}, 710 ± 30 ml·sec^{-2}, 64 ± 3 mm Hg, 430 ± 10 beats/min and 74 ± 4 dynes/sec/cm^{-5}·10^{-3}, respectively, in the DXR-treated group.

TPR, heart rate and the contractile index dF/dt max did not differ significantly between both groups.

Hemodynamic reactivity toward catecholamines in urethane anesthetized rats at day 25. In a separate acute flowmetric study the cardiovascular system was stimulated by norepinephrine and dobutamine 25 days after administration of either DXR (cumulative dose, 7 mg/kg) or saline in a same dose regimen as mentioned above. In figure 3 the dose-effect relationship for norepinephrine (1–10 μg/kg) toward the various hemodynamic variables is demonstrated clearly. The vasoconstrictor action of norepinephrine as reflected in the TPR and MAP increase, was significantly less in the DXR-treated group than in the control group. Moreover, the contractile index dF/dt max did not respond in a positive inotropic manner.

With respect to the positive inotropic drug dobutamine (5–30 μg/kg), it was seen that both the aortic flow and dF/dt max were significantly less increased within the DXR-treated animals whereas the vascular *beta*-2 receptor-mediated vasodilatation was not different for both groups (fig. 4).

Isolated hearts. Table 1 demonstrates no significant differences between the DXR- and saline-treated groups with respect to basal values for both the contractility indices and the coronary flow measured after a stabilization period of 30 min. In the 52-day experiment the number of animals used numbered to 16 as additional experiments with eight animals were performed in order to obtain perfusion pressure-flow relationships in both groups (data not shown). A stepwise elevation of the perfusion pressure from 60 up to 100 mm Hg did not show any difference between the pressure-flow curves for the different groups (data not shown).

Inotropic stimulation of the hearts with dobutamine gave rise to a dose-dependent increase in both contractile indices under fixed heart rate (300 beats/min). The dose-response curves obtained in hearts from both DXR- and saline-treated animals did not differ from each other in rats 24 days after DXR treatment (cumulative dose, 7 mg/kg). However, 52 days after DXR treatment (cumulative dose, 11 mg/kg) dobutamine revealed a significantly lower response within the DXR-treated group (fig. 5) with respect to the contractile indices.

Proteinuria, edema and hematocrit. From table 2 it can be seen that a slight decrease in body weight occurred in the DXR-treated group and an increase in the saline-treated group during the observation periods of 24 and 52 days. At the latter points of time body weights were signficantly different between both groups.

At day 24 and 52 the albumin concentration in urine mostly exceeded 10.0 g/l in the DXR-treated rats whereas the amounts of albumin in urine of the control group varied between 0.3 to 10 g/l. Qualitatively, all animals developed both ascites and hydrothorax which was most pronounced at day 52 for all DXR-treated Lou/M rats (table 2).

Systemic toxicity of DXR indicated by the decreased value for the hematocrit was time-dependent. At day 52 hematocrit was as low as 0.35 ± 0.01 (n = 16) which was significantly different from control values (0.50 ± 0.005, n = 16).

Discussion

In order to find procedures for increasing the therapeutic index of DXR, an animal model should be introduced in which cardiotoxicity and antitumor effect of DXR can be evaluated simultaneously. Recently, van Hoesel *et al.* (1984b) demonstrated that antineoplastic and histologically confirmed cardiotoxicity could be evaluated in one and the same model using the tumor-bearing Lou/M Wsl rat. However, to our knowledge scarce information is available about changes in myocardial

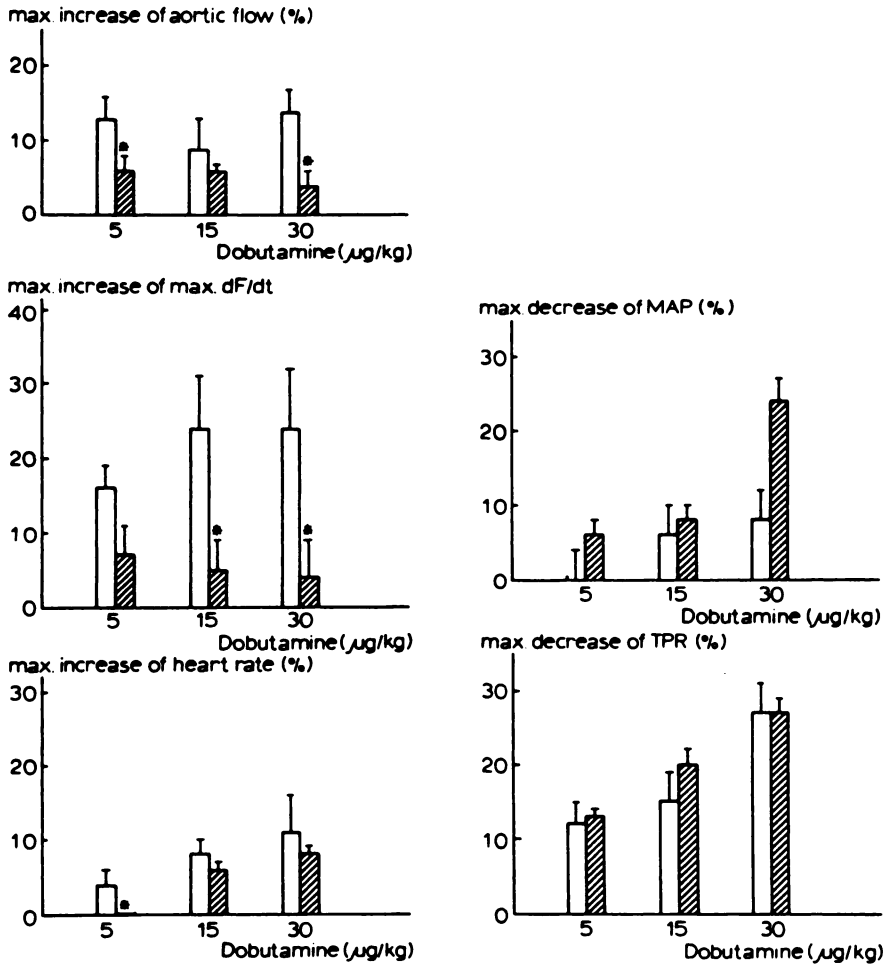

Fig. 4. Dose-response relationship of i.v. infused doses of dobutamine (1–15 μg/kg with respect to different hemodynamic variables in urethane-anesthetized Lou/M rats instrumented for acute flow measurements, 25 days after the start of administration of 0.9% saline (open bars) of DXR (1 mg/kg) (hatched bars) (i.v. administered on 5 consecutive days, then weekly one injection, cumulative dose of 7 mg/kg). Results are expresed as $\bar{x} \pm$ S.E.M. ($n = 6$). For initial values see legend to figure 3. *Significantly different from control ($P < .05$).

TABLE 1

Initial values for various variables 24 and 52 days, respectively, after treatment with saline or DXR (1 mg/kg, administered i.v. on 5 consecutive days and then one injection weekly) in isolated, retrogradely perfused and driven rat hearts

Treatment	Dose	*n*	Days	Wet Heart Wt.	Apico Basal Shortening	Velocity of Shortening	Coronary Flow
	mg/kg			*g*	*mm*	*mm/sec*	*ml/min*
DXR	7	8	24	0.95 ± 0.03	2.67 ± 0.12	93 ± 5	8.1 ± 0.6
	11	16	52	1.07 ± 0.03	2.32 ± 0.07	86 ± 3	7.7 ± 0.5
Saline, 0.9%		8	24	1.00 ± 0.03	2.71 ± 0.07	97 ± 5	9.2 ± 0.5
		16	52	1.04 ± 0.03	2.50 ± 0.09	92 ± 3	8.1 ± 0.6

and/or hemodynamic function due to DXR in experimental animals including Lou/M Wsl rats.

In the study of van Hoesel *et al.* (1984b), it was demonstrated that a cumulative DXR dose of 11 mg/kg i.v. (1 mg/kg on 5 consecutive days, followed by weekly injections) caused a morphologically assessed cardiomyopathy (scored grade III according to Billingham *et al.*, 1978) and a complete regression of an inoculated tumor at day 12 after the start of DXR administration.

Using this dose scheme during a period of 25 days (cumulative dose of 7 mg/kg), it was found that cardiac output was significantly reduced in conscious Lou/M Wsl rats (fig. 1). This observation might suggest a reduction in myocardial performance induced by DXR. The variable *dF/dt* max as index of myocardial contractility (de Wildt and Sangster, 1983) was not significantly decreased, although a tendency to a lower *dF/dt* max within the DXR-treated group was present. However, the results observed in the isolated rat hearts did not reveal an alteration in myocardial contractility under either basal or inotropic conditions 24 days after DXR treatment (fig. 5, table 1). Moreover, at that time cardiotoxicity seemed to be very low from a morphological point of view, *e.g.*, scored as grade I (van Hoesel *et al.*, 1985). Therefore, it seems justified to hypothesize that extracardiac phenomena are responsible for the reduction in cardiac output after a 25 day treatment (cumulative dose of 7 mg/kg).

Conclusions drawn from only one hemodynamic variable, *i.e.*, cardiac outut, is dangerous. From a practical point of view it was impossible to follow blood pressure during 25 days in conscious, flowmetry instrumented, animals. Therefore, complete hemodynamic measurement was performed at day 25 under urethane anesthesia. The results indicate the occurrence of strong cardiovascular compensatory mechanisms in the DXR-treated animals (fig. 2). It appeared that sufficient compensation toward urethane-induced cardiodepression was present in the DXR-treated animals, as the cardiac output was not changed in comparison with that observed in conscious animals. Furthermore, blood pressure within the DXR-treated animals was significantly higher than in control rats. Inasmuch as the calculated TPR did not differ for both groups, it appears that the increase in blood pressure was determined mainly by the increase of cardiac output.

Vascular and cardiac adrenergic receptor stimulation demonstrated a strongly disturbed adrenergic receptor functioning.

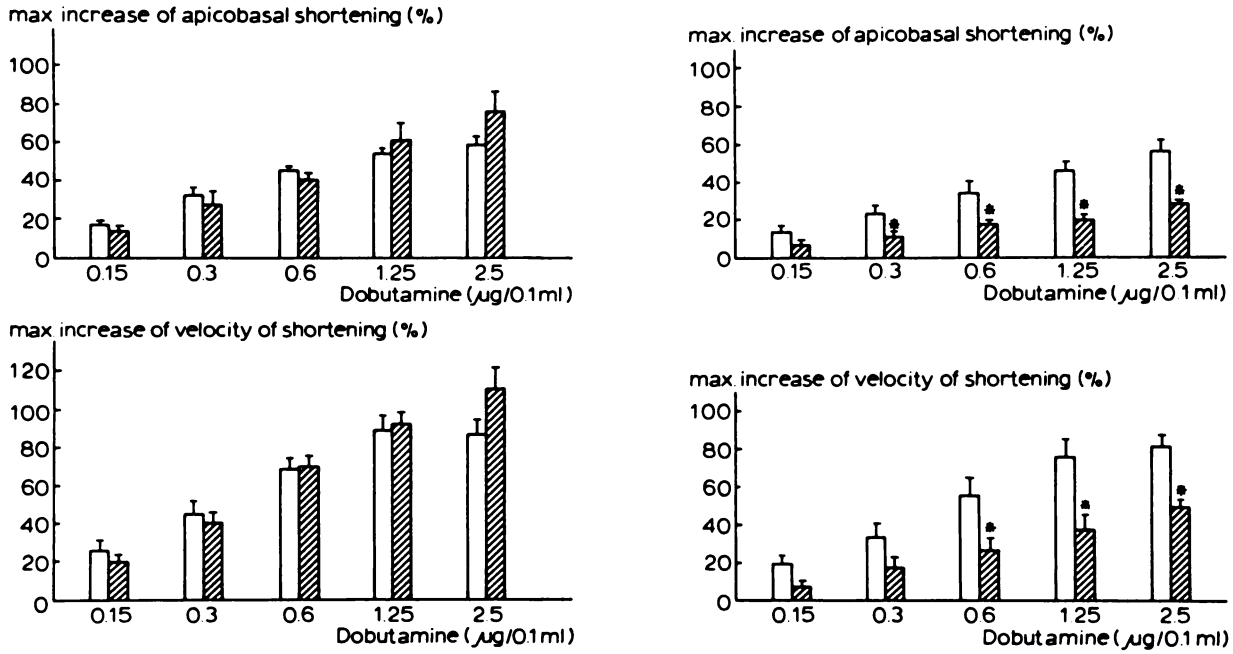

Fig. 5. Dose-response relationship for dobutamine with respect to maximal increase in apicobasal shortening and in velocity of shortening as percentage of the predose level in isolated, perfused and driven rat hearts. The open bars represent the saline-treated animals and hatched bars correspond with the DXR-treated (1 mg/kg) animals. Left side demonstrates the results after a 25-day treatment (cumulative dose of 7 mg/kg) and right side represents the results after a 52 days treatment (cumulative dose of 11 mg/kg). Results are expressed as $\bar{x} \pm$ S.E.M. ($n = 8$). *Significantly different from control ($P < .05$). Initial values for the various variables did not differ significantly for both groups (see also table 1).

TABLE 2

Effect of DXR (1 mg/kg, administered i.v. on 5 consecutive days then one injection weekly) in comparison with saline-treated animals upon body weight at the start (before) and the end (after) of the experiment; hematocrit (Ht), urinary albumin and edema development

Treatment	Dose	n	Days	B Wt.		Ht	Urinary Albumin[a]	Edema[b]
				Before	After			
	mg/kg			g				
DXR	7	8	24	275 ± 12	270 ± 13*	0.41 ± 0.02*	+++/++++	++ → +++
	11	16	52	276 ± 5	252 ± 4*	0.35 ± 0.01*	+++/++++	+++
Saline, 0.9%		8	24	288 ± 9	316 ± 9	0.48 ± 0.005	+	–
		16	52	286 ± 6	316 ± 5	0.50 ± 0.005	+	–

[a] +, 0.3 to 1.0 g/l; ++, 1.0 to 3.0 g/l; +++, 3.0 to 10.0 g/l; ++++, >10.0 g/l.
[b] ++, mild; +++, severe.
* Significantly different from saline, $P < .05$.

Although for technical reasons no plasma catecholamine levels could be assayed, it might be speculated that a decreased response toward catecholamines can be explained by receptor desensitization by continuous exposure to catecholamines (Lefkowitz *et al.*, 1983). Further indirect evidence for such a catecholamine interference in the myocardium of dogs after chronic exposure to DXR and daunorubicin is given by several authors (Bristow *et al.*, 1979, 1980, 1981; Soldani *et al.*, 1980).

Ultimately, both the altered hemodynamic status and the reduced cardiovascular reactivity toward catecholamines at day 25 might be the consequence of secondary phenomena rather than intrinsic cardiac actions of DXR upon the myocardium. An important and causal factor can be found in a shift of body fluid as DXR induces extensive renal damage leading to proteinuria and extreme hypoalbuminemia (Bertani *et al.*, 1982; Bristow *et al.*, 1981; Deprez-de-Campeneere *et al.*, 1982) in rats and rabbits. Recent studies in our institute (van Hoesel *et al.*, 1984a) demonstrated a dose- and time-dependent decrease in serum albumin levels within treated rats. A dose of 1 mg/kg according to the aforementioned dose scheme lowered serum albumin significantly from 41.3 to 21.6 g/l after 14 days. Both albuminuria and decrease in serum albumin levels were accompanied by a modest to severe nephropathy assessed by light microscopic and ultrastructural studies on kidneys (van Hoesel *et al.*, 1984a; 1985). These studies allowed us to take albuminuria in the present hemodynamic studies as an indicator of nephropathy. After 42 days albumin levels were beneath 10 g/l and a higher urinary albumin level was measured. In the present study also a strong elevation of urinary albumin was observed at day 25 (table 2), indicating the occurrence of renal damage. The loss of albumin and therewith the loss of plasma colloid osmotic pressure has consequences for the circulation. Therefore, the accumulation of body fluids, *e.g.* ascites and hydrothorax (table 2), has to be interpreted as a manifestation of hypoalbuminemia rather than chronic heart failure due to DXR-induced cardiomyopathy. At least this interpretation holds true after a cumulative DXR dose of 7 mg/kg (24 days) as neither loss of cardiac function in the isolated hearts can be observed or histological evidence of cardiac injury can be demonstrated at that time (van Hoesel *et al.*, 1984a). However, a

fluid accumulation at day 52 (cumulative dose of 11 mg/kg) might be a consequence of heart failure and hypoalbuminia. Because morphological studies (van Hoesel *et al.*, 1984a,b) demonstrated cardiac damage after 52 days, a functional test in the isolated heart was performed.

Considering that the renal damage and the strongly altered hemodynamic state of the animals already occurred 25 days after DXR treatment, a functional test *in vivo* was meaningless at day 52. After a cumulative dose of 11 mg/kg after 52 days a reduced response toward the inotropic drug dobutamine was found. This observation might be interpreted as a loss of reserve capacity due to cardiac injury by DXR, which is in agreement with the earlier found histological data of van Hoesel *et al.* (1984a,b). However, a reduced dobutamine response might also be considered as a consequence of receptor desensitization due to continuous exposure of compensatory released catecholamines.

In conclusion, the present data indicate that the earlier found histological evidence of cardiomyopathy could be supported by the assessment of cardiac function as loss of contractile reserve capacity was observed at day 52. However, considering the occurrence of compensatory mechanisms and reduced catecholamine responsiveness one cannot exclude the fact that secondary phenomena as a result of an early occurring nephrotoxicity contributed to the histologically or functionally proved cardiomyopathy shown 52 days after DXR treatment.

Furthermore the results demonstrate clearly that interpretation of cardiomyopathic changes due to DXR in rats must be done carefully as other actions of this compound, *e.g.* renal damage, might have influenced the cardiomyopathic process in this species apart from direct intrinsic cardiomyopathic toxicity of DXR. Whatever the clinical impact of the observed phenomena might be is not obvious as until now no renal damage has been reported in humans.

Acknowledgments

The authors are indebted to A. van de Kuil and N. J. P. Blokhuijzen for their excellent technical assistance.

References

Bazin, H., Deckers, C., Beckers, A. and Heremans, J. F.: Transplantable immunoglobulin secreting tumors in rats. General features of Lou/Wsl strain rat immunocytomas and their monoclonal proteins. Int. J. Cancer **10:** 568–580, 1972.

Bertani, T., Poggi, A., Pozzoni, R., Delaini, F., Sacchi, G., Thoua, Y., Mecca, G., Remuzzi, G. and Donati, M. B.: Adriamycin-induced nephrotic syndrome in rats, sequence of pathologic events. Lab. Invest. **46:** 16–23, 1982.

Billingham, M. E., Mason, J. W., Bristow, M. R. and Daniëls, J. K.: Antracycline cardiomyopathy monitored by morphologic changes. Cancer Treat. Rep. **62:** 865–872, 1978.

Bristow, M. R., Billingham, M. E. and Daniëls, G. R.: Histamine and catecholamines mediate adriamycin cardiotoxicity. Proc. Am. Assoc. Cancer Res. **20:** 118, 1979.

Bristow, M. R., Minobe, W. A., Billingham, M. E., Marmor, J. B., Johnson, G. A., Ishimoto, B. M., Sagemann, W. S. and Daniëls, J. K.: Anthracyclin-associated cardiac and renal damage in rabbits; evidence for mediation by vasoactive substances. Lab. Invest. **45:** 157–168, 1981.

Bristow, M. R., Sagemann, W. S., Scott, R. H., Billingham, M. E., Bowder, R. E., Kernoff, R. S., Snidow, G. H. and Daniëls, J. R.: Acute and chronic cardiovascular effects of doxorubicine in dogs; the cardiovascular pharmacology of drug-induced histamine release. J. Cardiovasc. Pharmacol. **2:** 487–515, 1980.

de Jong, W. H., Steerenberg, P. A., Vos, J. G., Bulten, E. J., Verbeek, F., Kruizinga, W. and Ruitenberg, E. J.: Antitumor activity, induction of cross resistance, and nephrotoxicity of a new platinum analogue, *cis*-1.1-diaminomethyl cyclohexane platinum (II) sulfate and *cis*-diamine dichloro platinum (II) in an immunocytoma model in the Lou/M rat. Cancer Res. **43:** 4927–4934, 1983.

Deprez-de-Campeneere, D., Jaerte, K. and Trouet, A.: Comparative cardiac and renal toxicity of daunorubicin in the rat and the rabbit. Cancer Treat. Rep. **66:** 395–397, 1982.

de Wildt, D. J., Hillen, F. C., Rauws, A. G. and Sangster, B.: Etomidate-anaesthesia with or without fentanyl compared with urethane anaesthesia in the rat. Br. J. Pharmacol. **79:** 461–469, 1983.

de Wildt, D. J. and Sangster, B.: An evaluation of derived aortic flow parameters as indices of myocardial contractility in rats. J. Pharmacol. Methods **10:** 55–64, 1983.

Fajardo, L. F., Elhingham, J. K., Stewart, J. R. and Klauber, M. K.: Adriamycin nephrotoxicity. Lab. Invest. **43:** 242–253, 1980.

Hu, S. T., Brändle, E. and Zbinden, G.: Inhibition of cardiotoxic, nephrotoxic and neurotoxic effects of doxorubicin by ICRF-159. Pharmacology (Basel) **26:** 210–220, 1983.

Jaenke, R. S.: An anthracycline antibiotic-induced cardiomyopathy in rabbits. Lab. Invest. **30:** 292–304, 1974.

Lefkowitz, R. J., Stadel, J. M. and Caron, M. G.: Adenylate cyclase-coupled beta adrenergic receptors: Structure and mechanisms of activation and desensitization. Annu. Rev. Biochem. **52:** 159–182, 1983.

Mettler, F. P., Young, D. M. and Ward, J. M.: Adriamycin-induced cardiotoxicity (cardiomyopathy and congestive heart failure) in rats. Cancer Res. **37:** 2705–2713, 1977.

Meyler, F. L., Bode, C. and Offerijns, F. G. J.: A simple method for the recording of the contractions of the isolated rat's heart, if necessary, together with the electrocardiogram. Arch. Int. Physiol. Biochem. **66:** 303–308, 1958.

Olson, H. M. and Capen, C. C.: Subacute cardiotoxicity of adriamycin in the rat. Biochemical and ultrastructural investigations. Lab. Invest. **37:** 386–394, 1977.

Poggi, A., Kornblihtt, L., Delaini, F., Colombo, T., Mussoni, L., Reyers, I. and Donati, M. B.: Delayed hypercoagulability after a single dose of adriamycin to normal rats. Thromb. Res. **16:** 639–650, 1979.

Praga, C., Beretta, G. and Vigo, P. L.: Adriamycin cardiotoxicity. A survey of 1273 patients. Cancer Treat. **63:** 827–834, 1979.

Smith, T. L. and Hutchins, P. M.: Central haemodynamics in the developmental stage of spontaneous hypertension in unanaesthetized rat. Hypertension **1:** 507–508, 1979.

Soldani, G., del Tacca, M. and Bernardini, C.: Noradrenaline and alpha blockers in daunomycin cardiotoxicity. Clin. Toxicol. **18:** 1435–1440, 1980.

Stam, H. and de Jong, J. W.: Sephadex-induced reduction of coronary flow in the isolated rat heart: A model for ischemic heart disease. J. Mol. Cell. Cardiol. **9:** 633–650, 1977.

Sternberg, S. S.: Cross-striated fibrils and other ultrastructural alterations in glomeruli of rats with daunomycin nephrosis. Lab. Invest. **22:** 39–51, 1970.

van Hoesel, Q. G. C. M., Steerenberg, P. A., Crommelin, D. J. A., van Dijk, A., van Oort, W., Klein, S., de Wildt, D. J. and Hillen, F. C.: Reduced cardiotoxicity and nephrotoxicity with preservation of antitumor activity of doxorubicin entrapped in stable liposomes in the Lou/M Wsl rat. Cancer Res. **44:** 3698–3705, 1984a.

van Hoesel, Q. G. C. M., Steerenberg, P. A., Vos, J. G., Hillen, F. C. and Dormans, J. A. M. A.: Antitumor effect, cardiotoxicity and nephrotoxicity of doxorubicin in the IgM solid immunocytoma bearing Lou/M Wsl rat. J. Natl. Cancer Inst. **72:** 1141–1150, 1984b.

van Hoesel, Q. G. C. M., Steerenberg, P. A., Dormans, J. A. M. A., de Jong, W. H., de Wildt, D. J. and Vos, J. G.: Time lapse study on doxorubicin induced nephropathy and cardiomyopathy in male and female Lou/M Wsl rats, J. Natl. Cancer Inst., in press, 1985.

Young, D. M.: Pathologic effects of adriamycin (NSC-123127) in experimental systems. Cancer Chemother Rep. **6/2:** 159–175, 1975.

Young, R. C., Ozols, R. F. and Myers, C. E.: The anthracycline antineoplastic drugs. N. Engl. J. Med. **305:** 139–153, 1981.

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