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Prevention of doxorubicin-induced myocardial and haematological toxicities in rats by the iron chelator desferrioxamine*

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Summary. Biochemical and histopathological evaluations of the protective effects of the iron-chelator desferrioxamine against the cardiac and haematological toxicities of doxorubicin in normal rats were carried out. A single dose of doxorubicin (15 mg/kg, i.v.) caused myocardial damage that manifested biochemically as an elevation of serum cardiac enzyme [glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH) and creatine phosphokinase (CPK)] and cardiac isoenzyme levels and histopathologically as a swelling and separation of cardiac muscle fibers. Doxorubicin caused severe leucopenia and decreases in red blood cell counts and haemoglobin concentrations at 72 h after its administration. Desferrioxamine treatment (250 mg/kg, i.p.) carried out 30 min before doxorubicin administration protected the heart and blood elements from the toxic effects of doxorubicin as indicated by the recovery of levels of cardiac enzymes and isoenzymes and of red blood cell counts to normal values and by the absence of significant myocardial lesions. The findings of this study suggest that desferrioxamine can potentially be used clinically to prevent doxorubicin-induced cardiac and haematological toxicities.

Introduction

Doxorubicin is widely accepted as a potent broad-spectrum chemotherapeutic agent against different types of tumours [4, 5, 17, 18]. Several reports have indicated that chronic administration of doxorubicin causes myocardial alterations and other toxic effects in rats [19, 20]. Furthermore, electrocardiogram changes have been observed at 24 h after the administration of doxorubicin analogues [31]. Bone marrow despression is one of the major adverse effects of doxorubicin and in certain cases, requires discontinuation of the treatment [7]. It has been suggested by many investigators that the myocardial cellular damage produced by doxorubicin is mediated by the formation of an iron-anthracycline complex that generates free radicals [6, 7, 22]. Desferrioxamine is an iron chelator that is mainly used to treat iron poisoning [15]. However, it has also been that desferrioxamine prevents the myocardial mitochondrial damage induced by ischemia-reperfusion in the rat [28]. Therefore the aim of the present study was to investigate the possible protective effects of desferrioxamine against doxorubicin-induced cardiac and haematological toxicities in normal rats. Cardiotoxicity was assessed by measurements of the common serum cardiac enzymes [glutamic exaloacetic transaminase (GOT), lactic dehydrogenase (LDH) and creatine phosphokinase (CPK) and cardiac isoenzymes and by histological examination of cardiac muscle tissue. Haematotoxicity was evaluated in terms of changes in the number of white blood cells (WBCs) and red blood cells (RBCs) and in the haemoglobin (Hb) concentration.

Materials and methods

Adult Wistar albino rats aged 12 weeks and weighing 250–300 g were used in this study. A total of 72 rats were divided into 4 groups of 18 animals each. Two groups were injected i.p. with desferrioxamine (250 mg/kg) and an equivalent volume of normal saline at 30 min prior to a single i.v. injection of doxorubicin (15 mg/kg) via the tail vein. Two additional groups were used as controls and received i.p. injections of either desferrioxamine or normal saline 30 min prior to an i.v. injection of normal saline. Desferrioxamine (Desferal, Ciba Geigy, Switzerland) and lyophilised doxorubicin (Farmitalia Carlo Erba, Italy) were dissolved in normal saline just before use and were injected at doses of 0.2 and 0.1 ml/100 g body weight, respectively.

At 24, 48 and 72 h after the last injection, six animals from each group were anaesthetised with ether and blood samples were taken by heart puncture. Part of the blood was collected into containers to which ethylene-diaminetetraacetic acid (EDTA) had been added for the determination of haematological parameters. Serum was separated for cardiac

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Table 1. Effect of desferrioxamine and doxorubicin on serum enzyme levels in the rat[a]

Time after treatment (h)	Treatment	Serum enzyme levels (U/l)[b]		
		GOT	LDH	CPK
	Saline[c]	79 ± 5	279 ± 20	226 ± 15
	Desferrioxamine	50 ± 4**	231 ± 34	221 ± 15
24	Doxorubicin	142 ± 17**	347 ± 19*	1293 ± 28**
	Desferrioxamine plus doxorubicin	77 ± 2.9	288 ± 28	165 ± 13**
48	Desferrioxamine	103 ± 7*	295 ± 3.5	211 ± 7.8
	Doxorubicin	61 ± 3	411 ± 30*	148 ± 29*
	Desferrioxamine plus doxorubicin	71 ± 5	235 ± 5	231 ± 6
72	Desferrioxamine	82 ± 6	270 ± 6	204 ± 6
	Doxorubicin	65 ± 5.8	287 ± 12	185 ± 29
	Desferrioxamine plus Doxorubicin	91 ± 3.9	238 ± 35	188 ± 12

[a] Desferrioxamine (25 mg/kg, i.p.) was given 30 min prior to doxorubicin (15 mg/kg, i.v.) administration
[b] All data represent mean values ± SEM ($n = 6-12$). Test of significance indicates experimental group vs control values
[c] Average of 12 animals, 4 at each time point
* $P < 0.05$
** $P < 0.01$

Table 2. Effect of desferrioxamine pretreatment and doxorubicin on cardiac isoenzyme levels in the rat[a]

Time after treatment (h)	Treatment	Isoenzyme (U/l)[b]	
		LDH-Iso	CPK-MB
	Saline	9 ± 1	30 ± 2.5
	Desferrioxamine	13.9 ± 2	32 ± 2.5
24	Doxorubicin	14.4 ± 3.7	33 ± 2.2
	Desferrioxamine plus doxorubicin	16 ± 1.4*	32 ± 7
48	Desferrioxamine	10.5 ± 2.6	32 ± 7
	Doxorubicin	26.5 ± 5.1*	50.3 ± 3.2*
	Desferrioxamine plus doxorubicin	14 ± 0.3	35 ± 0.9

[a] Desferrioxamine (250 mg/kg, i.p.) was given 30 min prior to doxorubicin (15 mg/kg, i.v.) administration
[b] All data represent mean values ± SEM ($n = 6-8$). Test of significance indicates experimental group vs control values
[c] Average of 8 animals, 4 at each time point
* $P < 0.01$

enzyme and isoenzyme assay. The heart was excised and fixed in 10% formaline for histopathological examination. LDH, CPK and GOT levels were measured according to the methods of Gruiber [12], Swanson and Wilkinson [27] and Reitman and Frankels [24], respectively. RBCs and WBCs were counted using a haemocytometer [9]. Hb was assayed colourimetrically [29], and LDH and CPK isoenzymes were determined according to the methods of Bergmeyer and Bernt [3] and Kachmar and Moss [14], respectively.

Table 3. Effects of desferrioxamine and doxorubicin on RBCs, WBCs and Hb concentrations in the rat[a]

Treatment	RBCs ($\times 10^{-6}$)[b]	WBCs ($\times 10^{-3}$)[b]	Hb (g/d)[b]
Saline	9.43 ± 0.83	7.9 ± 0.67	14.8 ± 0.74
Desferrioxamine	8.4 ± 0.23	9.5 ± 0.29	14.4 ± 0.23
Doxorubicin	5 ± 0.70[c]	0.57 ± 0.1[c]	10.6 ± 0.4[c]
Desferrioxamine plus doxorubicin	11.6 ± 0.55	2.1 ± 0.5[c]	11.6 ± 0.55[c]

[a] Desferrioxamine (250 mg/kg, i.p.) was given 30 min prior to doxorubicin (15 mg/kg, i.v.) administration
[b] Blood samples were obtained at 72 h after doxorubicin administration
[c] All data represent mean values ± SEM ($n = 6$). Test of significance indicates experimental group vs control values ($P < 0.01$)

Results

Cardiac enzyme and isoenzyme activities

Table 1 shows the effect of the iron chelator desferrioxamine (250 mg/kg) given i.p. 30 min before doxorubicin (15 mg/kg, i.v.) on the serum cardiac enzyme activities of normal rats. At 24 h after a single dose of doxorubicin, we observed a significant elevation of serum GOT, LDH and CPK levels by 80%, 24% and 472%, respectively, as compared with saline-treated controls. The maximal elevation of LDH levels reached 48% at 48 h after treatment. Desferrioxamine pretreatment prevented the increase in cardiac enzyme levels induced by doxorubicin (Table 1). Cardiac isoenzymes of LDH and CPK showed significant elevations (194% and 68%, respectively) at 48 h after doxorubicin treatment. These increased values were greatly reduced (56% and 17%) by pretreatment with desferrioxamine (Table 2).

Haematological changes

Table 3 shows the effect of desferrioxamine pretreatment on the haematological toxicity of doxorubicin. A single i.v. injection of doxorubicin resulted in severe leucopenia at 72 h after treatment (570 cells/mm^3). In the presence of desferrioxamine, the numbers of WBCs were restored to a great extent (2100 cells/mm^3). Doxorubicin treatment caused a 47% decrease in the numbers of RBCs, but pretreatment with desferrioxamine resulted in a complete restoration of the RBC count.

Myocardial pathology

Histopathological investigation of cardiac muscles at 24 h after doxorubicin treatment revealed swollen cardiac muscle fibres, interstitial oedema and variation in the size of nuclei, with few pyknotic nuclei being observed in the myocardium (Fig. 1a). Desferrioxamine pretreatment prevented doxorubicin-induced myocardiac lesions as compared with treatment with either saline or desferrioxamine alone (Fig. 1b, c).

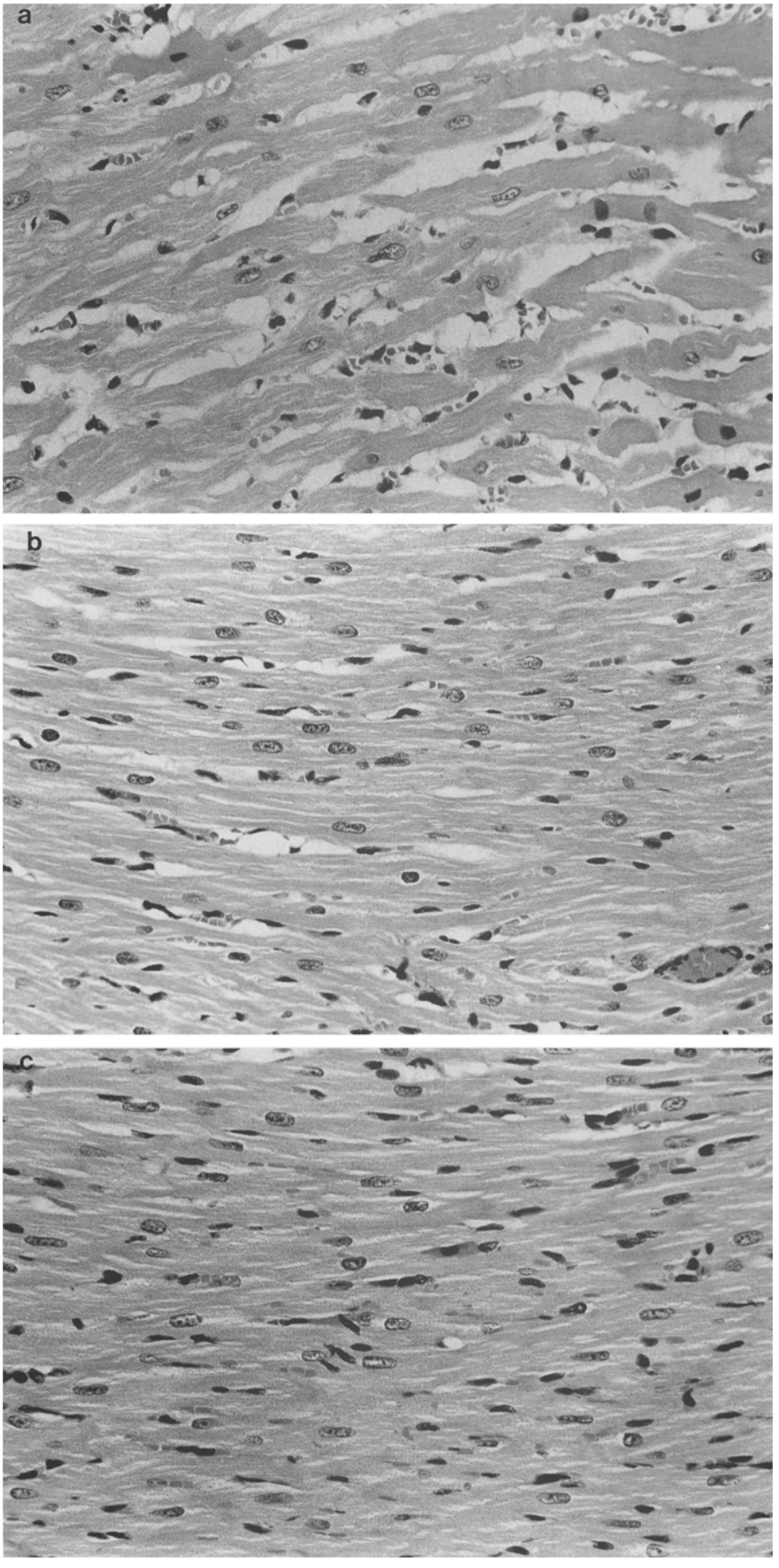

Fig. 1(a). Section of heart tissue obtained from a rat at 24 h after a single dose of doxorubicin (15 mg/kg, i. v.). The myocardial fibers are swollen and separated by oedema. H & E stain, × 100. (**b.**) Section of heart tissue obtained from a rat at 24 h after desferrioxamine treatment (250 mg/kg, i. p.) carried out 30 min prior to dosing with doxorubicin (15 mg/kg, i. v.). No myocardial lesion is apparent. H & E stain, × 100. (**c.**) Section of heart tissue obtained from a rat treated with saline only. H & E stain, × 100

Discussion

In the present study, the possible protective effects of the iron chelator desferrioxamine against the cardio and haematotoxicity of doxorubicin were investigated. A single dose of doxorubicin (15 mg/kg) caused significant elevations of serum GOT, LDH and CPK levels at 24 h after treatment. The maximal elevation of LDH levels was reached at 48 h after treatment.

It has been reported that serum GOT, LDH and CPK activities are elevated in some types of heart damage such as myocardial infarction or myocarditis and in heart failure, reaching maximal levels at 24–48 h post-injury [14]. Moreover, Singh et al. [26] have found increases in serum GOT, LDH and CPK activities following myocardial damage induced by isoproterenol sulfate in rats. On the other hand, Herman et al. [13] have observed non-significant changes in serum GOT, LDH and CPK activities after chronic administration of 1 mg/kg doxorubicin for 12 weeks in normotensive and hypertensive rats. This discrepancy could be due to the different schedules of doxorubicin administration used and to the interval between drug administration, heart damage and the enzyme assay. Desferrioxamine treatment consisting of a single i.p. dose of 250 mg/kg given 30 min before doxorubicin protected the heart from the damage induced by doxorubicin. This was clearly reflected in the nearly normal levels of cardiac enzymes and isoenzymes obtained at 48 h after the combination treatment (Tables 1, 2).

The mechanism of doxorubicin-induced cardiotoxicity has been reported by many investigators [6, 7, 16]. Our results may agree with those reported by Calabresi and Parks [7], who stated that the cardiotoxicity of doxorubicin may be attributable to free radicals, whose generation requires iron [21]. Since serum iron levels were decreased by the iron chelator desferrioxamine (data not shown) and because cardiac enzyme and isoenzyme activities returned to nearly normal levels following desferrioxamine treatment (Tables 1, 2), it seems that pretreatment with this iron chelator may prevent the formation of iron-doxorubicin complexes and thus decrease the liberation of the harmful free radicals that cause myocardial damage [16]. These interpretations are in harmony with the concept of El-Hage et al. [10, 11], who have shown that ICRF-187 can chelate iron and simultaneously reduce the severity of alloxan-induced diabetes, a toxic reaction that requires iron for the generation of free radicals.

The protective effect of desferrioxamine against doxorubicin-induced cardiac toxicity was clearly confirmed by histopathological examination of the myocardium, as desferrioxamine pretreatment prevented the histological changes induced by doxorubicin as compared with treatment with either saline or desferrioxamine alone (Fig. 1 a–c). Herman et al. [13] and Dardir et al. [8] have shown that the divalent cation chelator ICRF-187, which has the ability to chelate iron, prevents the cardiotoxic lesions induced by the chronic administration of doxorubicin and epirubicin in experimental animals. In support of our work, Van Jaarsveld et al. [28] have recently reported that desferrioxamine prevents the myocardial mitochondrial damage caused by ischemia-reperfusion but does not reverse the pre-existing damage.

With regard to the effect of doxorubicin on blood elements, doxorubicin treatment resulted in severe leucopenia and in decreases in both the number of RBCs and the Hb concentration. In support of this finding, Pannacciulli et al. [23] reported a decrease in haemopoietic progenitors of mice at 24 h after a single dose of doxorubicin (12 mg/kg). However, Yoda et al. [30] showed that doxorubicin (20 mg/kg) induced leucocytosis at 7 days after treatment in mice. In general, the effects observed for doxorubicin on the haematological parameters in the present study are in agreement with the general trend that doxorubicin treatment causes myelosuppression together with leucopenia, thrombocytopenia and anaemia [7]. However, desferrioxamine pretreatment resulted in restoration of the numbers of WBCs and RBCs and of the Hb concentration (Table 3).

Since iron is involved in the process of lipid peroxidation by a variety of different agents [20] and since desferrioxamine pretreatment induces a decrease in serum levels of iron (data not shown), it seems that desferrioxamine pretreatment protects the blood elements via the chelation of iron and thus prevents the liberation of harmful free radicals that not only are toxic to cardiac tissue [25] but also affect other tissues [2], including the erythrocyte ghost membrane [21]. The latter effect occurred via binding of the doxorubicin-iron complex to the erythrocyte ghost membrane, with the liberation of high concentrations of reactive oxygen species causing lysis of the ghost membrane; this reaction was completely blocked by EDTA via removal of the iron. In the present study, a similar reaction may have occured in different blood elements and desferrioxamine treatment may have prevented the doxorubicin-induced haematotoxicity.

The lack of parallel studies on tumour-bearing animals raises the important question as to whether desferrioxamine may increase or decrease the therapeutic efficacy of doxorubicin. This question has in some part been answered by Bristow [6], who reported an increase in the release of vasoactive substances after doxorubicin administration that were responsible for the cardiomyopathic effect, and this effect was completely independent of the antitumour activity of doxorubicin. On the other hand, Bachur et al. [1] have reported that the antitumour activity of quinone-containing anticancer drugs (doxorubicin and daunorubicin) is mediated via free-radical generation. This question needs further investigation and is currently being evaluated in our laboratory.

In conclusion, the present study shows that biochemical and haematological parameters could be used as markers for doxorubicin-induced cardio- and haematological toxicity and that the iron chelator desferrioxamine may be an interesting agent for the prevention of such harmful effects. Finally we suggest that iron chelators should be further investigated to enable a definitive assessment of their selectivity and of their possible future clinical exploitation.

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