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Effects of ICRF-187 on the cardiac and renal toxicity of epirubicin in spontaneously hypertensive rats

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Summary. A study was made of the protective effect of ICRF-187 against the cardiotoxicity and nephrotoxicity produced by epirubicin in spontaneously hypertensive rats (SHR). A total of 20 SHR were divided into 4 groups of 5 animals; the first group received i.v. injections of 1.5 mg/kg epirubicin; the second was treated with i.p. injections of 50 mg/kg ICRF-187 30 min before receiving 1.5 mg/kg epirubicin; the two remaining groups received ICRF-187 and saline, respectively, and served as controls. The experiment was terminated after 12 weekly injections (total cumulative dose of epirubicin, 18 mg/kg). Morphologic studies showed that severe cardiomyopathy manifested by myofibrillar loss and dilatation of the sarcoplasmic reticulum and nephropathy characterized by tubular dilatation and atrophy, protein casts in the lumina of renal tubules, and glomerular vacuolization occurred in SHR given epirubicin alone. Animals receiving the combination of ICRF-187 and epirubicin showed a marked reduction in the severity of cardiomyopathy and a moderate reduction in nephropathy. These changes, and their modification by ICRF-187, were similar to those we have previously observed in SHR treated with total cumulative doses of 12 mg/kg doxorubicin. Such pathologic changes were absent in animals receiving ICRF-187 or saline alone. The findings of this study suggest that ICRF-187 can be used clinically to prevent the cardiotoxicity of epirubicin, particularly in situations in which this drug may have to be given either in large doses or to patients at high risk of developing anthracycline cardiotoxicity.

Introduction

Among the anthracyclines, epirubicin (4′-epi-doxorubicin) is considered to be an effective antineoplastic agent that is less cardiotoxic than doxorubicin [1, 4, 13, 20, 21]. Nevertheless, clinically evident cardiotoxicity and cardiac lesions morphologically similar to those caused by doxorubicin have been reported in patients receiving high cumulative dose of epirubicin [17, 19, 21]. Animal studies have also demonstrated the cardiotoxic potential of this compound [2, 3, 6]. In previous investigations, we have demonstrated that spontaneously hypertensive rats (SHR) are more sensitive than normotensive Wistar-Kyoto rats (WKY) to the cardiotoxic and nephrotoxic effects of doxorubicin [11]. In addition, we have shown that, as in other species of animals [7–10], pretreatment of SHR and WKY with ICRF-187 [(+)-1,2-*bis* (3,5-dioxopiperazinyl-1-yl)propane] causes a significant reduction in the severity of duxorubicin-induced cardiac and renal lesions [12]. ICRF-187 has also been reported to produce a reduction in the incidence and severity of doxorubicin-induced cardiomyopathy in patients with carcinoma of the breast [18]. Nevertheless, it is not known whether ICRF-187 exerts protective effects against the cardiotoxicity induced by analogs of doxorubicin. Therefore, the present study was undertaken to evaluate this problem using epirubicin and the SHR model [11] of anthracycline cardiotoxicity.

Materials and methods

Adult male, spontaneously hypertensive rats (SHR) of the Aoki-Okamoto strain, 12 weeks of age and weighing 250–300 g, were obtained from Charles River Breeding Laboratories (Wilmington, Mass). The experiment commenced after a 1-week accommodation period. A total of 20 SHR were divided into 4 groups of 5 animals. One group received i.v. injections of epirubicin (1.5 mg/kg) via the tail vein once a week for 12 weeks. A second group was pretreated with 50 mg/kg ICRF-187 (i.p.) 30 min before epirubicin administration. Two additional groups were used as controls and received 12 weekly treatments with 50 mg/kg ICRF-187 (i.p.) and physiological saline, respectively. ICRF-187 (Drug Synthesis and Chemical Branch, NCI, NIH, Bethesda, Md) and lyophilized epirubicin (Adria Laboratories, Columbus, Ohio) were dissolved in normal saline just before use and injected in doses of 0.2 and 0.1 ml/100 g body weight, respectively. The animals were observed daily and weighed weekly throughout the 13-week experimental period. The dose of epirubicin used in this study was selected on the basis of reports indicating that the myelosuppressive dose of epirubicin corresponds approximately to 1.5 times that of doxorubicin [2].

Each animal was anesthetized with 45 mg/kg pentobarbital sodium 1 week after the 12th injection. A midline tracheal incision was made, and a carotid artery was isolated and cannulated with a catheter tipped with a 25-gauge needle. Systemic arterial pressure was monitored by attaching the catheter to a Hewlett-Packard 267B pressure transducer. Needle-tipped electrodes were placed s.c.

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in the appropriate limbs to record lead II of the ECG and the heart rate. Arterial pressure, lead II of the ECG, and the heart rate were recorded with a Hewlett-Packard multichannel polygraph.

Samples of left ventricular myocardium were obtained, fixed with glutaraldehyde, and prepared for electron microscopic examination as previously described in detail [8]. The remainder of the heart, one kidney, and samples of liver, lungs, and small intestine were excised from each animal and fixed in 10% neutral formalin. The hearts were embedded in glycol methacrylate plastic resin. Sections of the plastic-embedded heart tissue (1 μm thick) were stained with H & E and alkaline toluidine blue. All other tissues were embedded in paraffin and stained with H & E.

The frequency and severity of doxorubicin-induced cardiac lesions were assessed by light microscopic examination. These changes were graded on a scale of 0–4 on the basis of the number of muscle cells showing myofibrillar loss and cytoplasmic vacuolization: 0, no damage; 1, involvement of only an occasional cell; 4, severe involvement of ⩾50% of the cells; 2 and 3, intermediate degrees of involvement. Electron microscopic evaluation of the glutaraldehyde-fixed myocardial tissue was carried out using a JEOL 1200EX electron microscope. A scoring scale of 0–4 [12] was used to evaluate histologically the extent of renal damage, which was manifested by tubular dilatation and damage, deposition of protein casts within the tubular lamina, and glomerular vacuolization. All sections were evaluated without prior knowledge of the treatment given to the animals. A chi-square test was used to determine the significance of differences in the severity of cardiomyopathy and nephropathy scores among the different groups.

Results

General toxicity and weight changes

All animals survived until the experiment was terminated. All treatment groups gained weight during the first 6 weeks of treatment. After 6 weeks, the body weights of animals treated with epirubicin and with epirubicin plus ICRF-187 remained relatively constant, whereas those of animals given ICRF-187 alone or saline continued to increase (Table 1).

Blood pressure and heart rate

At the conclusion of the study, the mean systemic arterial pressure was significantly lower in animals given epirubicin alone than in the other groups (Table 2). No differences in heart rate were found among the four groups (Table 2).

Table 1. Change in body weights of SHR after 6 and 12 weekly doses of epirubicin (1.5 mg/kg), ICRF-187 (50 mg/kg) plus epirubicin (1.5 mg/kg), ICRF-187 (50 mg/kg), or saline

Treatment	Pretreatment weight ±SE	Change in body weight ±SE	
		6 weeks	12 weeks
Epirubicin	260± 2	312±6	304±10
ICRF-187/ epirubicin	263±10	309±6	300± 8
ICRF-187	263± 2	316±7	330± 8
Saline	273± 6	343±4	361± 7

Table 2. Mean arterial pressure and heart rate in pentobarbital-anesthetized SHR[a]

Treatment	Mean arterial pressure ±SE (mm Hg)	Mean heart rate ±SE (beats/min)
Epirubicin	102±16[b]	378±12
ICRF-187/epirubicin	158± 8	414±22
ICRF-187	160± 7	414±15
Saline	157± 6	420±17

[a] Determinations were made 1 week after the 12th weekly treatment with epirubicin (1.5) mg/kg), epirubicin (1.5 mg/kg) plus ICRF-187 (50 mg/kg), ICRF-187 (50 mg/kg), or saline

[b] Significantly different from saline-treated control ($P < 0.05$)

Gross anatomic changes

The only gross anatomic changes found were a pale discoloration in the kidneys of all animals given epirubicin alone and the accumulation of peritoneal and pericardial fluid in two animals in this group. The renal discoloration was less evident in animals given epirubicin together with ICRF-187 and was not found in animals given ICRF-187 or saline alone.

Myocardial pathology

The light and electron microscopic alterations in the hearts of animals treated with epirubicin were similar to those described following treatment with doxorubicin in humans

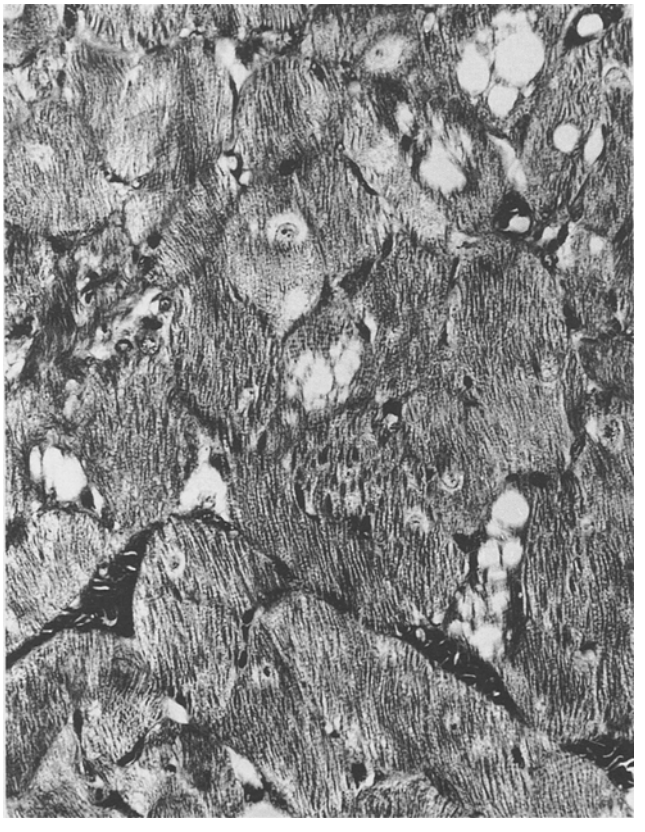

Fig. 1. Section of heart from SHR given a total cumulative dose of 18 mg/kg epirubicin. The myocytes show cytoplasmic vacuolization of moderate degree. Toluidine blue stain, X 700

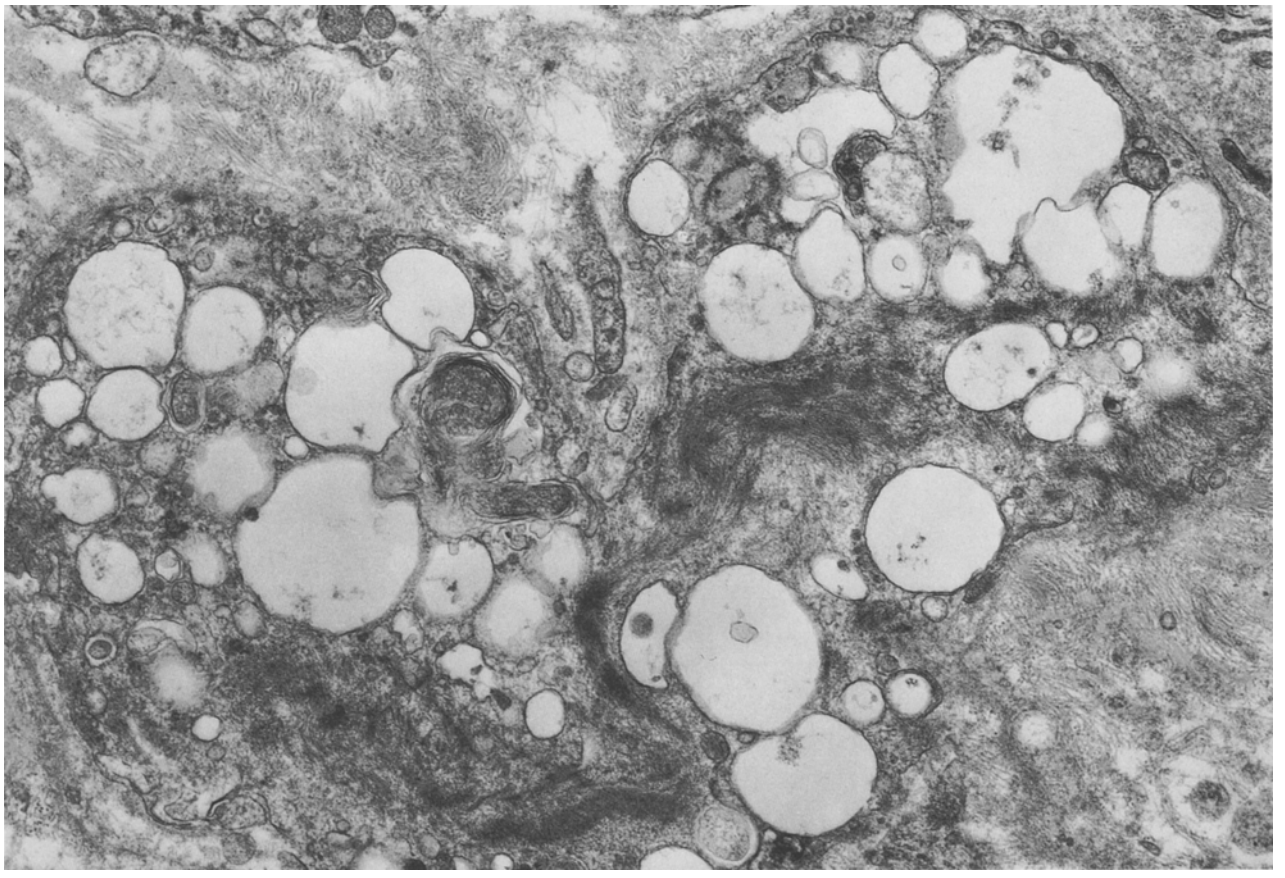

Fig. 2. Electron micrograph of heart from SHR given a total cumulative dose of 18 mg/kg epirubicin, showing complete myofibrillar loss and severe dilatation of the sarcoplasmic reticulum. Uranyl acetate and lead citrate stain, X 24,000

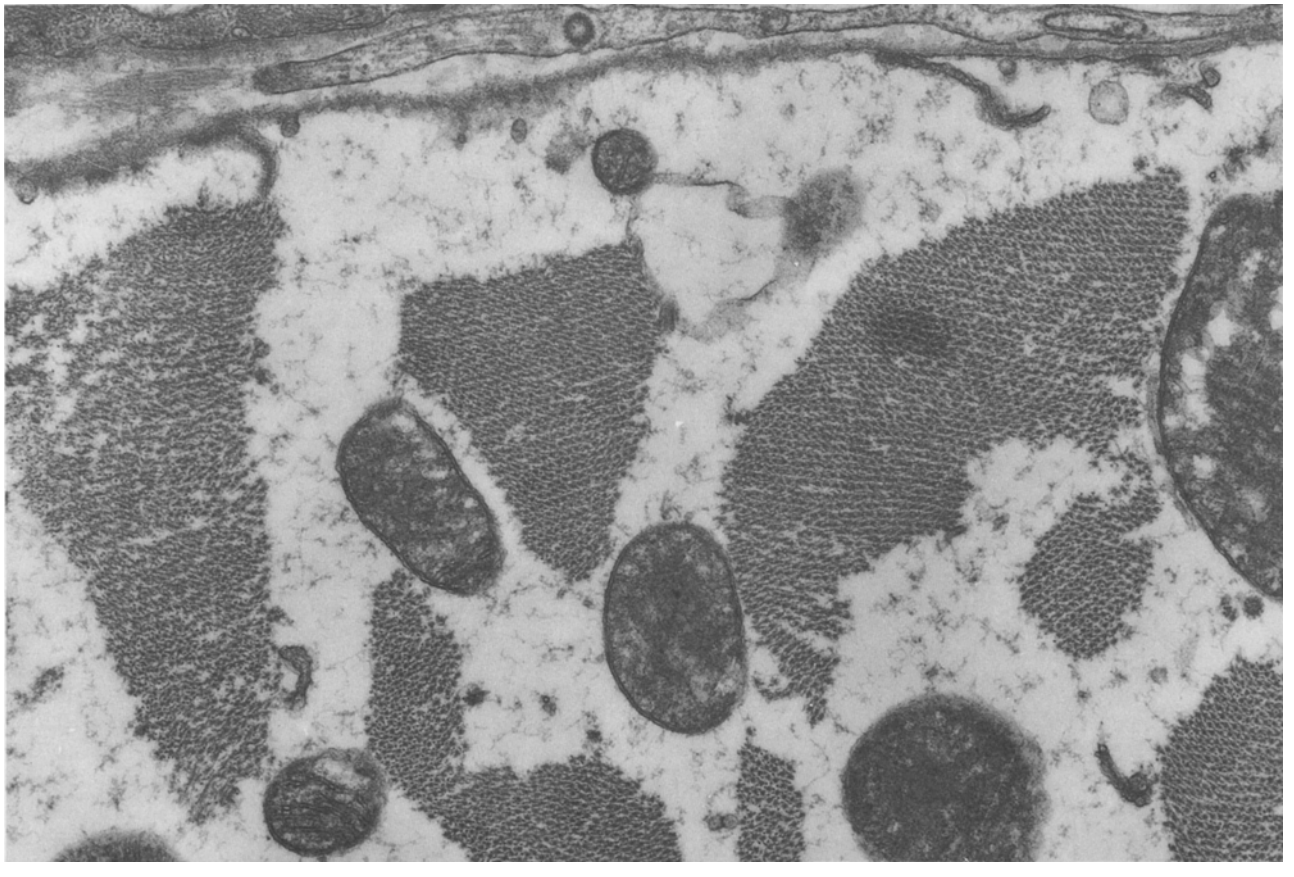

Fig. 3. Electron micrograph of heart from SHR given a total cumulative dose of 18 mg/kg epirubicin, showing marked cytoplasmic edema. Uranyl acetate and lead citrate stain, X 24,000

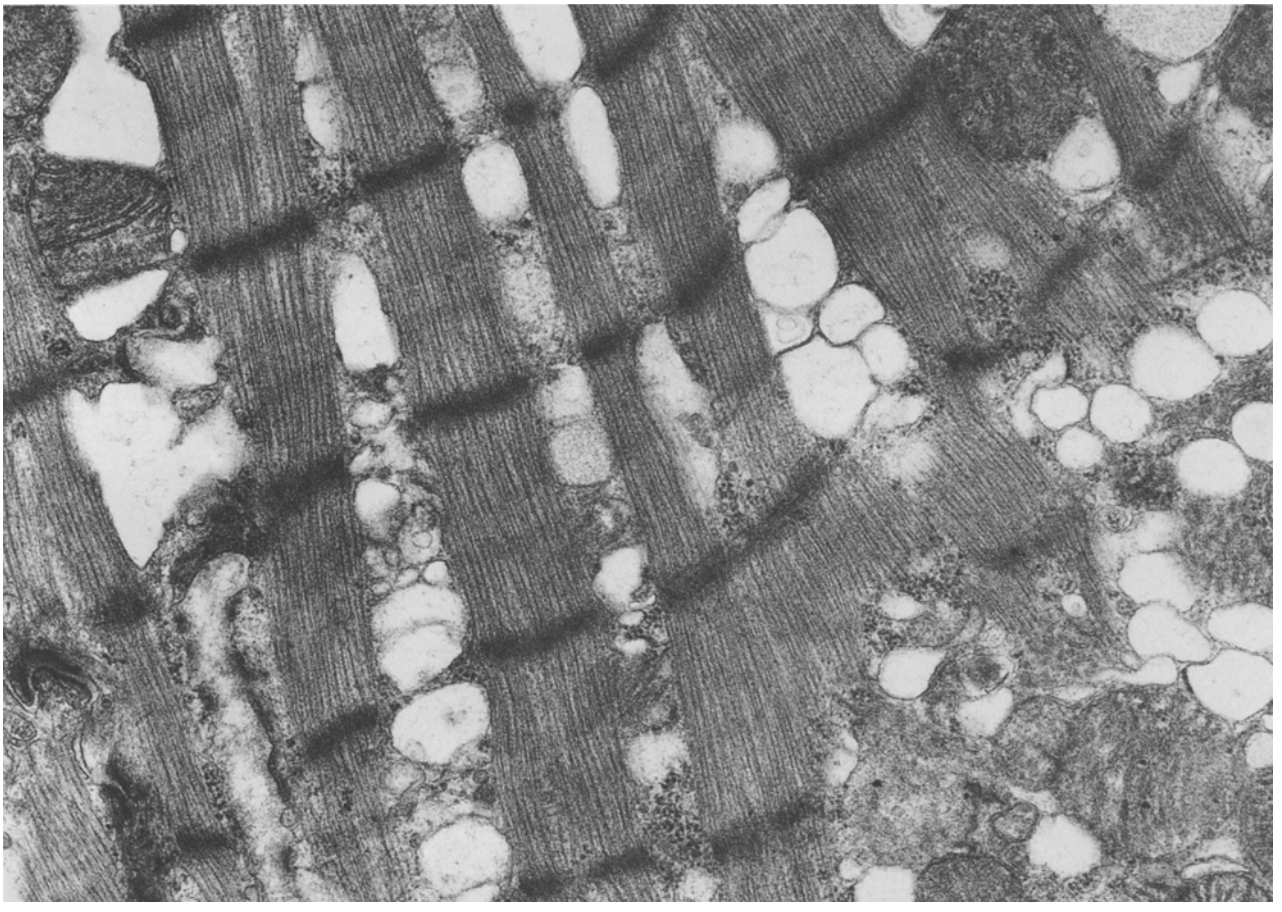

Fig. 4. Electron micrograph of heart from SHR given a total cumulative dose of 18 mg/kg epirubicin, showing moderate dilatation of the sarcoplasmic reticulum. Uranyl acetate and lead citrate stain, X 24,000

Fig. 5. Section of heart from SHR given 50 mg/kg ICRF-187 prior to dosing with epirubicin (total cumulative dose of 18 mg/kg), showing minimal cytoplasmic vacuolization (cf. Fig. 1). Toluidine blue stain, X 700

Table 3. Cardiomyopathy scores in SHR given 1.5 mg/kg epirubicin with or without 50 mg/kg ICRF-187 for 12 consecutive weeks

Treatment	Animals (*n*)	Cardiomyopathy score						Score ≤1
		0	0.5	1	2	3	4	
Epirubicin	5	0	0	0	2	3	0	0/5[a]
ICRF-187/epirubicin	5	1	3	1	0	0	0	5/5[b]
ICRF-187	5	5	0	0	0	0	0	5/5
Saline	5	5	0	0	0	0	0	5/5

[a] Where ratios are given, the numerator denotes the number of animals with a cardiomyopathy score of ≤1 and the denominator denotes the number of animals examined

[b] Lesion scores in SHR given the combination of epirubicin and ICRF-187 were significantly lower by chi-square analysis than those of SHR given epirubicin alone; $P < 0.05$

[14] and in experimental animals, including mice [6], SHR and WKY rats [11], dogs [7], rabbits [9], and pigs [8]. As previously described in detail, these alterations consisted of the loss of myofibrils, cytoplasmic vacuolization (Figs. 1 and 2), and cytoplasmic edema (Fig. 3). Cytoplasmic vacuolization was caused by dilatation of the sarcoplasmic reticulum (Fig. 4); these two changes often coexisted in the same cells. Data on the severity of the myocardial lesions in the four groups of animals are summarized in Table 3. Animals receiving the combination of epirubicin and ICRF-187 had myocardial lesions that were significantly less severe than those in animals receiving epirubicin alone ($P < 0.05$) (Figs. 5 and 6). Animals receiving ICRF-187 or saline alone had no myocardial lesions.

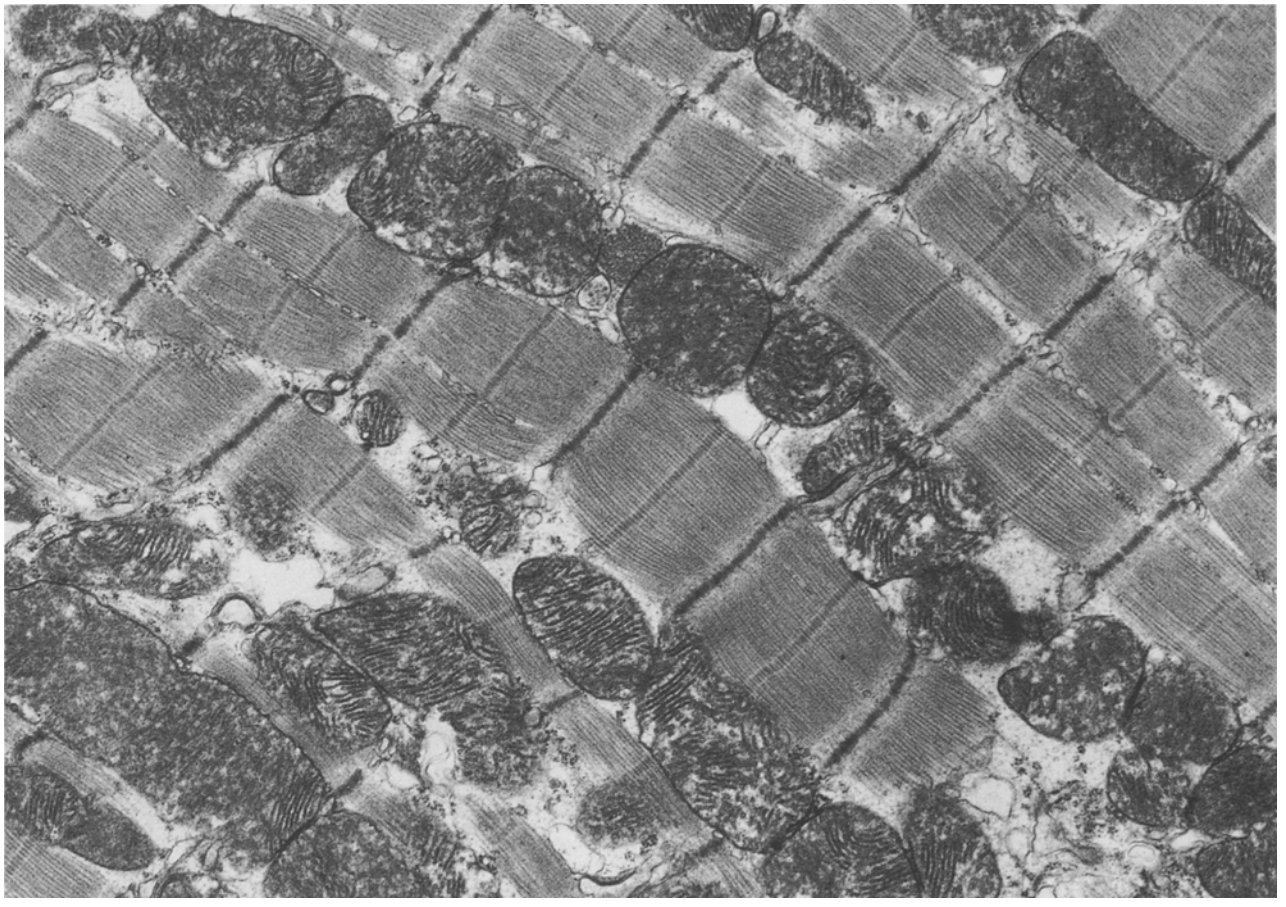

Fig. 6. Electron micrograph of heart from SHR given 50 mg/kg ICRF-187 prior to dosing with epirubicin (total cumulative dose of 18 mg/kg), showing minimal cytoplasmic edema (cf. Fig. 4). Uranyl acetate and lead citrate stain, X 15,000

Noncardiac pathology

Epirubicin caused severe renal alterations consisting of atrophy and dilatation of the tubules, protein casts in the lumina of renal tubules, and glomerular vacuolization. These changes (Fig. 7) were similar to those observed in SHR after the administration of doxorubicin under similar experimental conditions [12]. The severity of these changes in the four groups is indicated in Table 4. In comparison with the changes in animals given epirubicin alone, these renal alterations were significantly reduced ($P < 0.05$) in animals given ICRF-187 and epirubicin (Fig. 8). No lesions attributable to therapy with epirubicin were detected in other organ systems.

Discussion

Epirubicin is a new anthracycline analog that has not yet been approved for general clinical use in the United States. Results of experimental animal studies [2, 3, 6] and some human clinical trials [1, 4, 13, 20, 21] have been interpreted as indicating that, when given in equimolar doses, epirubicin is significantly less cardiotoxic than doxorubicin. The difference in cardiac toxicity is less evident when the relative myelosuppressive potencies of the two agents are taken into account. The myelosuppressive potency of epirubicin is estimated to be 70% that of doxorubicin [2]. From the standpoint of a therapeutic ratio, 1.81 mg epirubicin is considered to correspond to 1.09 mg doxorubicin in mice with P388 leukemia [3]. However, equal doses of epirubicin and doxorubicin have been found to have comparable effectiveness in patients with advanced carcinoma of the breast [13]. It has not been determined how the relative therapeutic potencies of the two drugs may differ for various other types of tumors.

Congestive heart failure has been reported in patients who received high doses of epirubicin [17, 21]. Furthermore, epirubicin has been demonstrated to induce the characteristic cardiac morphologic changes of anthracycline cardiac toxicity in both animals [2, 3, 6] and humans [19]. In the present study, we demonstrated the occurrence of severe morphologic changes in the hearts of SHR treated with a total cumulative dose of 18 mg/kg epirubicin. The cardiac histologic and ultrastructural lesions induced by this dose of epirubicin were comparable in severity with those produced by 12 mg/kg doxorubicin [11, 12]; this was also true of the severity of the histologic lesions induced in the kidney by these two drugs [11, 12].

The results of the present study also demonstrate that pretreatment with ICRF-187 protects against the cardiac toxicity and nephrotoxicity induced by epirubicin. The features of this protection are similar to those observed in SHR treated with doxorubicin, i.e., almost complete protection against cardiac lesions (myofibrillar loss and dilatation of the sarcoplasmic reticulum) and less complete protection against renal changes [11, 12]. These similarities between the reactivities of doxorubicin and epirubicin are

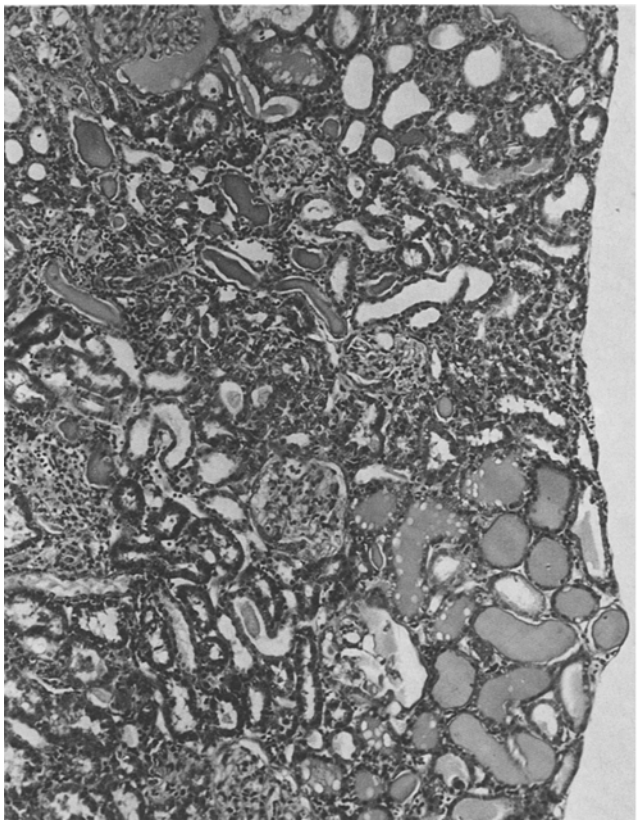

Fig. 7. Section of kidney from SHR given a total cumulative dose of 18 mg/kg epirubicin. Tubular dilatation and protein casts in tubular lumina are shown. H & E stain, X 100

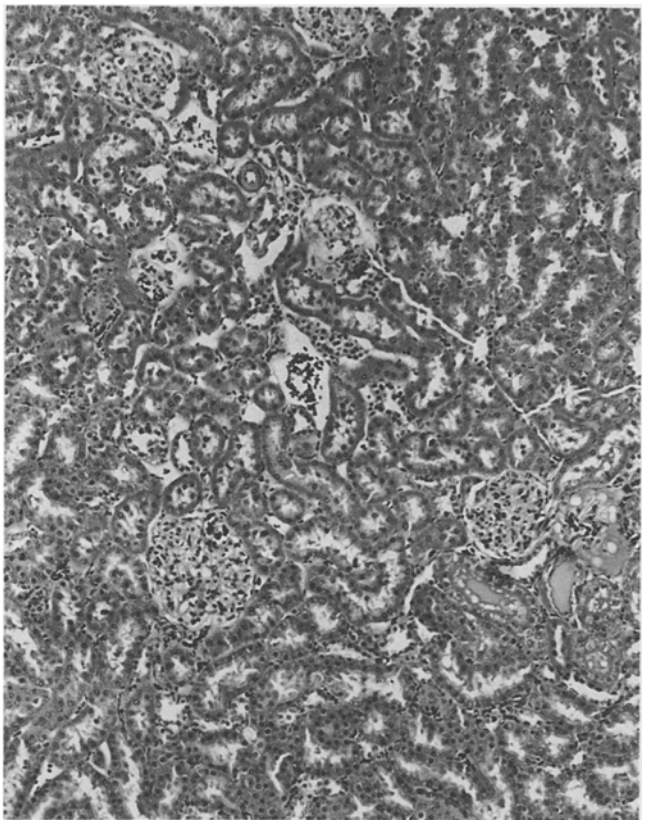

Fig. 8. Section of kidney from SHR given 50 mg/kg ICRF-187 before dosing with epirubicin (total cumulative dose of 18 mg/kg). Tubular lesions are much less severe than those shown in Fig. 7. H & E stain, X 100

Table 4. Nephropathy scores in SHR given 1.5 mg/kg epirubicin with or without 50 mg/kg ICRF-187 for 12 consecutive weeks

Treatment	Animals *(n)*	Nephropathy score					Score ≤2
		0	1	2	3	4	
Epirubicin	5	0	0	0	4	1	0/5[a]
ICRF-187/epirubicin	5	0	1	4	0	0	5/5[b]
ICRF-187	5	5	0	0	0	0	5/5
Saline	5	5	0	0	0	0	5/5

[a] Where ratios are given, the numerator denotes the number of animals with a nephropathy score of ≤2 and the denominator denotes the number of animals examined

[b] Lesion scores in SHR given ICRF-187 and epirubicin were significantly lower by chi-square analysis than those of SHR given epirubicin alone; $P < 0.05$

not surprising, in view of the close structural resemblance between these two drugs as well as the proposed mechanism (formation of cytotoxic free radicals) of their toxicities [14, 15]. The mechanism by which ICRF-187 exerts its cardioprotective effect is probably through prevention of the iron-mediated production of free radicals in cardiac myocytes [16]. A recent brief report concluded that ICRF-187 ameliorates the cardiomyopathy induced by epirubicin in mice [5]. The clinical usefulness of ICRF-187 in preventing doxorubicin-induced cardiotoxicity has been demonstrated in patients with carcinoma of the breast [18]. The findings of the present study clearly suggest that ICRF-187 can be used clinically to prevent epirubicin cardiotoxicity, particularly in situations in which this drug may have to be given either in large doses or to patients at high risk of developing anthracycline cardiotoxicity.

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