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The protective activity of ICRF-187 against doxorubicin-induced cardiotoxicity in the rat*

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Summary. The protective activity of the bisdioxopiperazine ICRF-187 against the cardiotoxicity of doxorubicin was evaluated in the rat using both functional and histological assays. Animals that had received a single i.v. dose of doxorubicin (4 mg/kg) alone were compared with those that had been pretreated with a single i.v. injection of saline or ICRF-187 (40 or 60 mg/kg). All rats showed a transient reduction in body weight during the first 3 weeks after drug administration. The greatest reduction (~16%) was observed in animals that had received a combination of ICRF-187 (40 or 60 mg/kg) and doxorubicin. Deaths related to cardiotoxicity were observed only in rats that had received doxorubicin alone and in those treated with saline; most of the deaths occurred at between 8 and 13 weeks after drug administration. Sequential assessments of heart function showed a persistent depression of cardiac output in animals that had received doxorubicin, with or without pretreatment with ICRF-187. The reduction in cardiac output observed in rats that had been pretreated with ICRF-187 (40 or 60 mg/kg) amounted to ~15% and ~30% after 12 and 20 weeks, respectively, indicating that cardioprotection was only partial. Nevertheless, this represented a marked improvement as compared with the ~35% reduction in cardiac output measured at 12 weeks in animals that had received doxorubicin but without pretreatment with ICRF-187. Histological examination of animals that had died during the course of the study and had received doxorubicin after pretreatment with saline revealed severe myocardial lesions typical of doxorubicin-induced damage. In contrast, animals that had been pretreated with ICRF-187 and survived for up to 20 weeks after treatment showed a marked amelioration of these lesions. The present findings may be interpreted as a true cardioprotection or a delay in the onset of the cardiotoxicity of doxorubicin resulting from pretreatment with the bisdioxopiperazine ICRF-187. Although prior and ongoing clinical trials clearly indicate that ICRF-187 protects patients well against doxorubicin-induced heart damage, further investigations are required before *high* doses of ICRF-187 can be used as a means of increasing the protective activity of this drug against doxorubicin-induced cardiotoxicity.

Introduction

Doxorubicin, an anthracycline antibiotic, has shown activity against a wide spectrum of tumours ranging from haematological malignancies to solid tumours [1]. However, its clinical use is often limited by its cardiotoxicity, which is delayed in onset and can be fatal [20]. The abrogation of this adverse late effect of anthracyclines presents a formidable task for biochemists, clinical pharmacologists and clinical oncologists.

A number of strategies have been suggested to reduce or abrogate the cardiotoxicity of anthracyclines. These include the synthesis of new analogues with reduced cardiotoxicity [28], the manipulation of the route or schedule of drug administration [12, 24], the modification of drug pharmacokinetics by the binding of the drug to appropriate drug carriers [23, 33] and the use of cardioprotectors [6, 17, 29]. All of these strategies are based on one important assumption, i.e. that the anti-tumour activity and the cardiotoxicity of anthracyclines occur via different mechanisms and that this difference could be exploited to improve the therapeutic ratio of anthracyclines.

Amongst these strategies, the use of cardioprotective drugs has potential advantages. It can offer a simple drug-administration system that can be used in conjunction with the bolus delivery of anthracyclines for which the pharmacokinetic properties of these drugs are well documented. A number of cardioprotective agents have been suggested

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[6, 17, 29], the most promising of which is the compound ICRF-187, a bisdioxopiperazine [6]. It is believed that as ICRF-187 is converted to a strong chelating agent following hydrolysis in vivo, it is capable of preventing the formation of doxorubicin-iron complexes, which are thought to be involved in one of the major mechanisms responsible for the cardiotoxicity of doxorubicin [7, 18, 19]. Over the last two decades, experimental studies have demonstrated the protective activity of ICRF-187 against the cardiotoxic effects of anthracyclines in a wide range of animal species [8, 9, 11, 14–16, 26]. A more recent clinical study has also indicated that ICRF-187 reduces the cardiotoxicity of doxorubicin in humans without affecting its anti-tumour activity [25]. In spite of these favourable results, a number of questions associated with the use of ICRF-187 as a cardioprotector remain to be answered. In particular, it is not known whether the time course of anthracycline-induced cardiotoxicity can be modified by the use of ICRF-187. It is also not certain whether the improvement observed reflects a delay in the onset of cardiotoxicity or true cardioprotection. Moreover, the degree of cardioprotection provided by ICRF-187 has not been quantified. The effect of ICRF-187 on the dose-response relationship of the heart to the cardiotoxic effects of anthracyclines remains largely unknown. Answer to these questions could improve the clinical application of doxorubicin and ICRF-187 and are important for patients undergoing doxorubicin therapy.

This paper describes initial investigations in which the cardioprotective activity of ICRF-187 was studied in the rat using an established animal model [30–32]. The body weight, cardiac output and heart rate of rats were measured sequentially for up to 20 weeks after the administration of doxorubicin with or without pretreatment with ICRF-187 or saline. Histological examinations were carried out on animals that died during the course of the study and on those that were killed at the end of the observation period. The severity of the lesions was assessed using an established histological scoring method [14].

Materials and methods

A total of 56 male Sprague-Dawley rats aged 14 weeks were divided into 7 groups containing a minimum of 6 animals each (Table 1). The rats in groups 1, 2 and 3 received an i. v. injection of saline (2 ml/kg) 15 min after the i. v. administration of either saline or 40 or 60 mg/kg ICRF-187, respectively. Animals in groups 4, 5 and 6 received an i. v. injection of either saline or 40 or 60 mg/kg ICRF-187, respectively, at 15 min prior to the i. v. administration of 4 mg/kg doxorubicin. A group of 11 rats (group 7) received a single i. v. injection of 4 mg/kg doxorubicin alone. The animals in group 1 served as age-matched controls for this study.

ICRF-187 (Adria Laboratories, USA) and doxorubicin (Farmitalia, Italy) were dissolved in saline (15 mg/ml) and water (2 mg/ml), respectively, for injection just prior to their use. Drugs were injected into the femoral vein at a rate of 2 ml/min. To minimise the possible effect of circadian rhythms on the toxicity of the drugs, drug administration was always carried out between 1330 and 1530 hours. During drug administration and the subsequent functional measurements, the animals were anaesthetised with chloral hydrate (300 mg/kg).

The methods used to assess the general acute toxicity and late cardiotoxicity in the rat have been described elsewhere [31]. Briefly, the body weights of animals were measured daily during the first 3 weeks following drug administration. The maximum reduction in body weight recorded over this period was used as an index to assess the general acute toxicity of the drug. The longer-term cardiotoxicity of the drugs was assessed by sequential 4-weekly measurements of cardiac output and heart rate for up to 20 weeks. Cardiac output was assessed using an external counting technique: each rat was anaesthetised and immobilised in a supine position, and a bolus of 0.4 mCi sodium pertechnetate Tc 99m (Amersham) was injected into the femoral vein. The activity-time curve over the precardial region was recorded for a period of 40 s using an NaI detector connected to a multi-channel analyser (ND-62, Nuclear Data) at a counting interval of 0.1 s. ECG and heart-rate measurements were carried out using a human ECG monitor (Hewlett Packard 7830A) coupled to a scope memory (Model VK-12-2, Seltek Instrument Ltd) and a chart recorder.

Postmortem examinations were carried out on animals that had died during the course of the study and on those that had been killed at the end of the experiment after 20 weeks. The heart tissue was fixed in 10% formal saline and embedded in Paraplast, after which 5-μm-thick sections were cut and stained with haematoxylin and eosin or Hughesdon's modification of Mallory's stain for collagen. Detailed histological examinations were carried out in four groups of animals, i.e. in those receiving saline plus doxorubicin (group 6), those receiving 40 or 60 mg/kg ICRF-187 plus doxorubicin (groups 4 and 5) and those receiving 60 mg/kg ICRF-187 plus saline (group 3). The heart was examined at three levels along with longitudinal axis, i.e. through the atria and through the upper and lower third of the ventricles, so as to permit evaluation of the septum as well as the left and right ventricular walls. Scoring of cardiac changes was based on criteria established in previous studies of anthracycline-induced myocardial damage in experimental animals (Table 2). The total lesion score assigned to each rat heart was the sum of the damage observed at the atria level and the average of that found at the two ventricular levels.

Table 1. List of the treatment groups used in the present study

Group	Animals (*n*)	Treatment[a]
1	6	Saline + saline
2	6	ICRF-187 (40 mg/kg) + saline
3	6	ICRF-187 (60 mg/kg) + saline
4	9	ICRF-187 (40 mg/kg) + DOX (4 mg/kg)
5	9	ICRF-187 (60 mg/kg) + DOX (4 mg/kg)
6	9	Saline + DOX (4 mg/kg)
7	11	DOX (4 mg/kg)

[a] All agents were given i. v., with 15 min elapsing between injections
DOX, Doxorubicin

Table 2. Histological scores and characteristics of doxorubicin-induced cardiac lesions in the rat[a]

Histological score	Characteristics of lesions
1	Scattered cardiac myocytes of normal diameter and density but with reduced sarcoplasmic myofilamentous density
2	Large numbers of contiguous myocytes showing changes characteristic for histological score 1
3	Many contiguous myocytes exhibiting a markedly reduced myofilamentous density leaving interfibrillary gaps containing sarcoplasmic granules ("adria cells")
4	Similar to 3 but myocytes reduced in diameter with an increase in intermyofibre distances
5	Majority of myocytes affected with sharply reduced myocyte diameter and prominent interfibre spaces; many "adria cells" present

[a] Modified from Jaenke [14]

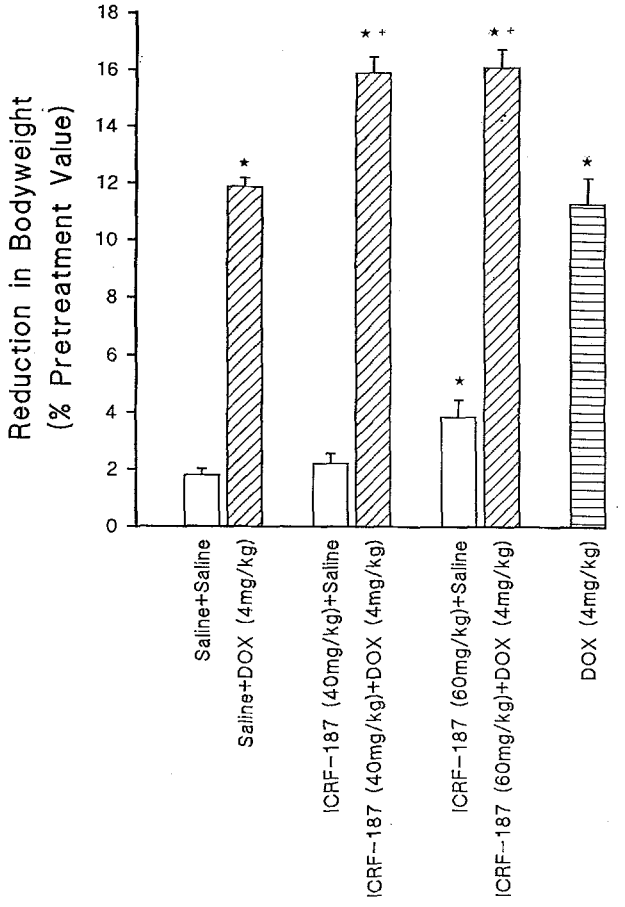

Fig. 1. Histogram showing the mean (± SE) maximum reduction in body weight shown by the different treatment groups during the first 2–3 weeks after drug administration (★, $P < 0.05$ vs Saline + Saline; +, $P < 0.001$ vs Saline + doxorubicin (*DOX*) or DOX alone

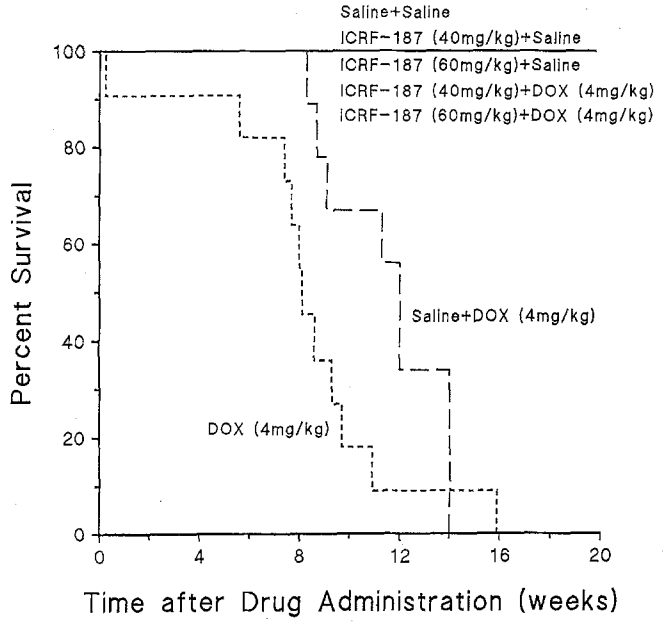

Fig. 2. Time-related changes in animal survival for up to 20 weeks after drug administration

Animals that had died during the course of the study were included in the final analysis only when they fulfilled the criteria of heart failure described elsewhere [31, 32]. In the present study, statistical differences between group means were analysed using Student's two-sided *t*-test.

Results

In the first 3 weeks after drug administration, we observed no apparent sign of acute toxicity except in one rat that had received doxorubicin alone. However, the daily weighing of animals over this period showed a transient reduction in body weight. Rats that had received two consecutive i.v. injections of saline 15 min apart (group 1) showed a small but non-significant reduction in body weight of ~2% (Fig. 1); the maximum reduction in body weight in these animals was observed on day 2, and recovery to pretreatment levels was noted on day 3 or 4. A similar pattern and magnitude of change in body weight was observed in rats that had received 40 mg/kg ICRF-187 and saline (group 2). All of the remaining animals showed a significant reduction in body weight over the initial 2-week period ($P < 0.05$), with the maximum reduction in body weight being observed between day 6 and day 7 and recovery to approximate pretreatment levels occurring at around day 14. The greatest reduction in body weight (~16%) was seen in rats that had received the combination of ICRF-187 (either 40 or 60 mg/kg) and doxorubicin (4 mg/kg); this decrease in body weight was significantly larger than that observed in animals that had received either saline and doxorubicin or doxorubicin alone ($P < 0.001$).

One rat that had received 4 mg/kg doxorubicin alone died on day 3 after drug administration and showed clinical signs of congestive heart failure, with haemorrhagic pleural effusion (~10 ml) and general subcutaneous oedema. The majority of cardiotoxicity-related deaths observed in the remaining animals that had been given doxorubicin alone occurred at between 7 and 13 weeks after drug administration (Fig. 2). Approximately 50% of these rats had died of heart failure by week 8. All other animals, i.e. those that had received the combination of 40 or 60 mg/kg ICRF-187 and doxorubicin (groups 4 and 5), age-matched control animals (group 1) and those that had been given ICRF-187 and saline (groups 2 and 3), survived over the course of the 20-week study.

The time-related changes in cardiac output, measured in treated rats and expressed as a percentage of age-matched controls values (group 1) are shown in Figs. 3 and 5. Age-matched control animals showed an age-related change in cardiac output amounting to 243.0±6.6, 217.1±6.5 and 205.2±6.5 ml min^{-1} kg^{-1} at the age of 18, 26 and 34 weeks, respectively (i.e., corresponding to postinjection intervals of 4, 12 and 20 weeks, respectively).

The rats that had received ICRF-187 (40 or 60 mg/kg) at 15 min prior to the administration of saline (groups 2 and 3) showed a transient reduction in cardiac output of ~13% after 4 weeks ($P < 0.001$; Fig. 3). Furthermore, sequential measurements of heart rate in these animals showed a progressive and significant increase from at 4 weeks after drug administration ($P < 0.01$; Fig. 4). The mean heart rate was increased by ~15% as compared with that measured in

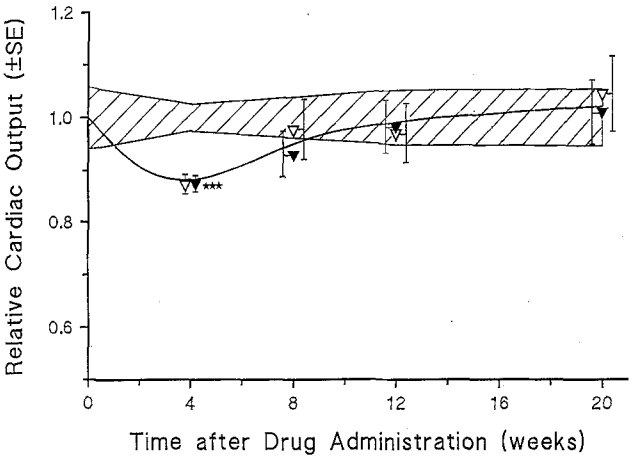

Fig. 3. Time-related changes in the relative cardiac output of rats (± SE) after treatment with 40 mg/kg ICRF-187 and saline (▽) or 60 mg/kg ICRF-187 and saline (▼). Results are expressed as a fraction of the levels shown by age-matched control animals. *Hatched area,* ±SE of age-matched control values. *** P <0.001 vs controls

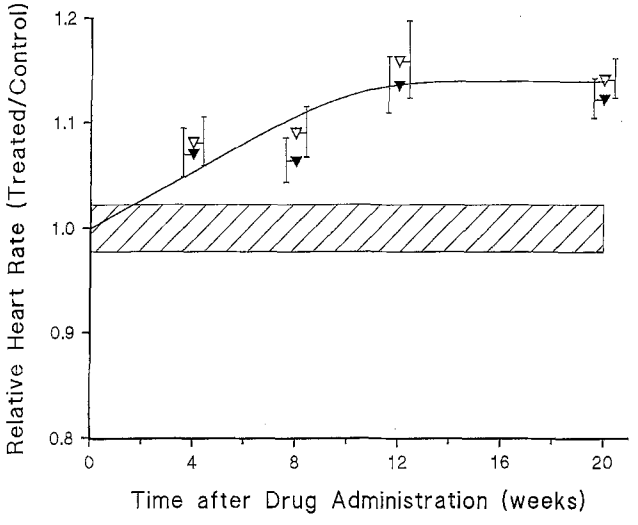

Fig. 4. Time-related changes in the relative heart rate of rats (±SE) after treatment (for key to symbols, see Fig. 3). Results are expressed as a fraction of the levels shown by age-matched control animals

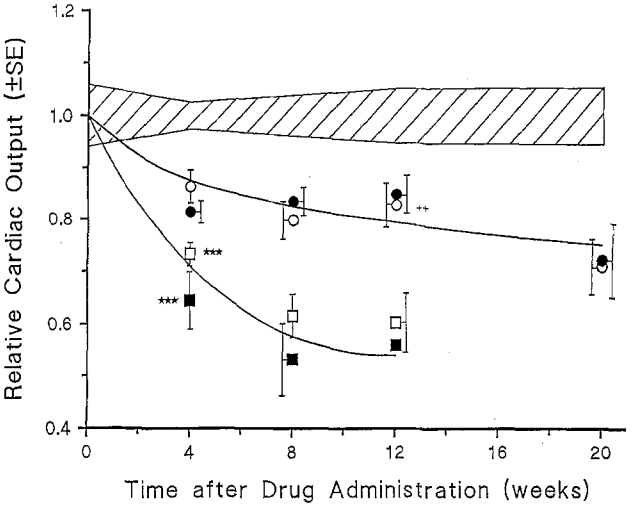

Fig. 5. Time-related changes in the relative cardiac output of rats (±SE) after treatment with 40 mg/kg ICRF-187 and 4 mg/kg DOX (○); 60 mg/kg ICRF-187 and 4 mg/kg DOX (●); saline and 4 mg/kg DOX (□); or 4 mg/kg DOX alone (■). Results are expressed as a fraction of the levels shown by age-matched control animals. *Hatched area,* ±SE of age-matched control values. *** P <0.001 vs controls; ++ P <0.05 vs controls

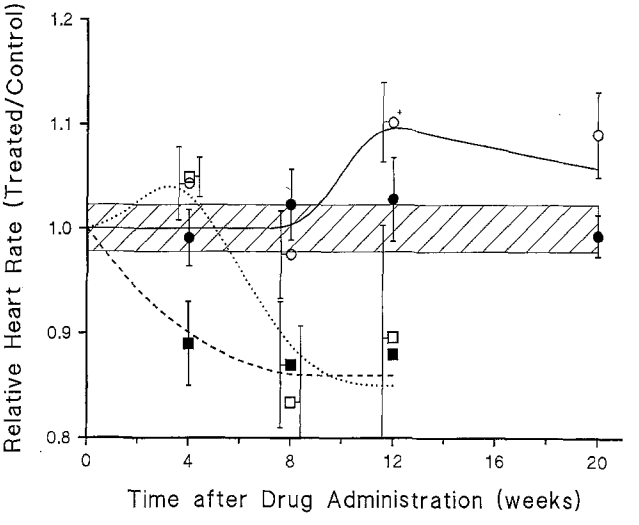

Fig. 6. Time-related changes in the relative heart rate of rats (±SE) after treatment (for key to symbols, see Fig. 5). Results are expressed as a fraction of the levels shown by age-matched control animals. + P <0.05 vs controls

age-matched controls at 12 weeks; the heart rate remained elevated at this level until week 20.

All animals that had received doxorubicin with or without pretreatment with ICRF-187 showed a significant reduction in cardiac output relative to age-matched control values by week 4 (P <0.001), which persisted throughout the course of the study (Fig. 5). However, pretreatment with ICRF-187 significantly reduced the magnitude of this effect as compared with that observed in rats that had been given doxorubicin alone or those that had been pretreated with saline prior to the injection of doxorubicin (P <0.02). The latter two groups, i.e. animals that had not been pretreated with ICRF-187, also exhibited a significant reduction in heart rate of ~10% at 8 and 12 weeks (P <0.05; Fig. 6). Pretreatment with ICRF-187 prevented this decrease in heart rate, although animals that had been pretreated with only 40 mg/kg ICRF-187 did show a transient increase in heart rate at 12 weeks (P <0.05; Fig. 6).

Histological examination of the hearts of rats that had died at between 8 and 14 weeks after having received saline 15 min prior to the i.v. administration of doxorubicin revealed varying degrees of cardiomyopathy, with typical anthracycline-induced cardiac myocyte changes. In most rats examined at between 8 and 12 weeks, the characteristic change was one of the progressive loss of myofilaments, first from a small number of myocytes located in random focal regions and then a more diffuse involvement of parenchymal cells that were seen to contain only eosinophilic granules. The latter myocytes have previously been designated as "adria cells" [15]. In severely affected areas, the myocyte diameter was markedly reduced and the interstitium appeared to be more prominent. Lesions were consistently more severe in the atria. Ventricular changes,

Table 3. Histological grading of cardiac lesions after treatment with doxorubicin and/or ICRF-187

Treatment schedule[a]	Atria	Ventricles
Saline + DOX (4 mg/kg)	3.57 (3–4)	2.1 (0.5–4)
ICRF-187 (40 mg/kg) + DOX (4 mg/kg)	0.25 (0–1)	0.5 (0–1)
ICRF-187 (60 mg/kg) + DOX (4 mg/kg)	0.67 (0–1)	0.0
ICRF-187 (60 mg/kg) + saline	0.0	0.0

[a] All agents were given i. v., with 15 min elapsing between injections Data represent the mean grades scored for lesions; the associated value ranges are shown in parentheses. DOX, Doxorubicin

although less severe, were in general similar at the base and apex of the heart and a similar severity and number were found in the free walls of both the left and the right ventricles as well as in the septum. Two rats that survived for 14 weeks exhibited moderately severe atrial lesions; however, the ventricular changes noted in these animals were quantitatively less severe than those observed in other doxorubicin-treated rats. The mean histological scores for the lesions seen in the atria and ventricles of the group of rats that had been treated with saline plus doxorubicin were 3.75 and 2.1, respectively (Table 3).

In contrast, the lesions in rats that had been pretreated with either 40 or 60 mg/kg ICRF-187 were markedly less severe. At 20 weeks after treatment, changes in myocytes were limited to a small number of scattered fibres that contained reduced numbers of myofilaments in both the atria and the ventricles. Little evidence of progressive damage was observed in the rats examined. The histological scores for lesions observed in the atria and ventricles of these two groups of animals were much lower than those for lesions observed in rats that had received saline and doxorubicin (Table 3).

Discussion

The mechanisms leading to cardiac damage after doxorubicin administration are not yet fully understood. However, recent experimental evidence has suggested that the cardiotoxicity of doxorubicin may be related to its ability to generate free radicals in heart tissue [3]. One of these pathways involves the formation of a complex of doxorubicin and iron [7, 18, 19]. It has been shown that this doxorubicin-iron complex can bind to vital cellular structures such as DNA and cell membranes and cause local oxidative destruction of these structures via a free-radical mechanism [4, 8, 19]. This observation suggests that iron could play an important role in doxorubicin-induced cardiotoxicity [18].

The compound ICRF-187, a bisdioxopiperazine, is a cyclic analogue of ethylenediaminetetraacetic acid (EDTA) that undergoes hydrolysis in cells [2]. One of its main hydrolysis product is a strong chelator of metal cations including iron [13]. Studies in humans have shown that ICRF-187 causes a marked increase in the clearance of iron and other transitional metal ions by the kidney [27]. On the basis of these observations, it has been suggested that ICRF-187 might be used to block the formation of a doxorubicin-iron complex, thereby reducing the cardiotoxic effects of doxorubicin. The results of experimental studies carried out over the last two decades have indicated that ICRF-187 provides protection against doxorubicin-induced cardiotoxicity in a number of animal species, including the mouse, rat, rabbit, dog and miniature pig [5, 8, 9, 11, 26]. The general conclusion drawn from these studies was that pretreatment with ICRF-187 increased animal survival and reduced the incidence and severity of doxorubicin-induced myocardial lesions. Furthermore, a recent randomised clinical study involving patients with advanced breast cancer demonstrated that ICRF-187 reduced the cardiotoxicity of doxorubicin without affecting its antitumour activity [25].

In the present study, the protective activity of a single i.v. dose of 40 or 60 mg/kg ICRF-187 against the cardiotoxicity produced by a single i.v. dose of 4 mg/kg doxorubicin (a drug-dose ratio of ICRF-187 to doxorubicin 10:1–15:1) was investigated. Using both functional and histological end points, we demonstrated that at these doses, ICRF-187 produced significant long-term protection against doxorubicin-induced cardiotoxicity. Sequential measurements of cardiac output over a period of 20 weeks in animals that had been pretreated with ICRF-187 showed a significantly reduced cardiac dysfunction as compared with those that had received doxorubicin alone or pretreated with saline ($P < 0.02$). This protective effect appeared to be only partial, since the cardiac output measured in these animals was consistently lower than that observed in age-matched controls ($P < 0.01$). Moreover, the histological examination of animals that had been pretreated with ICRF-187 revealed a marked reduction in doxorubicin-induced cardiac lesions. Long-term protective effects of ICRF-187 against daunorubicin-induced cardiotoxicity in rabbits have also been demonstrated in a recent study [10]. Rabbits receiving ICRF-187 prior to daunorubicin showed a marked reduction in both the incidence and the severity of cardiac lesions at 3 months after the last dose of a multiple-injection regimen.

The time course of changes in cardiac output observed over the 20 weeks of this investigation in the rat after doxorubicin administration did not seem to be altered by pretreatment with ICRF-187. After combined treatment with ICRF-187 and doxorubicin, animals showed an initial decline in cardiac output, which remained persistently depressed throughout the course of the study. The mean value obtained for cardiac output in rats at 20 weeks was ~70% of the age-matched control value. However, since there was a slight but significant ($P < 0.05$) decrease in cardiac output at 20 weeks as compared with that measured at 12 weeks, it was not known whether this level of reduction in cardiac output would be maintained in these animals beyond the 20-week study period. Thus, as judged from the results of these initial studies, it remains uncertain as to whether ICRF-187 produced only a delay in the onset of cardiotoxicity or provided true protection against doxoru-

bicin-induced cardiotoxicity. A longer follow-up period will be required to clarify this important point.

Combined treatment with ICRF-187 and doxorubicin produced a maximum reduction in body weight of ~16% during the first 3 weeks after drug administration, suggesting a more than additive effect for the acute toxicities caused by each of these substances as single agents. A further increase in the dose of ICRF-187 was limited by this combined acute toxicity of the two agents. However, this 'acute' period was followed by a marked improvement in the general condition of the rats. Following the initial delay, our measurements showed that these animals continued to grow at a rate similar to that of age-matched controls. Although these rats were ~10% lighter than the age-matched control animals, they appeared healthy and active over the 20-week study period. All of these animals survived over the whole period of the study, whereas rats that had received doxorubicin alone (or pretreated with saline) had died of heart failure by week 14.

The transient but significant ($P < 0.001$) 13% reduction in cardiac output measured after 4 weeks in animals that had received ICRF-187 and saline was somewhat unexpected. Moreover, in addition to the reduction in cardiac output, there was a significant increase in heart rate at 4 weeks ($P < 0.01$), which progressed to a value that was ~15% higher than control levels at 12 weeks and remained elevated at this level until the end of the experiment at 20 weeks. If these changes signify a subtle alteration in the heart, then they did not affect the general condition of the rats – these animals put on weight at the same rate as the controls and survived over the entire course of the study. Furthermore, histological investigations at 20 weeks did not reveal any significant change in the heart. Thus, it would appear to be important to study the effects of strong metal-ion chelating agents on the heart. Recent experiments have suggested that perturbation of the calcium concentration in the heart could impair cardiac function; this has been postulated as one of the mechanisms underlying doxorubicin-induced cardiotoxicity [16, 21, 22]. Although prior and ongoing clinical trials clearly indicate that ICRF-187 protects patients well against doxorubicin-induced heart damage, the present study shows that further investigations are required before *high* doses of ICRF-187 can be used as a means of increasing the protective activity of this drug against doxorubicin-induced cardiotoxicity.

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References

1. Blum RH (1975) An overview of studies with Adriamycin in the United States. Cancer Chemother Rep 6: 247
2. Dawson KM (1975) Studies on the stability and cellular distribution of dioxopiperazines in cultured BHK-21 cells. Biochem Pharmacol 24: 2245
3. Doroshow JH (1988) Role of reactive oxygen production in doxorubicin cardiac toxicity. In: Hacker MP, Lazo JS, Tritton TR (eds) Organ directed toxicities of anticancer drugs. Martinus Nijhoff, Boston, p 31
4. Eliot H, Gianni L, Myers CE (1984) Oxidative destruction of DNA by Adriamycin-iron complex. Biochemistry 23: 928
5. Filppi JA, Imondi AR, Wolgemuth RL (1988) Characterization of the cardioprotective effect of (S) (+)-4,4′-propylene-2,6-piperazinedione (ICRF-187) on anthracycline cardiotoxicity. In: Hacker MP, Lazo JS, Tritton TR (eds) Organ directed toxicities of anticancer drugs. Martinus Nijhoff, Boston, p 225
6. Green MD (1987) Rationale and strategy for prevention of anthracycline cardiotoxicity with the bisdioxopiperazine, ICRF-187. Pathol Biol 35: 49
7. Gutteridge JMC (1983) Adriamycin-iron catalysed phospholipid peroxidation: a reaction not involving Adriamycin or hydroxyl radicals. Biochem Pharmacol 32: 1945
8. Herman EH, Ferrans VJ (1983) ICRF-187 reduction of chronic daunomycin and doxorubicin cardiac toxicity in rabbits, beagle dogs and miniature pigs. Drug Exp Clin Res 9: 483
9. Herman EH, Ferrans VJ (1983) Influence of vitamin E and ICRF-187 on chronic doxorubicin cardiotoxicity in miniature swine. Lab Invest 49: 69
10. Herman EH, Ferrans VJ (1990) Examination of the potential long-lasting protective effect of ICRF-187 against anthracycline-induced chronic cardiomyopathy. Cancer Treat Rev 17: 155
11. Herman EH, Ferrans VJ, Myers CE, Vleet JF van (1985) Comparison of the effectiveness of (±)-1,2-bis(3,5-dioxopiperazin-1-yl)propane (ICRF-187) and *N*-acetylcysteine in preventing chronic doxorubicin cardiotoxicity in beagles. Cancer Res 45: 276
12. Hortobagyi GN, Frye D, Buzdar AU, Ewer MS, Fraschini G, Huo V, Ames F, Montague E, Carrasco CH, Mackay B, Benjamin RS (1989) Decreased cardiac toxicity of doxorubicin administered by continuous intravenous infusion in combination chemotherapy for metastatic breast carcinoma. Cancer 63: 37
13. Huang ZX, May PM, Quinlan KM, Williams DR, Creighton AM (1982) Metal binding by pharmaceuticals: 2. Interactions of Ca(II), Cu(II), Fe(II), Mg(II), Mn(II) and Zn(II) with the intracellular hydrolysis products of the antitumour agent ICRF-159 and its inactive homologue ICRF-192. Agents Actions 12: 536
14. Jaenke RS (1976) Delayed and progressive myocardial lesions after Adriamycin administration in the rabbit. Cancer Res 36: 2958
15. Jaenke RS, Fajardo LF (1977) Adriamycin-induced myocardial lesions. Am J Surg Pathol 1: 55
16. Jensen RA (1986) Doxorubicin cardiotoxicity: contractile changes after long term treatment in the rat. J Pharmacol Exp Ther 236: 197
17. Milei J, Boveris A, Llesuy S, Molina HS, Storino R, Ortesa D, Milei SE (1986) Amelioration of Adriamycin-induced cardiotoxicity in rabbits by prenylamine and vitamins A and E. Am Heart J 111: 95
18. Myers CE (1988) Role for iron in anthracycline action. In: Hacker MP, Lazo JS, Tritton TR (eds) Organ directed toxicities of anticancer drugs. Martinus Nijhoff, Boston, p 17
19. Myers CE, Gianni L, Simone CB, Klecker R, Greene R (1982) Oxidative destruction of erythrocyte ghost membranes catalyzed by the doxorubicin-iron complex. Biochemistry 21: 1707
20. Neri B, Cini-Neri G, Bandinelli M, Bartalucci S, Cialpio A (1989) Doxorubicin and epirubicin cardiotoxicity: experimental and clinical aspects. Int J Clin Pharmacol Ther Toxicol 27: 217
21. Olson RD, Mushlin PS (1990) Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. FASEB J 4: 3076
22. Rabkin SW (1983) Interaction of external calcium concentration and verapamil on the effects of doxorubicin (Adriamycin) in the isolated heart preparation. J Cardiovasc Pharmacol 5: 848
23. Rahman A, Schein PS (1988) Use of liposomes in cancer chemotherapy. In: Gregoriadis G (ed) Liposomes as drug carriers. John Wiley & Sons, Chichester, p 381
24. Shapira J, Gotfried M, Lishner M, Ravid M (1990) Reduced cardiotoxicity of doxorubicin by a 6-hour infusion regimen. Cancer 65: 870
25. Speyer JL, Green MD, Kramer E, Rey M, Sanger J, Ward C, Dubin N, Freeans V, Stecy P, Zeleniuch-Jacquotte A, Wernz J, Feit F, Slater W, Blum R, Muggia F (1988) Protective effect of the

bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. N Engl J Med 319: 745
26. Villani F, Galimberti M, Monti E, Cova D, Lanza E, Rozza-Dionigi A, Favalli L, Poggi P (1990) Effect of ICRF-187 pretreatment against doxorubicin-induced delayed cardiotoxicity in the rat. Toxicol Appl Pharmacol 102: 292
27. Von Hoff DD, Howser D, Lewis BJ, Holcenberg J, Weiss RB, Young RC (1981) Phase I study of ICRF-187 using a daily for 3 days schedule. Cancer Treat Rep 65: 249
28. Weiss RB, Sarosy G, Clagett-Carr K, Russo M, Leyland-Jones B (1986) Anthracycline analogs: the past, present and future. Cancer Chemother Pharmacol 18: 185
29. Yamanaka N, Kato T, Nishida K, Fujikawa T, Fukushima M, Ota K (1979) Elevation of serum lipid peroxide levels associated with doxorubicin toxicity and its amelioration by [D, L]-α-tocopheryl acetate or coenzyme Q_{10} in mouse. Cancer Chemother Pharmacol 3: 223
30. Yeung TK, Simmonds RH, Hopewell JW (1988) Time- and dose-related modifications in cardiac function in rats after single intravenous doses of epirubicin. Radiother Oncol 11: 263
31. Yeung TK, Simmonds RH, Hopewell JW (1989) A functional assessment of the relative cardiotoxicity of Adriamycin and epirubicin in the rat. Radiother Oncol 15: 275
32. Yeung TK, Simmonds RH, Hopewell JW (1989) The relative toxicity of intravenous and intraperitoneal doses of epirubicin in the rat. Cancer Chemother Pharmacol 24: 211
33. Yeung TK, Hopewell JW, Simmonds RH, Seymour LW, Duncan R, Bellini D, Grandi M, Spreafico F, Strohalm J, Ulbrich K (1991) Reduced cardiotoxicity of doxorubicin administered in the form of *N*-(2-hydroxypropyl)methacrylamide conjugates: an experimental study in the rat. Cancer Chemother Pharmacol 29: 105