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ORIGINAL ARTICLE

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Comparison of the protective effects of desferrioxamine and ICRF-187 against doxorubicin-induced toxicity in spontaneously hypertensive rats

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Abstract Since the iron-mediated formation of free radicals is considered to be a critical factor in the pathogenesis of the toxicity of doxorubicin (DXR), comparisons were made of the protective effects of two iron chelators, ICRF-187 and desferrioxamine (DFO), against the chronic cardiac and renal toxicity induced by DXR in spontaneously hypertensive rats (SHR). Two preparations of DFO were studied: DFO mesylate (DFO-M) and a polymeric form (DFO-P) in which DFO is conjugated to hydroxyethyl starch. Groups of 5 SHR each were given 12 weekly i.v. injections of 1 mg/kg DXR either alone or 30 min after the i.p. injection of 25 mg/kg ICRF-187, 50 mg/kg DFO-M, 50 mg/kg DFO-P, or 100 mg/kg DFO-P. A semiquantitative assessment was made of the cardiomyopathy (Billingham scale) and nephropathy. Renal protection was minimal with DFO-M and moderate with ICRF-187 and both doses of DFO-P. There was no cardiac protection with DFO-M. Both doses of DFO-P provided similar but modest degrees of cardiac protection. DXR-induced mortality was not prevented by either preparation of DFO. ICRF-187 provided a higher degree of protection against the cardiotoxicity and the mortality induced by DXR. Since both DFO and ICRF-187 are highly efficient chelators of iron in vitro, the differences in their in vivo protective effects are thought to be related to their cellular uptake and intracellular distribution and to the relative availability of different intracellular iron pools to these agents.

Key words DFO · ICRF-187 · Doxorubicin-induced toxicity · Iron chelation

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Introduction

The pathogenesis of the dose-dependent chronic cardiomyopathy that limits the clinical use of doxorubicin is thought to be related to multiple factors, the most important of which may be the formation of reactive oxygen radicals [8–10, 39]. Of special interest in this respect are studies showing that doxorubicin and iron form a complex that can react with oxygen to form superoxide and hydroxyl radicals [15]. ICRF-187, an iron chelator, has been shown to inhibit the doxorubicin-induced formation of hydroxyl radicals in isolated, perfused rat hearts [41]. This agent also has been found to decrease effectively the chronic cardiotoxicity of doxorubicin in several species of experimental animals as well as in patients undergoing cancer chemotherapy [25,42,43]. Because of its nonpolar nature, ICRF-187 diffuses rapidly into cells, where it undergoes hydrolysis to an open-ring derivative, ICRF-198 [11]. The latter compound functions as an effective intracellular chelator of iron and copper [20] and rapidly removes iron from the doxorubicin-iron complex. ICRF-187 and ICRF-198 also reduce the intracellular availability of iron by chelation from other sources such as ferritin [21].

Although ICRF-187 has proved to be clinically successful, several important questions remain concerning the use of this agent. These pertain to potentiation of hematological toxicity and to interference with the antineoplastic activity of doxorubicin [13]. The possibility that other chelators of iron may be useful in the prevention of doxorubicin-induced toxicity has been the subject of only limited studies. These have indicated that desferrioxamine (DFO) a nontoxic, highly effective chelator of iron and the only agent that has proved to be clinically useful for the treatment of iron overloading, offers some degree of protection against the cardiac and hematological toxicity of doxorubicin [1, 40, 45]. Therefore, the present study was undertaken to assess the influence of DFO on the chronic cardiomyopathy induced by doxorubicin in spontaneously hypertensive rats (SHR), an animal model that we have characterized extensively in studies of the cardiac toxicity

of anthracyclines [22, 23]. For this purpose, we used two preparations of desferrioxamine: desferrioxamine mesylate (DFO-M) and desferrioxamine polymer (DFO-P), a conjugate of DFO-M with hydroxyethyl starch (Pentastarch) that has a much longer plasma half-life than DFO-M.

Materials and methods

A total of 54 12-week-old male SHR (Aoki-Okamoto strain) weighing 200–250 g were obtained from the Charles River Breeding Laboratories (Wilmington, Mass.) and were allowed a 3-week accommodation period prior to the start of the study. The animals were divided into the following 6 treatment groups: group 1 (15 animals) received weekly injections of doxorubicin (1 mg/kg) via the tail vein for 12 weeks; group 2 (15 animals) was pretreated with 25 mg/kg ICRF-187 (i.p.) 30 min prior to each injection of doxorubicin; groups 3 and 4 (5 animals each) received 50 and 100 mg/kg, respectively, of DFO-P (i.p.) 30 min prior to the administration of doxorubicin; group 5 (5 animals) was pretreated with 50 mg/kg DFO-M (i.p.) 30 min prior to each dosing with doxorubicin; and group 6 (9 animals) received weekly i.p. and i.v. injections of normal saline. The rats in each of the DFO groups (groups 3–5) were dosed concurrently with 5 rats from groups 1 and 2 and with 3 rats from group 6.

Lyophilized doxorubicin (Adria Labs, Columbus, Ohio) and ICRF-187 (Drug Synthesis and Chemistry Branch, National Cancer Institute, NIH, Bethesda, Md.) were dissolved in normal saline prior to use and injected (i.v. and i.p., respectively) in volumes of 0.1 and 0.2 ml/100 g body weight. DFO-M and DFO-P were supplied by Biomedical Frontiers, Inc. (Minneapolis, Minn.) and were dissolved in normal saline. Both solutions contained DFO at 8.7 g/l (15.6 m*M*) and were injected in a volume of 0.58 ml/100 g body weight to achieve a dose equivalent to 50 mg/kg DFO. A second solution of DFO-P contained DFO at 10 g/l (17.6 m*M*) and was given in a volume of 1 ml/100 g body weight to achieve a dose of 100 mg/kg DFO. At 1 week after the last (12th) injection, all surviving animals were anesthetized with 45 mg/kg pentobarbital sodium. A midline incision was made over the trachea and a carotid artery was isolated and cannulated with a heparin-filled 25-gauge-needle-tipped catheter. The arterial pressure, lead II of the ECG, and the heart rate were monitored and recorded by means of a multichannel polygraph. The mean arterial blood pressure ($\pm$SD) was calculated for groups with sufficient numbers of survivors at the end of the experiment.

Blood samples were collected from each animal for hematological and clinical chemistry determinations. Serum levels of urea nitrogen, creatinine, glucose, total protein, albumin, globulin, total bilirubin, direct bilirubin, triglycerides, uric acid, cholesterol, sodium, potassium, phosphate, calcium, chloride, glutamic-pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase, glutamic-oxaloacetic transaminase, and γ-glutamyl transpeptidase were determined on each sample by Metpath, Inc. (Rockville, Md.). Student's two-tailed *t*-test was used to determine the significance of differences in body weight, arterial pressure, heart rate, and hematologic and clinical chemistry values. A P value of <0.05 was accepted as indicating a significant difference.

Animals were euthanized with additional pentobarbital (45 mg/kg) and necropsied. The entire heart, one kidney, and portions of the liver, lungs, diaphragm and small intestine were excised and fixed in 10% neutral-buffered formalin. Each heart was embedded in glycol methacrylate, and 1-μm-thick sections were stained with alkaline toluidine blue and hematoxylin-eosin. All other tissues were embedded in paraffin and stained with hematoxylin-eosin.

The frequency and severity of doxorubicin-induced myocardial alterations were evaluated semiquantitatively by light microscopic analysis of the sections and were scored from 0 to 3 according to the method of Billingham [2]. This scoring system is based on the percentage of myocytes displaying myofibrillar loss and cytoplasmic vacuolization as follows: 0, no alteration; 1, <5%; 1.5, 5%–15%; 2.0, 16%–25%; 2.5, 26%–35%; and 3, >35% of the myocardial cells showing damage.

Doxorubicin-induced renal damage was characterized by glomerular vacuolization and sclerosis, marked atrophy and dilatation of the tubules, protein casts in the tubular lumina, and interstitial lymphocytic infiltration. The severity of these alterations was graded semiquantitatively on a scale of 0 to 4 as follows: 0, no damage; 1, minimal damage; 2, mild damage; 3, moderate damage; and 4, severe damage [22, 23].

The Kruskall-Wallis ranking test [31] was utilized to assess differences in the severity of the cardiomyopathy and nephropathy scores obtained among the various treatment groups. A P value of <0.05 was accepted as indicating a significant difference. This study was approved by the Center for Drug Evaluation and Research, IACUC.

Results

General toxicity and body-weight changes

Of the 54 SHR, 16 died during the course of the study. No death occurred in the group receiving the combination of ICRF-187 and doxorubicin or in the saline-treated control group. The deaths occurred in the group receiving doxorubicin alone (8 of 15 animals) and in those given doxorubicin plus DFO-M (4 of 5 animals), 50 mg/kg DFO-P (1 of 5 animals) and 100 mg/kg DFO-P (3 of 5 animals). Of the SHR given doxorubicin alone, 1 died after the 3rd dose and was excluded from subsequent tissue analysis; the other deaths in this group occurred after the 9th to 12th doses of doxorubicin. The tissues obtained from 4 animals that died after receiving either doxorubicin alone (3 animals) or 100 mg/kg DFO-P and doxorubicin (1 animal) were not suitable for histological study.

Animals given doxorubicin alone had an average weight of 301 ± 32 g (mean $\pm$ SD) before treatment and 271 ± 31 g when the study was terminated. The average body weight increased from 317 ± 26 g to 327 ± 34 g in animals given the combination of ICRF-187 and doxorubicin. The only DFO pretreatment group with sufficient numbers of survivors was the DFO-P (50 mg/kg) group, and the average weights of these animals before treatment was 336 ± 14 g as compared with 294 ± 33 g at the end of the study. However, the changes observed in body weight from the beginning to the end of the study were not statistically significant in any of the doxorubicin treatment groups ($P > 0.05$). A significant increase in body weight was observed in animals treated with saline (from 299 ± 29 g to 376 ± 23 g at the end of the study; $P < 0.05$).

Blood pressure and heart rate

The mean arterial blood pressure values recorded at the end of the study were 127 ± 19 mmHg ($\pm$SD) for the group receiving doxorubicin alone, 162 ± 14 mmHg for the group given the combination of ICRF-187 and doxorubicin, and 194 ± 11 mmHg for the saline-treated control group. The mean arterial pressure was significantly higher in the control group than in either of the doxorubicin-treated groups ($P < 0.05$). Heart rates averaged between 345 and

360 beats/min and did not differ significantly among the groups.

Gross anatomic changes

Increased amounts of clear peritoneal and pericardial fluid were observed in the animals that received doxorubicin alone or DFO plus doxorubicin. Only small amounts of pericardial and peritoneal fluid were present in the animals given ICRF-187 plus doxorubicin. Pale kidneys were noted in all of the animals receiving doxorubicin alone or in any combination. Animals given saline had no gross abnormality.

Myocardial pathology

The myocardial cellular alterations associated with the administration of doxorubicin were similar to those reported in previous experimental and clinical studies [3, 22, 23, 32]. The affected myocytes displayed two characteristic light microscopic changes: myofibrillar loss and cytoplasmic vacuolization. As the lesions became more pronounced, more myocytes showed these changes. Data concerning the incidence, the severity, and the statistical significance of the myocardial abnormalities found in the various groups are presented in Table 1. The lesion scores from all animals in each group were included, even though the SHR that died did not receive the entire 12-mg/kg cumulative dose of doxorubicin.

No myocyte alteration was found in the control SHR receiving saline. The SHR given doxorubicin alone or together with DFO-M had the most severe lesions (Table 1). Of 11 animals given doxorubicin alone, 6 had lesion scores of 3, 3 had lesion scores of 2.5, and 2 had lesion scores of 2. A similar range of lesion scores was found in the group that received 50 mg/kg DFO-M plus doxorubicin: 2 SHR had a lesion score of 3, 1 had a lesion score of 2.5, and 2 had a lesion score of 2. There was no significant difference between the severity of the lesion scores in these two groups. The most severe lesion score found in the groups pretreated with either 50 or 100 mg/kg DFO-P was 2.5 (1 animal in each group). Other lesion scores were 1.5 (2 animals) and 2.0 (1 animal) in SHR receiving 100 mg/kg DFO-P and 2.0 (4 animals) in those given 50 mg/kg DFO-P plus doxorubicin (Table 1). There was no statistically significant difference in the lesion scores recorded for the groups receiving 50 versus 100 mg/kg DFO-P prior to doxorubicin (Table 1). However, in both groups the myocyte alterations were significantly less severe than those observed in the SHR receiving doxorubicin alone ($P<0.05$). The least severe myocyte alterations were found in the SHR pretreated with ICRF-187. In this group, 11 of the animals had lesion scores of 1 and the remaining 4 had scores of 1.5 (Table 1). These lesions were significantly less severe than those found in SHR given doxorubicin alone or the combination of DFO-M or DFO-P (50 or 100 mg/kg) and doxorubicin ($P<0.01$).

Pathology of noncardiac tissues

The kidney was the only noncardiac tissue found to be consistently altered by doxorubicin treatment. All 11 SHR given doxorubicin alone had severe lesions with scores of 4 (Table 2). Severe renal lesions (with a score of 4) were observed with less frequency in the groups receiving

Table 1 Cardiomyopathy scores obtained in SHR given doxorubicin weekly for 12 weeks with or without DFO-M, DFO-P, or ICRF-187

Drug/dose (mg/kg)[a]	Number of animals	Cardiomyopathy score					
		0	1	1.5	2.0	2.5	3.0
Doxorubicin (1)	11	0	0	0	2	3	6
DFO-P (100)[b] Doxorubicin (1)	4	0	0	2	1	1	0
DFO-P (50)[b] Doxorubicin (1)	5	0	0	0	4	1	0
DFO-M (50) Doxorubicin (1)	5	0	0	0	2	1	2
ICRF-187 (25)[b, c] Doxorubicin (1)	15	0	11	4	0	0	0
Saline	9	9	0	0	0	0	0

[a] Animals received DFO-P, DFO-M, or ICRF-187 30 min prior to each dose of doxorubicin

[b] Cardiomyopathy scores significantly lower than those of the group given doxorubicin alone ($P<0.05$, Kruskall-Wallis ranking test)

[c] Cardiomyopathy scores significantly lower than those of groups given 50 or 100 mg/kg DFO-P or DFO-M (50 mg/kg) and doxorubicin ($P<0.01$, Kruskall-Wallis ranking test)

Table 2 Nephropathy scores obtained in SHR given doxorubicin weekly for 12 weeks with or without DFO-M, DFO-P, or ICRF-187

Drug/dose (mg/kg)[a]	Number of animals	Nephropathy score				
		0	1	2	3	4
Doxorubicin (1)	11	0	0	0	0	11
DFO-P (100)[b] Doxorubicin (1)	4	0	0	1	1	2
DFO-P (50)[b] Doxorubicin (1)	5	0	0	1	3	1
DFO-M (50)[b] Doxorubicin (1)	5	0	0	0	3	2
ICRF-187 (25)[b, c] Doxorubicin (1)	15	0	1	8	5	1
Saline	9	9	0	0	0	0

[a] Animals were pretreated with DFO-M, DFO-P, or ICRF-187 30 min prior to each dose of doxorubicin

[b] Nephropathy scores significantly lower than those of the group given doxorubicin alone ($P<0.05$, Kruskall-Wallis ranking test)

[c] Nephropathy scores significantly lower than those of the group receiving DFO-M (50 mg/kg) and doxorubicin ($P<0.05$, Kruskall-Wallis ranking test)

Table 3 Mean WBC and RBC counts, hemoglobin concentration, and hematocrit values obtained in SHR given doxorubicin with or without DFO-P or ICRF-187 for 12 consecutive weeks (*Hb* Hemoglobin, *Hct* hematocrit)

Group	Drug dose (mg/kg)[a]	WBC ($\times 10^8$)	RBC ($\times 10^3$)	Hb (mg/dl)	Hct (%)
1	Doxorubicin (1)	9.2±4	3.0±0.6[b, c]	6.4±1[b, c]	19±3[b, c]
2	ICRF-187 (25) Doxorubicin (1)	9.3±4	6.0±1.5[b]	11.7±2.5[b]	31±7[b]
3	DFO-P (50) Doxorubicin (1)	5.7±1.3	3.3±1.3[b, c]	5.9±1.7[b, c]	19±6[b, c]
4	Saline	7.3±2.7	9.0±0.5	15.1±0.8	42±2

[a] Animals were pretreated with DFO-P or ICRF-187 30 min prior to each dose of doxorubicin. Data represent mean values ± SD from 4–10 animals surviving for 1 week after the 12th dosing

[b] Value significantly different from that recorded for saline-treated controls ($P<0.01$, Student's unpaired two-tailed *t*-test)

[c] Value significantly different from that recorded for SHR receiving ICRF-187 and doxorubicin ($P<0.01$)

Table 4 Mean serum concentrations of albumin, globulin, urea nitrogen, creatinine, iron, triglycerides and cholesterol measured in SHR given doxorubicin once a week for 12 weeks with or without DFO-P or ICRF-187

Group	Drug dose (mg/kg)[a]	Albumin (gm/dl)	Globulin (mg/dl)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	Iron (μg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
1	Doxorubicin (1)	1.7±0.1[b]	2.6±0.4[c]	51.4±5[b, c]	0.95±0.13[b]	13±9[b, c]	994±691[b, c]	285±72[b, c]
2	ICRF-187(25) DXR (1)	2.1±0.2[b]	4.5±0.8[b, c]	29.5±7[b]	0.91±0.17[b]	38±17[b]	2248±690[b]	394±55[b]
3	DFO-P (50) DXR (1)	1.6±0.1[b]	2.3±0.3[b, c]	58.5±5[b, c]	0.73±0.13	282±81[b, c]	607±477[b, c]	273±28[b, c]
4	Saline	3.6±0.2	2.8±0.1	22.1±5	0.63±0.16	186±22	46±9	37±4

[a] Animals were pretreated with DFO-P or ICRF-187 30 min prior to each dose of doxorubicin. Data represent mean values ± SD from 4–10 animals alive at 1 week after the 12th dosing

[b] Value significantly different from that recorded for the saline-treated control group ($P<.01$, Student's unpaired two-tailed *t*-test)

[c] Value significantly different from that recorded for the group receiving ICRF-187 and doxorubicin ($P<0.01$)

DFO-M or DFO-P pretreatment (1–2 animals/group; Table 2). The differences between the severity of the nephropathy scores obtained in SHR given doxorubicin alone and in those pretreated with the various combinations of doxorubicin and DFO or ICRF-187 were significant ($P<0.02$). The degree of renal protection provided by ICRF-187 was similar to that provided by each of the two doses of DFO-P but was significantly better than that provided by DFO-M.

Hematological determinations

The values (mean ± SD) recorded for white blood cell (WBC) counts, red blood cell (RBC) counts, hemoglobin concentration, and hematocrit in those groups containing more than 4 survivors are presented in Table 3. Doxorubicin treatment caused significant ($P<0.01$) decreases in RBC count, hemoglobin concentration, and hematocrit as compared with the values obtained in the saline-treated animals. The decline in each of these values was less severe in the group pretreated with ICRF-187 than in the groups given doxorubicin alone or DFO-P (50 mg/kg) and doxorubicin ($P<0.01$). No significant difference in the WBC count was detected between the various experimental groups and the saline-treated control group.

Clinical chemistry determinations

The clinical chemistry data were compared in the groups in which 3 or more animals survived until the end of the experiment (Table 4). Significant decreases in the serum albumin occurred in all three groups given doxorubicin. Serum iron concentrations declined markedly in the groups given doxorubicin alone or in combination with ICRF-187. The mean serum iron concentrations determined in these two groups were only 6%–20% of that measured in the saline-treated control group (Table 4). In contrast, the mean serum iron concentration obtained in SHR given 50 mg/kg DFO-P and doxorubicin was significantly higher than that measured in the saline-treated control animals (282±81 versus 186±22 μg/dl; $P<0.05$; Table 3). The blood urea nitrogen concentrations determined in the SHR given doxorubicin alone or in combination with DFO-P were over 2 times higher than that found in the saline-treated control group (Table 4). Significant increases in serum triglycerides and cholesterol were found in all groups receiving doxorubicin. The serum concentrations of triglycerides and cholesterol were increased 13–50 and 7–10 times, respectively, as compared with the control values (Table 4). The largest increases occurred in the animals receiving the combination of ICRF-187 and doxorubicin. Doxorubicin treatment did not lead to significant changes in the results of other clinical chemistry determinations.

Discussion

The results of the present study provide a comparison of the protective effects of ICRF-187 and two preparations of DFO against the toxicity induced by the chronic administration of doxorubicin in the SHR model. These toxic effects included increased mortality, cardiomyopathy, ne-

phropathy characterized by a nephrotic syndrome with elevations in concentrations of blood urea nitrogen as well as serum triglycerides and cholesterol, anemia, and decreases in iron concentrations.

Cardiac protection

Moderate to marked cardiac alterations were found in all SHR given doxorubicin alone. ICRF-187 exerted significant cardioprotective activity as shown by a marked decrease in the cardiomyopathy scores (Table 1). The severity of the cardiotoxicity induced by doxorubicin and the degree of protective activity exerted by ICRF-187 in the present investigation were similar to those reported in previous studies in SHR [23, 47].

The administration of DFO-M (50 mg/kg) did not alter the severity of the lesions induced in myocytes by doxorubicin. At the same pretreatment dose (50 mg/kg), DFO-P provided a modest degree of protection against doxorubicin cardiotoxicity (Table 1). The degree of protection did not increase significantly when the pretreatment dose of DFO-P was increased to 100 mg/kg. With both doses of DFO-P, the degree of cardiac protection was significantly lower than that observed after pretreatment with ICRF-187. The higher degree of cardioprotection observed with ICRF-187 was accompanied by protection against mortality. Thus, no death occurred in the group pretreated with ICRF-187, whereas significant mortality occurred in all other groups except that pretreated with 50 mg/kg DFO-P.

Renal and hematological protection

A similar modest degree of renal morphological protection was obtained with ICRF-187 and with the two doses of DFO-P (Table 2). The hypertriglyceridemia and hypercholesterolemia induced by doxorubicin were worsened by ICRF-187 (Table 4). The high mortality occurring in the groups of animals pretreated with DFO-M and DFO-P (100 mg/kg) precluded the evaluation of hyperlipidemia and hematological changes in these animals. The serum cholesterol and triglyceride concentrations measured in the animals given 50 mg/kg DFO-P did not differ significantly from those obtained in animals given doxorubicin alone. Nephrotoxic effects of doxorubicin have been observed mostly in rodents [36, 46], and interpretation of data concerning this toxicity is hampered by our lack of information on its basic mechanism.

ICRF-187 provided better protection against doxorubicin-induced anemia than did the 50-mg/kg dose of DFO-P (the only group of DFO-treated animals in which sufficient numbers of animals were available for comparison). Doxorubicin-induced reduction in serum iron concentration was not modified by pretreatment with ICRF-187. In contrast, however, DFO-P (50 mg/kg) induced a significant increase in serum iron levels as compared with the values obtained in control rats. This finding was interpreted as possibly being due to prolonged retention of the iron chelate of DFO-P in the vascular compartment.

DFO, ICRF-187, and iron chelation

A comparison of the cardioprotective effects of DFO and ICRF-187 against anthracycline toxicity under similar experimental conditions has not been reported previously. Hershko et al. [26] studied the interrelationships between iron loading, iron chelation, and doxorubicin cardiotoxicity (evaluated by changes in contractility and by release of lactic dehydrogenase) in cultures of neonatal rat cardiac myocytes. Prior loading with iron resulted in a marked increase in doxorubicin toxicity. Treatment of iron-loaded cells with DFO caused a marked decrease in this toxicity. However, treatment of control myocytes with DFO had no protective effect against doxorubicin toxicity. Thus, these results show that iron loading potentiates the cardiotoxicity of doxorubicin and that treatment with DFO abrogates this increased toxicity but does not protect myocytes with a normal iron content. These observations are of potential clinical relevance, because certain patients receiving doxorubicin treatment may be overloaded with iron due to multiple blood transfusions and bone marrow failure.

Only limited in vivo studies have been made of the protective activity of DFO against doxorubicin toxicity. Al-Harbi et al. [1] evaluated the effects of treatment with DFO (250 mg/kg, i.p.) 30 min before the administration of a single i.v. dose of 15 mg/kg doxorubicin to Wistar rats. This dose of doxorubicin caused increases in the serum levels of various cardiac enzymes and myocardial damage within 48 h of treatment. These alterations were attenuated by pretreatment with DFO. Osman et al. [40] reported that cardiac ultrastructural changes induced in Swiss albino mice by the administration of five doses of 2 mg/kg doxorubicin (given i.p. every other day) were abrogated by the simultaneous administration of 100 mg/kg DFO-M (i.p.). However, the cumulative dose (10 mg/kg) of doxorubicin used in this study would be expected to induce relatively mild subacute cardiotoxicity (as evaluated 7 days after the termination of treatment). In a chronic toxicity study, male Wistar rats were treated i.p. with 100 mg/kg DFO daily for 3 days prior to repeated administration of doxorubicin (3 mg/kg, i.v.) up to a cumulative dose of 12 mg/kg [45]. The animals pretreated with DFO showed decreased cardiac damage but suffered increased mortality from noncardiac causes. The degree of cardioprotection observed in the studies cited above was assessed only qualitatively. The semiquantitative data obtained in the present study indicate that DFO-P offers some limited protection against doxorubicin cardiotoxicity; however, this protection is significantly weaker than that observed with ICRF-187.

The differences in the cardioprotective activity of DFO and ICRF-187 may be related to differences in their patterns of uptake and distribution in tissues rather than to their iron-chelating abilities. These highly effective chelators of iron would be expected to interfere with the

iron-catalyzed generation of reactive oxygen species, which have been implicated in the pathogenesis of the toxicity induced by doxorubicin [10, 15, 38, 39]. As mentioned earlier in this report, ICRF-187 is known to diffuse rapidly into cells, where it is converted to ICRF-198 [11], which functions as an open-ring chelator with a high affinity for iron (stability constant, 10^{23}). DFO-M has an even higher affinity for iron (stability constant, 10^{31}) [28]. Both ICRF-198 and DFO have been shown to remove iron rapidly from the doxorubicin-iron complex in vitro [20]. However, the ability of DFO-M to enter into myocytes is very limited as suggested by the finding that tissue levels of DFO-M and its metabolites detected at 5 h after the administration of DFO-M to dogs are at least 5- to 10-fold higher in the liver, kidney, spleen, and plasma than in the heart [28]. The plasma half-life of DFO-M is only 5.5 min (due to rapid renal excretion), whereas that of DFO-P is much longer (85 min) [17].

In CDF-1 mice given 100 mg/kg of ICRF-187 (i.v.), the plasma concentration peaked at 108 μg/ml at 5 min and decreased rapidly to 1.6 μg/ml at 2 h [36], with the plasma half-life being 26 min. ICRF-187 concentrated maximally in the kidney and the liver, with these concentrations peaking at 296 and 171 μg/g tissue, respectively, at 5 min and decreasing to 146 and 99 μg/g tissue, respectively, at 10 min. The cardiac concentrations of 85 μg/g tissue observed at 5 min declined to 4 μg/g tissue in 2 h. In beagle dogs given a single i.v. dose of 12.5 mg/kg ICRF-187, maximal drug concentrations of 100 and 32 μg/g tissue were found at 2 h in the liver and kidney, respectively [37]. The cardiac concentration measured at 2 h was 6 μg/g tissue. These data suggest that the differences in the cardiac protection produced by ICRF-187 and DFO-M are not direct consequences of differences in their plasma half-lives, both of which are very short, or in their myocardial uptake, which for both agents is much lower than the hepatic and renal uptake. Moreover, the longer plasma half-life of DFO-P may result in increased myocardial uptake, thereby leading to greater myocardial protection with this agent than with DFO-M.

The intracellular compartmentalization of iron pools and the availability of such pools to chelators is also considered to be of importance in determining the efficacy of these agents. It has been suggested that ferritin is the source of the iron that is to be chelated in the process of reducing the cardiotoxicity of doxorubicin [15]. Other studies have concluded that the most immediate source of intracellular chelatable iron is constituted by a low-molecular-weight protein (1200 Da) in which iron is complexed with glutamate and aspartate residues [7]. The iron thus complexed is exchangeable with that of ferritin and is readily chelatable. Thus, this protein is considered to be the most direct source of iron available for rapid intracellular chelation. ICRF-187 is a smaller and more lipid-soluble molecule than either DFO-M or DFO-P and, thus, might be more widely distributed within different cellular compartments. Because of its polar nature, DFO-M is taken up by cells by pinocytosis and not by transmembrane diffusion [34]. Similar considerations would be expected for DFO-P, which is a large molecule. This pinocytotic/endocytic uptake of DFO is slow and leads to entry and accumulation of this agent into the lysosomal compartment (due to fusion of the endocytic vesicles with lysosomes) where ferritin is localized [34]. Because of the impermeability of lysosomal membranes to DFO-M, this agent is thought to remain localized within the lysosomal compartment [34]. The longer half-life of DFO-P in plasma would allow for greater pinocytotic uptake of this agent as compared with that of DFO-M and, therefore, might account for its greater protective ability.

Penetration of different iron chelators into the core of the ferritin molecule is also considered to be an important determinant of their chelating ability. Hasinoff and Kala [21] have shown that ICRF-198, the open-ring hydrolysis product of ICRF-187, is very efficient in removing iron from ferritin. In contrast, DFO removes iron from ferritin very slowly [6, 30].

Other effects of iron chelators

Other properties of iron chelators and their complexes with iron must be taken into account in the evaluation of their protective effects. DFO is a hexadentate chelator that binds to the six coordination sites of the iron atom by means of three hydroxamic acid groups [28]. The iron in the DFO-Fe(III) complex is relatively resistant to reduction to Fe(II) by O_2 or cellular electron-transport systems [4, 5, 16], and this prevents the iron in this complex from catalyzing redox reactions. Under certain in vitro conditions the DFO-Fe complex has been found to generate hydroxyl radicals [29]; however, other studies have suggested that DFO-M can also function directly as a free-radical scavenger [18, 27].

In contrast to DFO, ICRF-198 is a bidentate chelator. Therefore, the iron chelated by this agent has coordination sites that remain available for participating in redox reactions. Thomas et al. [44] have shown that the ICRF-198-iron complex, like the ethylenediaminetetraacetic adid (EDTA)-iron complex [33], can generate hydroxyl radicals in several Fenton-reaction systems. Nevertheless, the protective activity of ICRF-187 against tissue injury induced by reactive oxygen species has been observed not only in doxorubicin cardiomyopathy but also in the pulmonary damage resulting from hyperoxia [14], the pulmonary fibrosis caused by bleomycin [24], and the pancreatic β-cell damage induced by alloxan [12]. Furthermore, we found no tissue damage in animals given ICRF-187 alone. Thus, it seems very unlikely that the administration of ICRF-187 would result in the generation of reactive oxygen species and subsequent tissue damage in vivo.

In conclusion, under the experimental conditions employed in the present study, ICRF-187 was considerably more protective than DFO against the chronic cardiotoxic effects of doxorubicin in spontaneously hypertensive rats. Since both DFO and ICRF-187 are highly efficient chelators of iron in vitro, the differences in their in vivo protective effects are thought to be related to their cellular uptake and intracellular distribution and to the relative

availability of different intracellular iron pools to these agents.

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