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Pretreatment with ICRF-187 provides long-lasting protection against chronic daunorubicin cardiotoxicity in rabbits

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Summary. The long-term protective effect of ICRF-187 against chronic daunorubicin cardiotoxicity was examined. Rabbits were given 3.2 mg daunorubicin/kg, with or without pretreatment with 25 mg ICRF-187/kg, once every 3 weeks over an 18-week period (6 doses). The experiment was terminated 3 months after the last treatment. At this time, all seven rabbits given daunorubicin alone had evidence of myocardial alterations ranging from minimal (2 animals) to mild (5 animals). Pretreatment with ICRF-187 caused a significant reduction in both the incidence and the severity of cardiac lesions. Hearts from the majority (5 of 7) of animals given the combination of ICRF-187 and daunorubicin were normal; myocardial alterations were minimal in the remaining rabbits treated with ICRF-187. In previous studies ICRF-187 was found to cause a reduction in cardiotoxicity 1–3 weeks after the final anthracycline dose. The results of the present study demonstrate that pretreatment with ICRF-187 provides prolonged protection against the cardiomyopathy, as opposed to producing only a delay in the appearance of cardiac alterations.

Introduction

Dose-related chronic cardiotoxicity constitutes a major limitation to the optimal use of anthracyclines such as daunorubicin and doxorubicin in the treatment of patients with a variety of neoplastic diseases [2, 21, 22]. Considerable effort is being directed toward reducing the toxicity of these agents while at the same time maintaining their therapeutic efficacy. Certain compounds alter the extent of anthracycline-induced cardiac toxicity. One of these, ICRF-187 [(+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane], has been examined in a number of different animal models of anthracycline cardiac toxicity. Pretreatment with this agent has led to a significant reduction in both the incidence and the severity of chronic anthracycline-induced cardiomyopathy in rabbits [10], dogs [7], and miniature pigs [8]; however, it has not been determined whether this action is long-lasting. The animals in these studies [7, 8, 10] were killed 1–3 weeks after the last anthracycline injection, and it is conceivable that the toxicity might have appeared at a later time. The present investigation, performed in rabbits chronically treated with daunorubicin, was initiated to address this question. In this study, the interval between the final treatment and sacrifice for myocardial examinations was extended to 3 months.

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Materials and methods

Twenty-four male New Zealand white rabbits (2.3–3.3 kg) were divided into four groups. Rabbits in group 1 (7 animals) received 25 mg ICRF-187/kg by IP injection, followed 30 min later by IV administration of 3.2 mg daunorubicin/kg. Rabbits in group 2 (9 animals) were given normal (0.9%) saline IP 30 min before administration of daunorubicin (3.2 mg/kg). Group 3 (4 rabbits) received ICRF-187 IP (25 mg/kg), followed 30 min later by an IV injection of saline. Group 4 (4 rabbits) received saline IP, followed 30 min later by saline IV. ICRF-187 and daunorubicin (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute), were dissolved in saline just before use and were injected in dose volumes of 10 and 5 mg/ml, respectively. Animals were treated six times at 3-week intervals over an 18-week period.

Blood samples for biochemical and hematological analysis were obtained from the marginal ear vein before the first treatment (control) and at 3-week intervals thereafter. The last sample was taken just before termination of the study, 3 months after the sixth drug injection. A complete blood count and serum determinations of urea nitrogen, creatinine, glucose, total protein, albumin, globulin, total bilirubin, direct bilirubin, total lipids, triglycerides, uric acid, cholesterol, sodium, potassium, phosphorus, calcium, chloride, iron, serum glutamic-pyruvic transaminase (SGPT), lactic dehydrogenase (LDH), alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), and creatine kinase (CK) were performed by Vet-Path Laboratories, Silver Spring, Md. A 2-tailed, paired-sample statistical analysis was used to determine treatment-related differences in biochemical and hematologic results.

Three months after the sixth daunorubicin dose (total dose 19.2 mg/kg), the rabbits were anesthetized with pentobarbital sodium and the entire heart and samples of liver, kidney, lung, small intestine, and skeletal muscle were excised. All tissues were fixed in 10% buffered neutral formalin. Blocks of tissue from the free walls of all four cardiac chambers and from the ventricular septum were embedded in paraffin and glycol methacrylate plastic resin.

Sections of plastic-embedded tissues were stained with hematoxylin-eosin or with toluidine blue. All other tissues were embedded in paraffin and stained with hematoxylin-eosin.

The frequency and severity of daunorubicin-induced light microscopic alterations were assessed in the left ventricle, where the lesions were most severe. Alterations 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% or more cells; 2+ and 3+ represent intermediate degrees of involvement. Sections were evaluated without prior knowledge of the treatment given to the animals. A χ^2 test was utilized to determine the significance of differences in the severity of cardiomyopathy scores between groups.

Results

General toxicity and weight change

During the study, a total of five rabbits died or were in poor condition and were killed. Two rabbits receiving daunorubicin alone died 1 week and 5 weeks after the final (sixth) dosing. Although these two animals had moderate myocardial lesions, their cardiomyopathy scores were not included in the subsequent comparisons of daunorubicin cardiotoxicity. One rabbit receiving the combination of ICRF-187 and daunorubicin died less than 1 week before termination of the study and was included in the final analysis of cardiotoxicity with the rest of the animals similarly treated. The cause of death in this rabbit and the two rabbits receiving daunorubicin only appeared to be severe pulmonary infection. Two control animals (one given ICRF-187 and one given saline) became injured by struggling in a restraining box during blood collection and were killed. The average body weight increased to a similar degree in all four groups of animals over the 30-week experimental period (Table 1).

Myocardial pathology

The cellular alterations observed were similar to those previously found in daunorubicin-treated rabbits [10, 11, 12, 23, 27]. The affected cells were not restricted to any particular layer of the myocardium and displayed mainly cytoplasmic vacuolization and myofibrillar loss (Fig. 1). These alterations were found in the hearts of all seven rabbits killed 3 months after the sixth daunorubicin dose (19.2 mg/kg cumulative dose) (Table 2). The severity of the lesions in these hearts ranged from 1 to 2 (average 1.7). In contrast, no cardiac lesions were observed in five of the seven rabbits given the combination of ICRF-187 and daunorubicin (Fig. 2). The remaining two animals in this group showed minimal alterations (1+). The difference in both the incidence and the severity of cardiomyopathy between the animals given daunorubicin alone and those given the combination of ICRF-187 and daunorubicin was highly significant ($P<0.01$) (Table 2). No cardiac lesions were present in rabbits receiving ICRF-187 or saline without daunorubicin.

Pathology of noncardiac tissues

Minimal to mild renal tubular dilatation was found in four rabbits given daunorubicin alone and in four rabbits treated with the combination of ICRF-187 and daunorubicin. No histological lesions attributable to daunorubicin or ICRF-187 were found in the lung, small intestine, liver, or skeletal muscle. Scattered mononuclear inflammatory cells were noted in the livers of five rabbits given daunorubicin alone and of four rabbits given the combination of ICRF-187 and daunorubicin. A similar degree of hepatic inflammation was seen in control animals treated with ICRF-187 and saline.

Clinical chemistry and hematologic determinations

No consistent changes were found in serum concentrations of glucose, creatinine, urea nitrogen, bilirubin, total lipids, triglycerides, uric acid, cholesterol, sodium, potas-

Table 1. Body weights, white blood cell counts, red blood cell counts, and hemoglobin concentrations in rabbits given daunorubicin and ICRF-187[a]

Treatment group	Time of measurement[b]	No. of rabbits	Body weight (kg)	White blood cell count (X 10^3/mm^3)	Red blood cell count (X 10^6/mm^3)	Hemoglobin (g/100 ml)
ICRF-187 + daunorubicin	I	7	2.6 ± 0.2	7.6 ± 1.6	6.56 ± 1.6	14.4 ± 1.2
	E/T	7	3.3 ± 0.3	7.7 ± 2.5	5.63 ± 0.32	12.3 ± 0.9
	F	6	3.2 ± 0.2	11.1 ± 3.3	6.08 ± 0.62	13.3 ± 1.7
Daunorubicin	I	9	2.7 ± 0.3	8.5 ± 2.9	6.52 ± 0.83	14.8 ± 1.9
	E/T	9	3.3 ± 0.3	9.8 ± 7.9	6.00 ± 0.77	12.5 ± 2.0
	F	7	3.3 ± 0.4	8.1 ± 4.0	5.60 ± 1.53	12.4 ± 3.0
ICRF-187 control	I	4	2.3 ± 0.2	9.1 ± 2.3	5.67 ± 0.41	12.1 ± 0.4
	E/T	3	3.0 ± 0.2	9.1 ± 5.1	6.10 ± 0.95	12.5 ± 1.2
	F	3	3.2 ± 0.2	8.4 ± 4.5	5.80 ± 0.53	12.0 ± 0.7
Saline control	I	4	2.4 ± 0.4	9.1 ± 0.7	6.36 ± 0.64	14.1 ± 1.0
	E/T	3	3.2 ± 0.2	10.4 ± 2.2	6.30 ± 0.65	13.6 ± 1.3
	F	3	3.4 ± 0.1	9.6 ± 2.6	5.88 ± 0.32	13.1 ± 0.7

[a] Values given are means ± standard errors

[b] I, initial (control) value before treatment; E/T, value 3 weeks after the sixth treatment (21 weeks); F, final value 3 months after the sixth treatment (30 weeks)

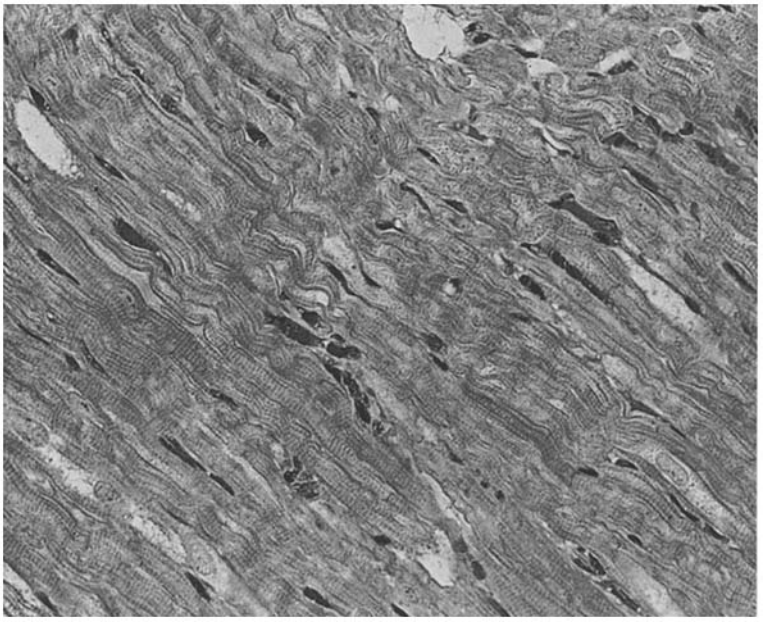

Fig. 1. Light micrograph of heart from rabbit, taken 3 months after receiving six injections, given at 3-week intervals, of 3.2 mg daunorubicin/kg (19.2 mg/kg total dose). Vacuolization and loss of myofibrils are shown in longitudinally sectioned myocytes. Section of plastic-embedded tissue 1 μm thick; alkaline toluidine blue stain; ×300

Table 2. Effect of ICRF-187 pretreatment on daunorubicin-induced chronic cardiomyopathy in rabbits, determined 3 months after the last treatment

Treatment group	Incidence		Cardiomyopathy score				
	Deaths	Lesions	0	1	2	3	≤ 1[a]
ICRF-187 + daunorubicin	1/7	2/7[b]	5	2	0	0	7/7[c]
Daunorubicin	2/9	7/7	0	2	5	0	2/7
ICRF-187 control	1/4	0/3	3	0	0	0	3/3
Saline control	1/4	0/3	3	0	0	0	3/3

[a] The numerator denotes the number of rabbits with a cardiomyopathy score of 1+ or less and the denominator, the total number of rabbits examined

[b] Significantly less than in the daunorubicin group according to χ^2 test ($P < 0.01$)

[c] Significantly greater than in the daunorubicin group according to χ^2 test ($P < 0.01$)

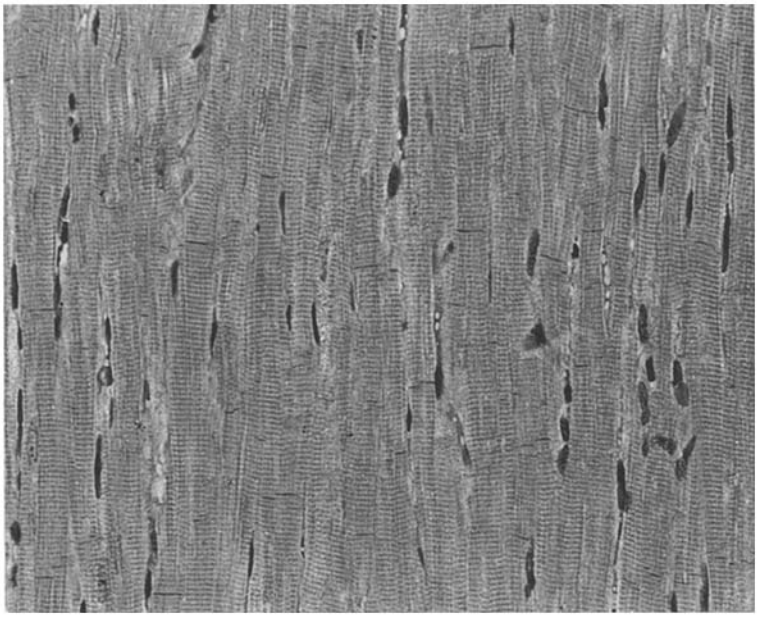

Fig. 2. Light micrograph of heart from rabbit taken 3 months after receiving six injections, given at 3-week intervals, of 25 mg ICRF-187/kg and 3.2 mg daunorubicin/kg. Myocytes appear normal. Section of plastic-embedded tissue 1 μm thick; alkaline toluidine blue stain; ×300

sium, calcium, iron, SGOT, SGPT, CK, or alkaline phosphatase. No statistical significant differences were found in the white blood cell count, red blood cell count, or hemoglobin concentration between any of the groups at any time during the study (Table 1).

Discussion

The rabbit has been used as a model in a number of studies for examining the characteristics of anthracycline cardiotoxicity. Myocardial alterations induced by chronic daunorubicin administration in the rabbit were first reported by Moral et al. [16]. Subsequently, Jaenke [11, 12] found that similar cardiac alterations could be induced by doxorubicin. These findings have been confirmed in the rabbit by other studies with daunorubicin [10] and doxorubicin [14, 23]. Both drugs induce cytoplasmic vacuolization and myofibrillar loss, lesions which are similar to those observed in patients treated with daunorubicin or doxorubicin [4].

There is abundant evidence to indicate that anthracycline cardiotoxicity is dose-dependent. At low doses the incidence of fatal congestive heart failure in patients is less than 1%, while at higher doses the incidence can approach 30% [24, 25]. The time course of overt anthracycline cardiotoxicity is variable. In a number of instances, patients have not developed congestive heart failure until some time after therapy has been concluded [24, 25]. Based on the results of his studies, Jaenke [12] has suggested that myocyte damage continues to increase even if treatment is terminated. These clinical and experimental observations are taken to indicate that anthracycline-induced cardiomyopathy is both delayed and progressive. In the present study, rabbits received a cumulative dose of 19.2 mg daunorubicin/kg over an 18-week period and were examined 3 months after the last treatment; at this time, all animals had cardiac lesions [five mild (2+) and two minimal (1+)]. We utilized a similar dosing regimen in an earlier study, which differed in two features from the present one: the rabbits received only 16 mg daunorubicin/kg (five doses) and the experiment was terminated 3 weeks after the last treatment [10]. The incidence and severity of the myocardial lesions found in animals treated with daunorubicin in both studies were almost identical. Friedman et al. [5] reported that if doxorubicin treatment is terminated after detection of a decrease in left ventricular function the level of dysfunction remains stable. Furthermore, abnormal left ventricular function may persist for extended periods of time without the development of overt heart failure [6]. These results suggest that myocyte damage does not progress in the absence of continuing drug administration. The present study supports this contention.

Attempts to resolve the problem of anthracycline cardiotoxicity have included the administration of a variety of potential myocardial protective agents [20]. Vitamin E has been utilized in a number of studies because of evidence linking the cardiotoxicity to toxic free radicals formed from reactions catalyzed by anthracyclines [17–19]. Myers et al. [17] reported that vitamin E offered significant protection against acute high-dose doxorubicin lethality in mice. Later, vitamin E was found to delay, rather than prevent, the lethal effects of doxorubicin [15]. In other studies of acute and chronic cardiotoxicity, vitamin E protection has also proved to be inconsistent. The compound decreased the cardiotoxic effect of acute high doses of doxorubicin in mice, rats, and rabbits [11, 17, 21, 26], but afforded little or no protection against chronic cardiotoxicity in animal or human studies [1, 8, 13].

ICRF-187 is another agent that has provided protection in a number of animal models. This compound reduces the toxicity of acute high doses of daunorubicin for prolonged periods of time [9]. Likewise, the protective effect of ICRF-187 against chronic anthracycline cardiotoxicity has been observed in three different animal species [7, 8, 10]. The possible mechanism of this protective activity has been discussed previously [7, 8, 10]. Briefly, it has been postulated that much of the cellular damage produced by anthracyclines is mediated by the formation of an iron-anthracycline complex which is capable of generating free radicals [19], and that ICRF-187 interferes with this process by chelating iron. In accord with this concept is the observation that ICRF-187 also attenuates the severity of alloxan-induced diabetes, a toxic reaction which requires iron for the formation of free radicals [3].

In the present study, pretreatment with ICRF-187 caused a significant decrease in anthracycline cardiotoxicity. As in previous studies, both the incidence and the severity of the lesions were reduced. An important aspect of the protection was that hearts from a majority (5 of 7) of the rabbits given the combination of ICRF-187 and daunorubicin were normal. This observation is all the more significant since myocyte alterations were noted in all seven animals given daunorubicin alone. Vacuolization and myofibrillar loss were minimal in the remaining two animals treated with ICRF-187. In previous studies, the reduction in cardiotoxicity was evaluated 1–3 weeks after the last anthracycline dose [7, 8, 10]. Thus, the results of the present study clearly show that ICRF-187 provides true protection against anthracycline cardiomyopathy, as opposed to causing only a delay in the onset of the toxicity of these agents.

The attenuation in myocyte damage may have additional advantages. When daunorubicin was given chronically to rabbits pretreated with ICRF-187, the degree of myocardial toxicity was reduced both at 3 weeks [10] and at 3 months (present study) after the last treatment. However, even though a higher cumulative daunorubicin dose was given to the animals observed at 3 months than to those observed at 3 weeks [10] (19.2, as against 16 mg/kg), 71% (5 of 7) of the hearts were normal at 3 months, while only 33% (4 of 12) were normal at 3 weeks. These differences strongly suggest that sc egression of the lesions had taken place by 3 months. In another study, vacuolization was observed in hearts from hamsters 2 weeks after they were pretreated with ICRF-187 and given a single high dose of daunorubicin [9]. However, a return to normalcy also appeared to occur subsequently in these animals, because after 10 weeks there was no morphologic difference between myocytes from surviving hamsters given ICRF-187 and daunorubicin and those from saline-treated control animals. Therefore, it is possible that ICRF-187 pretreatment, by attenuating anthracycline-induced cardiac damage, may also allow time for the myocytes to recover their cellular integrity.

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