Effect of Pretreatment with ICRF-187 on the Total Cumulative Dose of Doxorubicin Tolerated by Beagle Dogs

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ABSTRACT

Studies were made of the influence of ICRF-187 on the functional and morphological effects of very large cumulative doses of doxorubicin given over a prolonged period of time. Adult beagles of either sex (6.2-11.6 kg) were given doxorubicin (1.75 mg/kg i.v.) either alone or 15 min after ICRF-187 (25 mg/kg, i.v.) at 3-week intervals. Control dogs received ICRF-187 (25 mg/kg, i.v.) or 0.9% saline without doxorubicin. Of eight animals receiving doxorubicin alone, five died; two after a total dose of 12.25 mg/kg and three after 14 mg/kg; three others were in poor condition at the time of euthanasia after 14 mg/kg. Of eight animals receiving both ICRF-187 and doxorubicin, four died; two after 35 mg/kg, one after 43.75 mg/kg, and one after 52.5 mg/kg; two other dogs were euthanized after 43.75 mg/kg because of difficulties encountered in giving i.v. injections, and two dogs survived a total dose of 52.5 mg/kg. All control dogs survived. None of the treatment or control groups developed consistent echocardiographic changes or alterations in mean arterial pressure. By 300 days after onset of treatment, dogs given ICRF-187 and doxorubicin developed significant prolongation of the PQ interval; by 550 days, surviving dogs in this group developed ventricular premature contractions. Each animal receiving doxorubicin alone had severe myocardial lesions (lesion score 3+). Of the animals given ICRF-187 and doxorubicin, one that received 35 mg/kg doxorubicin had no lesions; of four given 43.75 mg/kg, three had no lesions and one had minimal lesions (lesion score 1+); of three given 52.5 mg/kg, one had minimal (lesion score 1+), and two had moderate (lesion score 2+) lesions. Control animals had no myocardial lesions. Thus, ICRF-187 provided significant protection when administered with doxorubicin over a period of 90 weeks, and made it possible to give doses of doxorubicin which otherwise would have been lethal.

INTRODUCTION

Doxorubicin and other anthracyclines exert a broad spectrum of antitumor activity. The potential clinical effectiveness of these compounds is limited by a serious dose-related cardiomyopathy (1-3). A variety of efforts have been made to reduce the cardiotoxicity of anthracyclines without compromising their antitumor activity. An approach has been to administer sub-toxic doses of anthracyclines in combination with other agents (4). One agent that holds promise is ICRF-187 (5-7). ICRF-187 exerted long-term protection against the cardiotoxic effects of doxorubicin. The most useful agent for this purpose is ICRF-187. Pretreatment with this compound has led to a significant reduction in the incidence and the severity of anthracycline-induced chronic cardiomyopathy in a number of different animal models (11-14). This protection occurs whether doxorubicin is given in doses of 1 mg/kg once a week (12) or 1.75 mg/kg every 3 weeks (14), and is not associated with a decrease in antitumor activity (15, 16).

In addition, recent studies in rabbits have demonstrated that ICRF-187 exerts long-term protection, i.e., as opposed to only producing a delay in the onset of cardiotoxicity: anthracycline-induced lesions do not develop even at 3 months after termination of dosing with both daunorubicin and ICRF-187 (17).

The cardioprotection produced by ICRF-187 would be expected to be accompanied by an increase in the tolerated cumulative dose of doxorubicin. However, this important question has not been addressed, because in previous experimental studies the total dose of doxorubicin has been limited to the equivalent of a standard course of therapy. The present study was initiated to examine the influence of ICRF-187 on the cardiac functional and morphological effects of very large cumulative doses of doxorubicin given over a prolonged period of time.

MATERIALS AND METHODS

Twenty-seven beagle dogs (1 to 1.5 years old) of either sex, weighing between 6.2 and 11.6 kg, were divided into two groups of eight animals (Groups 1 and 2), one group of five animals (Group 3), and one group of six animals (Group 4). The dogs in Groups 1 (five males, three females) and 2 (five males, three females) received 1.75 mg/kg doxorubicin (i.v.) (Adria Laboratories, Columbus, OH) at 3-week intervals. The animals in Group 2 were pretreated with 25 mg/kg ICRF-187 (i.v.) (Drug Synthesis and Chemistry Branch, National Cancer Institute) 15 min before receiving each dose of doxorubicin. Dogs in Group 3 received 25 mg/kg ICRF-187 (i.v.), followed in 15 min by 5 ml physiological saline (i.v.), every 3 weeks. The animals in Group 4 received two i.v. injections of saline, 15 min apart, every 3 weeks. Both ICRF-187 and toxophorol doxorubicin were dissolved in 0.9% physiological saline just prior to use and were injected in dose volumes of 20 and 5 mg/ml, respectively. Animals were returned to their pens, observed daily, and weighed weekly.

Studies of Cardiovascular Function. Certain cardiovascular functions were monitored by noninvasive means during the course of the study. Baseline values for each function were obtained on two separate occasions, 3 weeks apart, prior to commencing drug administration. Once the study began, the measurements were repeated prior to each dosing at 3-week intervals. The dogs were awake and unsedated during these procedures.

Systolic, diastolic, and mean arterial pressure and heart rate were measured by placing a neonatal pneumatic cuff around a shaved area at the base of the tail. The pneumatic cuff was connected to an oscillometric device (DINAMAP research monitor, model 1255; Critikon, Inc., Tampa, FL) which at 60-s intervals inflated the cuff to a suprasystolic pressure and then permitted the cuff to deflate over 20 to 40 s. The pressure within the cuff and the oscillations generated in the cuff by each arterial pulsation were registered by the device. Measurements were repeated three to five times and a single average value was utilized for statistical evaluation.

The echocardiogram was used as a noninvasive test to monitor left ventricular function. Echocardiograms of the left ventricle and aortic value were obtained with the dogs positioned in left lateral recumbency, using a Hoffrel System 201 monitor (Hoffrel Instruments, Norwalk, CT) and a 5.0-MHz transducer with a focal length of 12 mm. Both systolic time intervals and ventricular dimensions were measured from the echocardiograms and simultaneous recordings of electrocardiographic lead II. The PEP of the left ventricle was taken as the interval between...
between the onset of the QRS complex and the opening of the aortic valve on the aortic root echocardiogram. ET was calculated as the time between opening and closing of the aortic valve leaflets on the aortic root echocardiogram. PEP/ET was then calculated from these measurements. The PEP and PEP/ET have been shown to correlate inversely with contractility (18–20). Ventricular dimensions were obtained from the echocardiogram of the left ventricle. EDD was taken as the distance between the surface of the left side of the ventricular septum and the endocardial surface of the left ventricular free wall, measured at a time corresponding to the peak of the R wave in lead II. ESD was taken as the distance between these two surfaces, measured when the free wall made its greatest anterior excursion toward the interventricular septum. Fractional shortening (FS = EDD − ESD/EDD) and velocity of circumferential fiber shortening (Vv = FS/ET) serve to monitor ventricular function (21–23).

Mean values and standard deviations for the various parameters were calculated for each group before commencing drug administration (control) and before each subsequent treatment period. Mean values were compared by two-way ANOVA with repeated measures design on group and time. When a significant statistic was observed, specific mean values were compared by Tukey’s post hoc analysis.

Hematology and Serum Chemistry Determinations. Blood samples for hematological and serum chemical determinations were collected from the jugular vein on two separate occasions prior to the beginning of the treatment period and at 3-week intervals thereafter. Complete blood counts and measurements of serum levels of urea nitrogen, creatinine, glucose, total protein, albumin, globulin, total bilirubin, direct bilirubin, total lipids, triglycerides, uric acid, sodium, potassium, phosphorus, calcium, chloride, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, lactate dehydrogenase, and creatine kinase were performed by Vet Path Laboratories (Teterboro, NJ). The values for each individual dog at each of four treatment time points (3, 7, 15, and 30 doses) were compared to the average of their two pretreatment values. A two-tailed paired-sample statistical analysis was then used to determine treatment-related differences in biochemical and hematological results.

Necropsies. Necropsies were performed within 12 h after death on the nine animals which died during the course of the study. At the end of the study, surviving animals were euthanized with an overdose of pentobarbital sodium. The entire heart and samples of liver, kidney, small intestine, diaphragm, and lung were excised from all animals and fixed in 10% neutral formalin. Additional tissue samples, including stomach, pancreas, spleen, adrenal glands, thyroid, skeletal muscle, ovary, uterus, testes, bladder, bone marrow, bone, breast, and skin, were obtained from animals euthanized at the end of the study. Blocks of tissue from each heart were embedded in glycol methacrylate plastic resin. Sections from the left ventricular free wall, left ventricular papillary muscles, and ventricular septum were prepared and stained with hematoxylin and eosin and with alkaline toluidine blue. All other tissues were embedded in paraffin and stained with hematoxylin and eosin.

The frequency and severity of doxorubicin-induced cardiac lesions were assessed by light microscopic examination of left ventricular tissue. The changes were graded on a scale of 0 to + + on the basis of the number of muscle cells showing myofibrillar loss and cytoplasmic vacuolation, in which 0 = no damage; 1+ = involvement of only an occasional cell (<5%); 4+ = severe involvement of 50% or more cells in the visual field; and 2+ and 3+ = intermediate degrees of involvement (6–25% and 26–49%, respectively). A single score was given after evaluation of all three myocardial sections. Sections were evaluated without prior knowledge of the treatment given to the animals. Differences in the lesion severity scores between the groups were analyzed by the χ² method. A lesion score of 2 was arbitrarily chosen as the level for determining whether or not significant protective effects were obtained.

RESULTS

Clinical Signs and Weight Changes. Five of the eight beagle dogs given doxorubicin alone died during the course of the study (Table 1). Two of these animals had received seven doxorubicin doses (12.25 mg/kg cumulative dose) and three had been given eight doses (14 mg/kg cumulative dose). The other three animals in this group were euthanized after the eighth dose of doxorubicin because they were in poor condition.

Four of eight animals given the combination of ICRF-187 and doxorubicin died during the study (Table 1): one after the 20th dose (35 mg/kg cumulative doxorubicin dose); two after the 25th dose (43.75 mg/kg cumulative doxorubicin dose), and one after the 30th dose (52.5 mg/kg cumulative doxorubicin dose). It became very difficult to continue i.v. dosing in two animals being treated with ICRF-187 and doxorubicin and these dogs were euthanized after 25 injections. Two additional dogs survived all 30 dosings.

None of the animals given ICRF-187 alone (five dogs) or saline (six dogs) died during the study. Two saline-treated (control) animals were euthanized after the eighth dose and three animals treated with ICRF-187 were euthanized after the 25th dose. The rest of the saline-treated (N = 3) and ICRF-187-treated (N = 3) animals were euthanized 3 weeks after the 30th dose.

Alopecia was observed in dogs receiving doxorubicin. The loss of hair began on the legs and snout after the third dose of doxorubicin. As dosing continued, the alopecia spread to the head, trunk, and tail. Similar effects were observed in the dogs given the combination of ICRF-187 and doxorubicin. Animals receiving ICRF-187 alone did not show alopecia.

A temporary reduction in food consumption was observed during the first 2 days after administration of doxorubicin, either alone or in combination with ICRF-187. This effect was not noted in animals given ICRF-187 or saline alone.

During the first 12 weeks of the study, decreases in body weight occurred in all four groups of animals. After 21 weeks, the three remaining groups had regained some weight. During the remainder of the study, the body weights of dogs treated with the combination of ICRF-187 and doxorubicin remained relatively constant; during the same period (75 weeks) animals given only ICRF-187 or saline showed slight increases in body weight.

Electrocardiographic and Echocardiographic Changes. Electrocardiographic changes occurred in dogs given the combination of doxorubicin and ICRF-187. By 300 days after onset of treatment, these animals developed significant prolongation of the PQ interval, and this change continued to be present until either death or euthanasia (Fig. 1). Occasionally, this pro-

<table>
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<tr>
<th>Treatment</th>
<th>Doxorubicin cumulative dose (mg/kg)</th>
<th>No. of animals</th>
<th>No. of deaths</th>
<th>Cardiomyopathy score</th>
</tr>
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<tbody>
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<td>ICRF-187 plus doxorubicin</td>
<td>35</td>
<td>4</td>
<td>3</td>
<td>0 1 2 3 4</td>
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<tr>
<td>Total</td>
<td>43.75</td>
<td>3</td>
<td>1</td>
<td>0 1 2 3</td>
</tr>
<tr>
<td>Saline control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

* Numerator, number of animals with a cardiomyopathy score of 2 or less; denominator, number of animals examined.

### Table 1

Effect of ICRF-187 pretreatment on incidence and severity of doxorubicin-induced chronic cardiomyopathy in dogs
progressed to high grade, second degree AV block or to complete AV block. After 425 days of treatment, other changes were observed which consisted of prolongation, decrease in amplitude, and development of “high-frequency” components in the QRS complex. When intraventricular conduction disturbances were severe, J-point deviation and large T waves, both in a direction opposite to that of the major QRS vectors, occurred. Finally, after 550 days of treatment, all surviving dogs receiving both doxorubicin and ICRF-187 developed ventricular premature depolarizations that occurred either singly or in short paroxysms of ventricular tachycardia. The configuration of QRS complexes in ectopic depolarizations suggested that the focus was in the specialized conducting system of the left ventricle. Dogs receiving doxorubicin and ICRF-187 showed prolongation of the QT interval at 350 to 400 days of treatment; however, this prolongation did not achieve statistical significance.

No group developed echocardiographic changes either in dimensions (e.g., EDD, ESD, FS) or systolic time intervals (e.g., PEP, ET, PEP/ET). Occasionally, individual dogs receiving doxorubicin and ICRF-187 showed a greater PEP/ET ratio, reduced motion of the left side of the ventricular septum and decreased FS. These changes, reflecting a reduction in left ventricular function, occurred during the later stages of treatment. They occurred episodically, and they did not correlate with either the general appearance of the dog or the frequency or ventricular premature depolarizations. An apparent difference in PEP/ET between dogs receiving doxorubicin and ICRF-187 and control dogs reflected more a reduction in that parameter for controls and consistency in the group receiving the antineoplastic agents. There was no significant difference in either heart rate or mean systemic arterial pressure in the four groups of dogs; therefore, it appears that neither the cardiac output nor the double product (i.e., stroke volume times mean systemic arterial pressure), a corollary of myocardial oxygen consumption, changed.

Gross Anatomic Changes. Relatively few gross anatomic changes were found in the eight dogs given doxorubicin alone. Two or the five animals that died spontaneously in this group had fluid accumulation in the peritoneal cavity and pericardium. Three of the four animals which died while receiving ICRF-187 and doxorubicin showed mild intestinal hemorrhage. No gross alterations were found in animals given only ICRF-187 or saline.

Myocardial Alterations. The myocardial lesions observed in the present study (Fig. 2) were typical of those described previously in humans (1-3) and in a variety of animal species (4-9) treated with doxorubicin. The alterations displayed two prominent characteristics: cytoplasmic vacuolization and myofibrillar loss. The vacuolization involved the formation of multiple, clear, membrane-limited vacuoles that filled the cytoplasm of the affected cells and often caused them to appear larger than normal. The loss of myofibrils resulted in a pale but nonvacuolated appearance of the cytoplasm. In many instances, vacuolization and myofibrillar loss occurred in the same cells. Both changes were seen with greater frequency as the lesions increased in severity. Data on the incidence and severity of myocardial lesions are summarized in Table 1. Myocardial lesions of marked degree (lesion scores, 3+) were observed in all eight animals given doxorubicin alone (cumulative dose, 12.25 to 14 mg/kg) (Table 1 and Fig. 2A). Dogs pretreated with ICRF-187 received much higher total cumulative doses of doxorubicin (35-52.5 mg/kg) and showed significant decreases (P < 0.05) in both the incidence and the severity of cardiac lesions (Table 1 and Fig. 2, B and C). No cardiac lesions were found in four of the five dogs pretreated with ICRF-187 and given cumulative doses of 35 to 43.75 mg/kg doxorubicin (Table 1). The severity of myocardial lesions in the remaining four dogs pretreated with ICRF-187 was 1+ in two animals given cumulative doxorubicin doses of 43.75 mg/kg and 52.5 mg/kg and 2+ in two animals given cumulative doses of 52.5 mg/kg. No cardiac lesions were present in any dogs receiving ICRF-187 (Fig. 2D) or saline without doxorubicin.

Noncardiac Tissue Alterations. Alterations in the liver, small intestine, lung, and kidney were found in the eight dogs given doxorubicin alone. The most consistent finding was hepatic congestion (ranging from slight to severe) in six of these animals. Alterations were also present in the small intestine of five
Fig. 2. Photomicrographs of toluidine blue-stained, 1-μm-thick sections of dog left ventricular myocardium. A, dog given eight injections of doxorubicin alone (total cumulative dose, 14 mg/kg) shows marked vacuolization in cytoplasm of myocytes (x 300); B, dog given 25 injections of doxorubicin with ICRF-187 (total cumulative dose of doxorubicin, 43.75 mg/kg) shows no vacuolization (x 300); C, dog given 30 injections of doxorubicin with ICRF-187 (total cumulative dose of doxorubicin, 52.5 mg/kg) shows only moderate, focal vacuolization. Compare with the more severe morphological changes, shown in A, after 8 doses of doxorubicin alone (x 300); D, dog given 30 doses of ICRF-187 without doxorubicin shows normal structure (x 200).
dogs. The intestinal changes consisted mainly of loss of epithelial cells, especially at the tips of the villi. An inflammatory reaction was associated with the loss of epithelial cells in three of these animals. Pulmonary changes consistent with the initial stages of pneumonia were found in three animals, while severe airway inflammation with neutrophil infiltration was found in a fourth animal. Renal congestion was found in two doxorubicin-treated dogs (minimal in one and moderate in one).

The animals pretreated with ICRF-187 received a higher cumulative dose of doxorubicin and showed a variety of tissue alterations. The most frequent morphological change was a marked decrease in lymphoid tissue (white pulp) in the spleen. Hepatic and renal congestion were found infrequently (together in one animal and separately in one animal). Of the four animals in the ICRF-187-doxorubicin group that died spontaneously during the course of the study, three had small intestinal ulceration and inflammation and were considered to have died of sepsis; the other had evidence of pulmonary infection. Additional tissues (pancreas, stomach, adrenal, thyroid, skeletal muscle, testes, ovary, bladder, bone, and bone marrow) were examined in animals that survived until the study was terminated. Testicular atrophy was found in the two dogs that had received 30 injections of ICRF-187 and doxorubicin. The tubules appeared atrophic and the epididymis was devoid of any sperm cells. There were no consistent abnormalities in any of the tissues taken from animals that received up to 30 injections of ICRF-187 or saline without doxorubicin.

**Clinical Chemistry and Hematological Determinations.** A number of serum chemical and hematological determinations were made at 3-week intervals during the entire experimental period. A majority of these, including serum levels of glucose, creatinine, urea nitrogen, bilirubin, albumin, globulin, total protein, triglycerides, uric acid, cholesterol, sodium, potassium, calcium, phosphorus, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, lactate dehydrogenase, creatine kinase, and alkaline phosphatase were not influenced by any particular drug treatment.

The concentration of CK in serum of dogs receiving ICRF-187 and doxorubicin became elevated toward the end of the study (156.57 I.U./L. control versus 336.82 I.U./L. after 30 doses). Red blood cell count, hemoglobin concentration, and hematocrit were significantly reduced in animals given doxorubicin alone. The decrease was first noted after the third treatment period (Table 2). A significant decrease in these three hematological indices was also observed after 7 dosings in animals given the combination of ICRF-187 and doxorubicin (Table 2). These differences were not apparent after 15 doses. By 30 doses, only three dogs from this group were available for sampling. After 30 doses, the red blood cell count and the hemoglobin and hematocrit values were not significantly different from those obtained from the same three animals during the control (pretreatment) period (Table 2).

**DISCUSSION**

The results of the present study confirm and extend previous observations showing that ICRF-187 has cardioprotective properties against doxorubicin-induced toxicity (11-14, 17). These results show that very large cumulative doses of doxorubicin can be given in combination with ICRF-187 over long periods of time, i.e., up to 2 years. Severe cardiomyopathy has been observed previously in beagle dogs treated chronically with a total cumulative dose of 12 to 15 mg/kg doxorubicin (6-8), and pretreatment with 25 mg/kg of ICRF-187 before each dosing has provided significant protection against this cardiomyopathy (12, 14). These findings were confirmed in the present study, in which the hearts of all eight animals given 12.25 to 14 mg/kg of doxorubicin alone had severe myocyte damage (lesion score, 3+); five of these animals died spontaneously, and the remaining three were euthanized. The most prominent features of these cardiac lesions, intracellular vacuolization and myofilbrillar loss, had been observed previously after treatment with doxorubicin in humans (1-3) and in a variety of animal species (11-14, 24-26). Several observations in the present study made it apparent that pretreatment with ICRF-187 causes a marked attenuation of doxorubicin-induced cardiotoxicity. A major finding was that cardiac lesions were absent in four or five dogs pretreated with ICRF-187 and given cumulative doses of 35 to 43.75 mg/kg of doxorubicin. A second indication of protection was noted in the incidence and severity of myocyte alterations in the remaining four animals treated with ICRF-187 and doxorubicin. Among these animals, vacuolization and myofilbrillar loss were minimal (lesion score, 1+) in two dogs which were given 43.75 and 52.5 mg/kg of doxorubicin, respectively, and mild (lesion score, 2+) in two other dogs which were given the maximum amount of doxorubicin (52.5 mg/kg). Such a dose of doxorubicin would have been lethal to animals not pretreated with ICRF-187. Thus, in all instances the degree of myocyte damage observed in animals given the combination of ICRF-187 and doxorubicin was significantly less than that occurring in animals given much lower cumulative doses of doxorubicin alone.

The most obvious general toxic effect caused by doxorubicin in dogs was alopecia. The loss of hair began after the third injection (5.25 mg/kg cumulative dose) and was not attenuated by ICRF-187. In the present study, most animals given doxorubicin alone had died by the 7th or 8th injection (12.25 to 14 mg/kg cumulative dose). In other investigations, slightly more doxorubicin could be tolerated when small individual doses of 1 mg/kg were given once a week instead of 1.75 mg every 3 weeks (12). However, severe myocyte damage was produced by both dose schedules (12-14). Damage to the kidney has been reported to occur in rats and rabbits treated chronically with doxorubicin (24-26). Alterations in renal function are rare when doxorubicin is used therapeutically in human patients (27). Renal toxicity did not develop in the present study, nor has it been observed in other investigations in which dogs were dose chronically with doxorubicin. Renal and hepatic congestion were apparent in some animals given doxorubicin alone. This effect was thought to be secondary to cardiomyopathy. Gastrointestinal toxicity was a major problem in three animals. Except for anemia, treatment with seven or eight doses of doxorubicin caused no apparent alteration in any of the other extracardiac tissues examined. This observation is in accord with the finding that negligible changes occurred in the majority of blood chemistry determinations in these animals.

In the present study, pretreatment with ICRF-187 allowed a highly significant, fourfold escalation in cumulative dose of doxorubicin. Since four of the eight animals pretreated with ICRF-187 died spontaneously, it is possible that giving high cumulative doses of doxorubicin could ultimately lead to severe noncardiac tissue alterations that are not apparent at lower doses. Examination of a number of organs from high-dose animals identified the testes, lymphoid tissue, and small intestine as sites of tissue toxicity. Sepsis resulting from gastrointestinal lesions probably was responsible for at least three of these four deaths; death of the fourth animal was attributed to pulmonary infection.
ICRF-187 PRETREATMENT EFFECT ON DOXORUBICIN DOSE TOLERATED

Table 2. Mean RBC count, hematocrit, and hemoglobin concentration in beagle dogs treated at 3-week intervals with doxorubicin (DXR) (1.75 mg/kg), ICRF-187 (25 mg/kg) and doxorubicin (1.75 mg/kg), ICRF-187 (25 mg/kg), or saline

All data are presented as mean ± SD.

<table>
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<tr>
<th>Pretreatment</th>
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<th>7</th>
<th>15</th>
<th>30</th>
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<tr>
<td>RBC (10^6/mm³)</td>
<td>7.01 ± 0.32</td>
<td>6.28 ± 0.4</td>
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<td>DXR</td>
<td>7.01 ± 0.30</td>
<td>6.84 ± 0.53</td>
<td>6.68 ± 0.30</td>
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<td>ICRF-187/DXR</td>
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<td>ICRF-187</td>
<td>7.4 ± 0.44</td>
<td>7.45 ± 0.77</td>
<td>7.2 ± 0.44</td>
<td>6.71 ± 1.13</td>
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<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>17.5 ± 1.1</td>
<td>15.1 ± 1.2</td>
<td>16.4 ± 1.7</td>
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<td>DXR</td>
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<td>15.8 ± 0.6</td>
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<td>ICRF-187/DXR</td>
<td>17.0 ± 0.4</td>
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<td>Saline</td>
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<td>17.6 ± 1.9</td>
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<td>Hematocrit (%)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>DXR</td>
<td>50.2 ± 2.5</td>
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<td>51.2 ± 2.6</td>
<td>47.7 ± 7.6</td>
</tr>
</tbody>
</table>

* Significantly different from control value (P < 0.05) as determined by a two-tailed paired t-test.

Echocardiographic studies of dogs receiving doxorubicin alone were not particularly useful for detecting cardiac toxicity effects, whereas electrocardiographic monitoring of dogs receiving both doxorubicin and ICRF-187 did detect changes in the PQ interval and in the duration of the QRS complex. It seems likely that those alterations were seen only in the animals pretreated with ICRF-187, because the doxorubicin toxicity in these animals evolved more slowly over a longer time. Prolongation of the PQ interval and of the duration of the QRS complex reflects a negative dromotropic effect of doxorubicin (28-30). ECG changes consisting of arrhythmias, prolongation of the QRS complex and ST segment, and T wave changes have been observed in other animal models of anthracycline toxicity (29, 31). Prolongation of the PQ interval was the first demonstrable alteration and the most consistent electrocardiographic change found in dogs given ICRF-187 and doxorubicin. However, this is well known to be a nonspecific alteration.

Ventricular premature depolarizations occurred in dogs receiving both doxorubicin and ICRF-187 but not in the other groups. A reentry mechanism does not seem to be responsible, since this arrhythmia occurred in the absence of prolongation of QRS, splintering of QRS, or late oscillations extending into the ST segment (32). Rather, its origin may have been related to increased automaticity (slope of Phase 4 depolarization) or triggered activity (oscillatory afterpotentials) (33). Evidence from this study indicates that sudden death in animals receiving doxorubicin may result from either ventricular arrest (as in the dogs with high grade, second degree AV block with ventricular escape) or ventricular fibrillation (for which paroxysmal ventricular tachycardia may be prodromal).

Despite the rather large doses of doxorubicin, the absence of echocardiographic changes appeared related, at least to some extent, to a protective action of ICRF-187. Reduction in ventricular function might have been episodic and brief, which could explain why consistent increases in the PEP:ET ratio and decreases in ventricular systolic motion were not found. In addition, the methods used to monitor ventricular function were relatively insensitive. Results of studies of ventricular function in human patients receiving doxorubicin have been inconsistent. Certain studies of adults (34) and children (35, 36) receiving doxorubicin have not been able to demonstrate significant changes in ventricular function by the noninvasive methods used in this study. In contrast, other investigators found systolic time intervals (37-39) and ventricular dimensional changes (40) to be useful in detecting changes in ventricular function in a dose-response relationship with doxorubicin. At the termination of the experiment, surviving dogs were not in congestive heart failure and had normal systemic arterial pressure.

Doxorubicin-induced myocardial alterations have been attributed to the formation of reactive oxygen radicals and subsequent lipid peroxidation (4). At least two mechanisms have been identified by which doxorubicin can initiate free radical formation. In the first of these, the drug is reduced by certain flavin-dependent reductases to a semiquinone radical which, by donating the electron to oxygen, can initiate the formation of superoxide anions, hydrogen peroxide, and hydroxyl radicals (41, 42). The second free radical generating mechanism involves the formation of a complex between doxorubicin and iron; this complex is capable of catalyzing the transfer of electrons from sulphydryl compounds (such as N-acetylcysteine and glutathione) to oxygen with the subsequent formation of reactive oxygen species (43). Because of the possible role of free radicals, attempts to resolve the problem of anthracycline cardiotoxicity have included the administration of a variety of potential free radical scavenging agents. In acute studies of doxorubicin toxicity, free radical scavengers such as vitamin E and N-acetylcysteine increased survival and decreased cardiac alterations (41, 42). The lack of any significant cardioprotection by vitamin E or N-acetylcysteine under these conditions suggests that the reduction of doxorubicin to a semiquinone radical is not the primary mechanism responsible for the chronic toxicity.

As mentioned above, it has been postulated that significant cellular damage produced by anthracyclines may be mediated by the formation of an iron-anthracycline complex which is capable of generating toxic oxygen radicals. ICRF-187 could interfere with this process by chelating iron. The fact that the hydrolysis product of ICRF-187 binds iron and that ICRF-187 also attenuates the severity of alloxan diabetes, a toxic reaction which requires iron for the formation of free radicals, supports this concept (44). An ICRF-187 induced decrease in intracellular iron could limit the formation of toxic free radicals and thereby attenuate myocyte damage. ICRF-187, in contrast to
vitamin E and N-acetylcysteine, exerts significant protective activity in various animal models, whether the anthracycline is administered acutely in high doses or in low doses over prolonged periods of time (present study). The protective activity demonstrated experimentally is relevant to the clinical situation, as shown by the fact that ICRF-187 has been recently found to exert cardioprotective activity in patients receiving doxorubicin for treatment of breast cancer (45, 46). These results document for the first time the ability of an agent to selectively attenuate doxorubicin-induced chronic cardiotoxicity in a clinical setting.

In summary, the results of the present study indicate that pretreatment with ICRF-187 makes it possible to administer much higher cumulative doses of doxorubicin than are tolerable without this treatment. The doses of doxorubicin that were given together with ICRF-187 to beagle dogs (up to 52.5 mg/kg) were greatly in excess of those (12–14 mg/kg) which produced severe cardiomyopathy when given without ICRF-187. Thus, these results confirm and extend our previous observations concerning the effectiveness of ICRF-187 against doxorubicin-induced cardiomyopathy. They also suggest that the use of ICRF-187 with doxorubicin in clinical treatment regimens will attenuate cardiotoxicity to the extent of allowing the safe administration of much higher doses of doxorubicin to human patients than is possible at the present time.

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REFERENCES

ICRF-187 PRETREATMENT EFFECT ON DOXORUBICIN DOSE TOLERATED


Effect of Pretreatment with ICRF-187 on the Total Cumulative Dose of Doxorubicin Tolerated by Beagle Dogs

E. H. Herman, V. J. Ferrans, R. S. K. Young, et al.


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