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The Effect of Mitoxantrone Treatment in Beagle Dogs Previously Treated With Minimally Cardiotoxic Doses of Doxorubicin

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Low-grade chronic cardiotoxicity as determined by myocardial biopsy specimens was induced in beagle dogs after four courses of doxorubicin hydrochloride (1.64 mg/kg, 34.0 mg/sq m) given intravenously once every 3 weeks. After this initial treatment, these dogs were separated into three groups. Two groups received six courses of mitoxantrone (0.25 mg/kg, 5 mg/sq m) commencing at 7 weeks or 19 weeks after the final doxorubicin treatment. The third group was treated with six additional courses of doxorubicin after an interval of 7 weeks. Up to seven sequential endomyocardial biopsies were performed to monitor the myocardial changes which were observed after the initial doxorubicin treatment. The low-grade cardiotoxic changes progressed for at least 7–11 weeks without any additional doxorubicin treatment, and stabilized or even partially reversed after 19 weeks of a treatment-free period. Dogs that received additional doxorubicin demonstrated progressive cardiotoxicity, associated with clinical signs, that resulted in death after a total of seven to ten courses of treatment (12–16 mg/kg, 238–340 mg/sq m cumulative dose). In dogs treated with doxorubicin followed by mitoxantrone after a 19-week treatment-free period, myocardial changes were shown to have stabilized and/or partially regressed. This study indicated that in beagle dogs four courses of doxorubicin (7 mg/kg, 136 mg/sq m cumulative dose) are the threshold dose at which non-life-threatening cardiotoxicity occurs. Residual toxic effects of doxorubicin may be erroneously interpreted as adverse findings attributable to other agents given subsequently during the susceptible period, ie, prior to stabilization of the myocardium. Mitoxantrone given after stabilization of doxorubicin-induced low-grade myocardial changes did not show additive or synergistic effects. (Am J Pathol 1987, 128:121–130)

MITOXANTRONE, 1,4-dihydroxy-5,8-bis [[2-[(2-hydroxyethyl) amino] ethyl]-amino]-9,10-anthracenedione, is an antitumor compound currently under development by the Medical Research Division of American Cyanamid; it is currently marketed in numerous countries. Mitoxantrone has shown antitumor activity comparable to that of doxorubicin against experimental tumors in mice.[1,2] The effectiveness of mitoxantrone in the treatment of human cancer[3–5] has been reported. The only consistent limiting toxic effect of mitoxantrone is myelosuppression, in the form of leukopenia, and to a lesser degree thrombocytopenia. However, it is generally of short duration and is readily reversible.[6,7]

Mitoxantrone did not produce cardiotoxicity in animal models such as dogs and monkeys.[6,8,9] In contrast, doxorubicin caused cardiotoxic effects comparable to those observed in humans in dogs and monkeys[6,8,9] at a cumulative dose of above 200 mg/sq m. In humans treated with doxorubicin, cardiotoxicitv has been observed after a cumulative dose of between 350 and 500 mg/sq m.

Because mitoxantrone is also being used as a second-line treatment in breast cancer patients who have previously received cardiotoxic doses of doxorubicin, this study was conducted to determine whether or not subsequent treatment with mitoxantrone might add to the cardiotoxic effects produced by doxorubicin. The doses of these two drugs (0.25 mg/kg of mitoxantrone and 1.64 mg/kg of doxorubicin) used in this study were equivalent, on the basis of similar myelosuppressive responses in dogs and monkeys.[6,9] Doses

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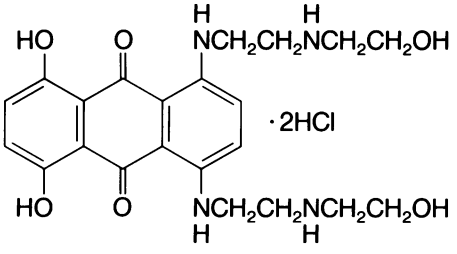
were given once every 3 weeks, which is consistent with the clinical dosing schedule. These doses, given at 3-week intervals, are approximately half the lethal dose for dogs with both compounds and are considered to be tolerated in terms of reversible myelosuppression. The doses and dosing schedule are therefore consistent with the clinical situation.

Cardiotoxicity associated with chronic anthracycline administration can be monitored successfully in patients with sequential endomyocardial biopsies,[10,13] and the progressive changes observed in the myocardium are a useful predictor of heart failure. The transvenous endomyocardial biopsy procedure was demonstrated to be a useful technique in beagle dogs for monitoring cardiotoxicity produced by doxorubicin.[8]

This study was designed to monitor myocardial changes using sequential endomyocardial biopsies in beagle dogs. The important points to be addressed in this report include 1) myocardial changes after a threshold minimally cardiotoxic dose of doxorubicin and the behavior of the myocardial changes during an extended treatment-free period, 2) the response of dogs with prolonged doxorubicin treatment, and 3) the effect of varying the treatment-free period between administration of doxorubicin and mitoxantrone on the response of the dogs.

Materials and Methods

The structure of mitoxantrone is



The experimental design is given in Table 1. A total of 24 beagle dogs (12 males, 12 females) were used. At the start of the study 6 beagle dogs (3 males, 3 female) received the vehicle (0.9% sodium chloride) intravenously, and 18 dogs (9 males, 9 females) were treated identically with doxorubicin for inducing myocardial changes just below the threshold of irreversible cardiotoxicity. The threshold dose of doxorubicin was determined in a previous study to be 7 mg/kg in dogs.[8] The dogs were given a total of four courses of doxorubicin intravenously once every 3 weeks. After the fourth course, there was a 7-week interval prior to the continuation of treatment. After the resting period, dogs treated with doxorubicin were assigned to Groups 2, 3, and 4. The dogs were distributed across the three groups according to comparable morphologic and clinical findings prior to the continuation of the study. The dogs in Group 1 were given the vehicle. In Group 2, mitoxantrone was administered upon demonstration by sequential endomyocardial biopsies that the cardiotoxicity induced by doxorubicin had stabilized (after a 19-week treatment-free period). Group 3 dogs were treated with six courses of mitoxantrone, and Group 4 dogs were treated with six additional courses of doxorubicin at 3-week intervals.

Table 1—Experimental Design

Group	Treatment and Dose (mg/kg)*	Number of doses	Number of dogs	
			Males	Females
1	Saline	10	3	3
2	Doxorubicin (1.64)	4	3	3
	Mitoxantrone (0.25)†	6		
3	Doxorubicin (1.64)	4	3	3
	Mitoxantrone (0.25)‡	6		
4	Doxorubicin (1.64)§	10	3	3

*Intravenous administration, once every 3 weeks.

†Mitoxantrone treatment started 19 weeks after the four courses of doxorubicin.

‡Mitoxantrone treatment started 7 weeks after the four courses of doxorubicin.

§The subsequent six courses of doxorubicin treatment started 7 weeks after the initial four courses.

All dogs were observed daily for clinical signs of toxicity. Blood samples were obtained weekly for hemograms and 24 hours after each dosing for clinical chemistry, including creatine phosphokinase (CPK) isoenzymes. Electrocardiographic (ECG) and blood pressure measurements were obtained at least once during each course. All during life monitoring methods have been described previously.[6] The animals were sacrificed 15 weeks after they received the last course of treatment.

Endomyocardial biopsy specimens were taken from the right side of the ventricular septum.[6,8] Usually two to three samples of approximately 1 cu mm were obtained from each biopsy specimen for evaluation. During the initial doxorubicin treatment, all the dogs underwent biopsy once after the third course of doxorubicin and twice (2 weeks and 5 weeks) after the fourth course for monitoring myocardial changes. Furthermore, dogs in the control group as well as those that received either mitoxantrone or doxorubicin after 7 weeks of intermission underwent biopsy after the sixth and seventh courses. The biopsy schedule is given in Table 2. The dogs in Group 2 treated with six courses of mitoxantrone 19 weeks after the initial doxorubicin treatment underwent biopsy after the seventh and tenth courses. Myocardial samples from dogs that were sacrificed were taken as close as possible to the biopsy sites. For sacri-

Table 2—Biopsy Schedule

Biopsy	Day of study	Number of doses		Group*†
		Doxorubicin	Mitoxantrone	
1	56–57	3	—	2,3,4
2	77–78	4	—	2,3,4
3	99–100	4	—	2,3,4‡
4	141	4	—	2
		4	2	3
		6	—	4
5	168	4	—	2
		4	3	3
		7	—	4
6	225	4	3	2
7	322	4	6	2

*Control animals in Group 1 underwent biopsy by the same schedule.
†All biopsies were done 2 weeks after treatment unless otherwise specified.
‡Biopsy 3 was taken 5 weeks after treatment.

ficed animals, a minimum of ten samples of approximately 1 cu mm were examined. Samples were fixed in 2.5% buffered glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon 812. Sections (1 μ) were stained with toluidine blue 0 and basic fuchsin for light microscopy. A modification of the grading system described by Billingham et al[10] was used for quantitating myocardial lesions observed in these sections.

The slides were assigned a special number and presented to the pathologist for scoring without any identification of the animal or dosage group. The modified grading system is based upon the prevalence of morphologically altered cells with secondary emphasis on the severity of histologic alteration of individual cells. A grade of 1 represents the presence of a single cell or occasionally a few morphologically altered cells (usually containing small vacuoles) widely spaced within a specimen. A 1+ grade was assigned when small vacuoles coalesced and became more prominent in one or two of the involved cells within the specimen. Grades 1 and 1+ were assigned a score value of 1 for calculation of mean grade. A grade of 2 is represented by groups of cells with definite morphologic alterations which are more pronounced and distinctive. Often a few of these altered cells are clustered in a group, and two or three clusters of altered cells may be detected in one section. These morphologically altered cells are usually isolated against a background of morphologically intact myocardium. A grade of 2+ represents clusters of altered cells involving a larger area than Grade 2 with marked cytoplasmic vacuolation resulting from coalescence of small vacuoles. Grades 2 and 2+ were assigned a score value of 2 for calculation of the mean grade. Grade 3 lesions demonstrate diffuse involvement of numerous cells (one-third or more cells in a high-power field), with severe morphologic alterations such as large coalesced vacuoles occupying most of the cytoplasmic space. The intercellular spaces may be widened, with evidence of edema or fibrosis. The grade given to an animal during a sampling period was the highest score of all the individual samples evaluated.

Ultrathin sections of selected blocks were stained with uranyl acetate and lead citrate and examined in a JEOL 100CX-II electron microscope. In addition, samples of heart as well as other organs were taken at necropsy, fixed in 10% buffered formalin, routinely processed, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for light-microscopic evaluation.

Results

Mortality is summarized in Table 3. There were two deaths during the initial doxorubicin treatment resulting from respiratory infection secondary to myelosuppression. These 2 dogs were not included in the assessment of cardiomyopathy. All of the remaining 5 dogs in the group that received doxorubicin alone were either found dead or sacrificed moribund after seven to ten courses of doxorubicin (12–16 mg/kg, 238–340 mg/sq m cumulative dose). Death in these dogs was generally preceded by a progressive decrease in mean arterial blood pressure and changes in the electrocardiogram. The blood pressure decreased from a predose mean of 148 mm Hg to a mean of 104 mm Hg within 10 days prior to death (a drop of 29%). A decrease in blood pressure was apparent up to 2 months prior to death. The last three blood pressure readings taken from animals that died indicated a steady drop of 17%, 21%, and 29%, respectively. The ECG changes included alteration in the QRS complex, inverted T waves, axis deviation, and premature ventricular complexes. Collectively these findings are indicative of cardiac changes. One dog that received four courses of doxorubicin and three courses of mitoxantrone had ECG changes and decreased blood pressure similar to those of the dogs in the doxorubicin treatment group. The death of this dog was attributed to the residual effect of the cardiomyopathic dose of doxorubicin. One female that received four courses of doxorubicin and six courses of mitoxantrone was found dead soon after the last biopsy. This death was a result of complications related to the biopsy procedure; there were no clinical findings to suggest any relationship to treatment.

CPK-MB isoenzyme was present only in the dogs treated with doxorubicin and the 1 dog that was sacrificed moribund after four courses of doxorubicin and

Table 3—Mortality Table

Group	Sex	n	Found dead	Moribund sacrifice	Schedule* sacrifice
1	M	3	0	0	3
	F	3	0	0	3
2 (doxorubicin/19 week	M	3	1‡	0	2
intermission†/mitoxantrone)	F	3	1§	0	2
3 (doxorubicin/7-week	M	3	0	1	2
intermission†/mitoxantrone)	F	3	0	0	3
4 (doxorubicin/7-week	M	3	2‡	1	0
intermission†/doxorubicin)	F	3	0	3	0

*Treated animals were allowed a recovery period of 15 weeks prior to terminal sacrifice during which time concurrent vehicle-treated animals were sacrificed.
†The intermission period is the time interval between the completion of the doxorubicin pretreatment and the initiation of the subsequent treatment.
‡One animal from these groups died during treatment with doxorubicin.
§Death associated with complications during the biopsy procedure.

three courses of mitoxantrone. This isoenzyme was not present in any of the other treated dogs. There were no changes in serum chemistry that could be related to treatment with either mitoxantrone or doxorubicin.

Grossly cardiac and pulmonary changes were seen in dogs that died with clinical signs suggestive of cardiac failure. The findings included dilatation of the cardiac ventricles with rounding of the apex and/or enlargement of the heart, thickened margins of the cardiac valves, paleness of the heart, and pulmonary edema.

The grading of cardiac changes is summarized in Table 4. Microscopic examination of 1-μ sections of myocardium revealed morphologic changes in dogs pretreated with doxorubicin, followed by doxorubicin or mitoxantrone. These changes consisted of vacuolation of myocytes and occasionally pale myocytes. The vacuolated myocytes showed scattered individual small vacuoles or large, clear vacuoles in the cytoplasm, and a few scattered individual myocytes or clusters of myocytes were involved. The pale myocyte is a cell with light-staining homogeneous cytoplasm that occurred rarely in dogs.

Table 4—The Effect of Mitoxantrone in Dogs Pretreated With Doxorubicin: Grading of Heart Findings (1-μ Sections)

Treatment group	Baseline cardiac changes after four doses of doxorubicin‡	Recovery			Additional treatment dose§			Postmortem
		5 weeks	11 weeks	15 weeks	2	3	6	
Doxorubicin/ 19-week recovery/ mitoxantrone								
Mean grade*	0.8	1.4	1.2	0.6		0.4	1.0	1.0‖
Incidence†	4/5	5/5	4/5	3/5		2/5	3/4	4/4
Doxorubicin/ 7-week recovery/ mitoxantrone								
Mean grade*	0.7	1.5			1.8	1.8		1.3‖
Incidence†	4/6	6/6			4/4	5/5		6/6
Doxorubicin/ 7-week recovery/ doxorubicin								
Mean grade*	0.8	1.2			2.0	1.8		2.8¶
Incidence†	4/5	4/5			4/4	4/4		4/4

*Modified grading system based upon prevalence of morphologically altered cells with secondary emphasis on severity of histologic alteration of individual cells. 1, occasional morphologically altered cells usually widely spaced (earliest detection of myocardial involvement, with no clinical symptoms or significance); 2, groups of cells with definite morphologic alterations, which are more pronounced and distinctive (altered cells often occurred in small clusters isolated against a background of morphologically intact myocardium with no clinical symptoms); 3, diffuse involvement of numerous cells with most severe morphologic alterations (large coalesced vacuoles replacing most of the cytoplasmic space, presence of clinical symptoms of irreversible cardiomyopathy, always associated with cardiac related mortality). Mean grade, total grades in the group per number of animals with evaluated sample.
†Incidence, number of animals with gradable lesions/number of animals with available sample.
‡1.64 mg/kg.
§Biopsies taken 2 weeks after dosing with additional doses of mitoxantrone (0.25 mg/kg) or doxorubicin (1.64 mg/kg).
‖Animals were sacrificed 15 weeks after the sixth (additional treatment) dose of mitoxantrone.
¶All animals died or were sacrificed moribund after a total of seven to ten doses of doxorubicin.

After the initial doxorubicin treatment myocardial changes were not uniformly distributed among the dogs. Gradable myocardial changes of 0 to 1+ (Figure 1) were observed in 12 of the 16 treated dogs 2 weeks after the fourth course. A subsequent biopsy at 5 weeks after the fourth course showed that the severity of the cardiac changes progressed in the absence of additional doxorubicin treatment. During this period, cardiac lesions progressed to a grade of 2 in 7 of 16 dogs, indicating that a low-grade cardiotoxic change was produced by four courses of doxorubicin.

After ten courses of doxorubicin, the myocardial changes progressed with time and accumulative dose to a grade of 2+ or 3 at death (Figures 2 and 3). Death in these animals was related to cardiac failure and/or pulmonary complications.

In the group with a 7-week treatment-free period prior to administration of mitoxantrone, the biopsy and necropsy specimens demonstrated no progression in severity of the cardiac changes observed after the initial treatment with doxorubicin. One male from this group was sacrificed moribund after four courses of doxorubicin and three courses of mitoxantrone had an increase in severity from grade 2 to 2+ as a result of the residual effect of doxorubicin.

In dogs given six courses of mitoxantrone 19 weeks after the initial doxorubicin treatment, sequential biopsies demonstrated that despite additional mitoxantrone treatment, there was stabilization of the myocardial changes. Furthermore, the safety of additional mitoxantrone treatment was established, because at sacrifice, the grading of the myocardial changes was either lower or unchanged when compared with the grading after the initial doxorubicin treatment.

Heart sections stained with H&E demonstrated that all dogs treated with doxorubicin alone had moderate to marked vacuolation of myocardial fibers, with slight to moderate interstitial edema and fibrosis. In addition, moderate degeneration of myocytes was seen in 4 dogs, and myxoid degeneration of the cardiac values was seen in 3 dogs. In dogs that received mitoxantrone 7 weeks after initial doxorubicin treatment, moderate myocytic vacuolation was seen in 2 dogs, 1 of which was sacrificed moribund, and slight myocytic vacuolation was seen in 2 other dogs. Slight interstitial edema and/or fibrosis were seen occasionally in these dogs. In addition, the dog sacrificed moribund also showed moderate myxoid degeneration of the heart valve and slight to moderate degeneration of myocytes. In the dogs that received six courses of mitoxantrone 19 weeks after doxorubicin, minimal interstitial edema and myocytic vacuolation were seen in 1 dog.

Slight to moderate endocardial fibrosis was seen focally or diffusely in most animals of all groups and most often at the terminal sacrifice. Other findings included moderate, diffuse pulmonary edema, often seen associated with intraalveolar hemorrhage in the animals that died. In addition, 2 of the moribund animals that received doxorubicin alone had bronchopneumonia or chronic interestitial pneumonia.

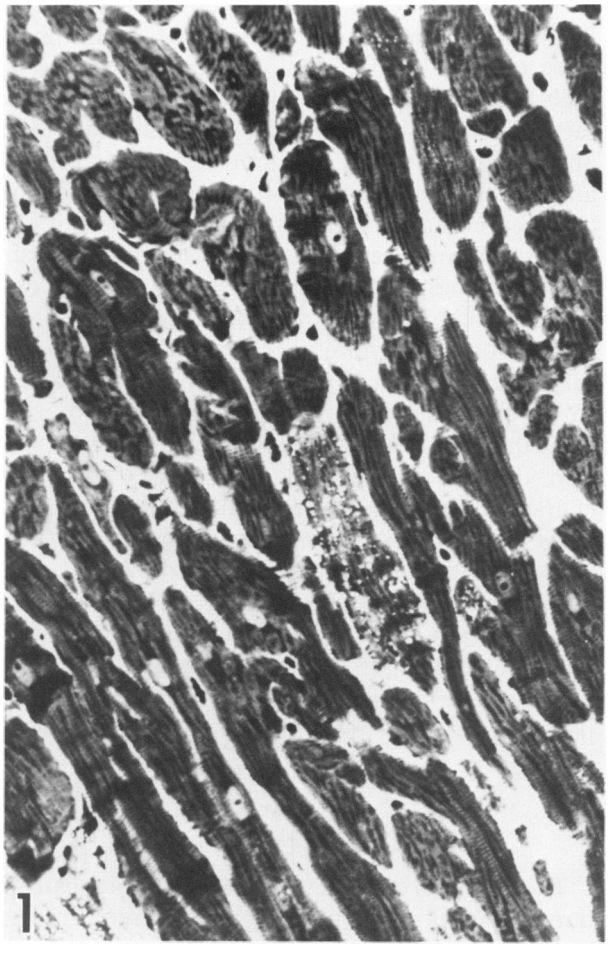
Electron-microscopic examination of heart samples from dogs treated with doxorubicin or with doxorubicin followed by mitroxantrone revealed two distinct lesions. The more prevalent lesion consisted of dilatation of the sarcoplasmic reticulum, progressing to the formation of aggregates of vacuoles derived from the sarcotubular system (Figure 4), which were also visible with the light microscope. This lesion characteristically was confined to individual myocytes. The second lesion was disorientation and loss of myofibrils in individual myocytes, in the absence of dilatation of the sarcoplasmic reticulum. This change, which was not observed until the third biopsy period, is illustrated in Figure 5, which shows a ribosome-rich myocyte that has lost its myofibrils, and which appeared as a pale myocyte at the light-microscopic level.

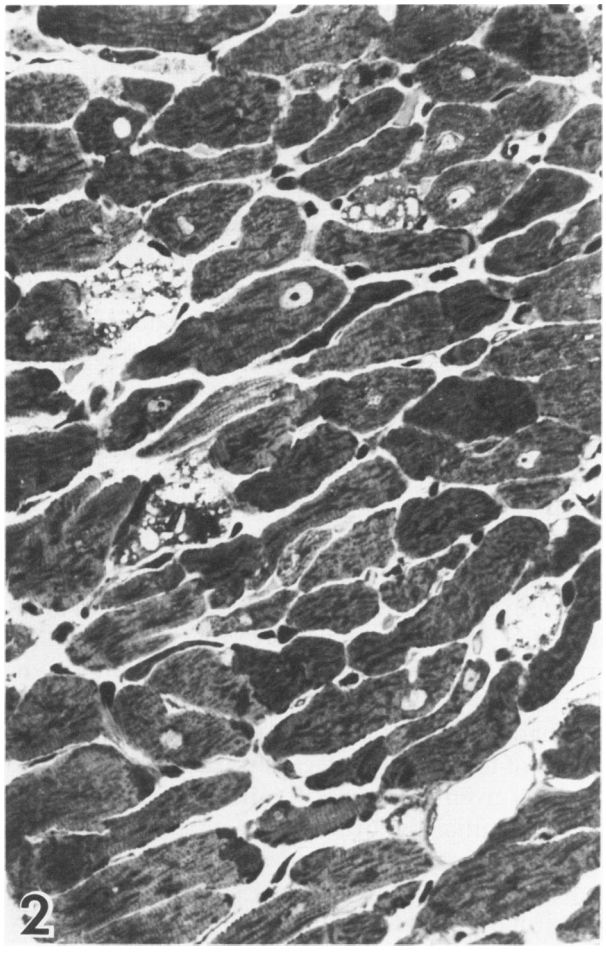
Discussion

The potential of a drug to cause cardiomyopathy in humans usually does not become apparent until the later phases of clinical trials. In addition, during the early phases of clinical trials, it is difficult to assess the impact of previous drug treatments on both clinical and morphologic changes induced by the current therapy.

In the study of drug-induced cardiotoxicity in humans, it is essential to use an animal model that closely resembles the human situation. The specifications for an ideal model were proposed by Young,[11] and the beagle dog was found to be an appropriate model.[6,8,9] In this study the dog model was used for evaluation of the potential additive cardiotoxic effect of mitoxantrone following doxorubicin, because subsequent treatment with mitoxantrone may be beneficial to patients heavily pretreated with doxorubicin. In the clinic it was observed that mitoxantrone does not have crossover resistance with anthracycline antibiotics in the treatment of some tumors.[3]

Billingham et al[10] demonstrated that endomyocardial biopsy specimens rapidly fixed and embedded in plastic provide excellent artifact-free samples suitable for both conventional microscopy and ultrastructural examination. In contrast, cardiac samples fixed routinely in buffered formalin and stained with H&E





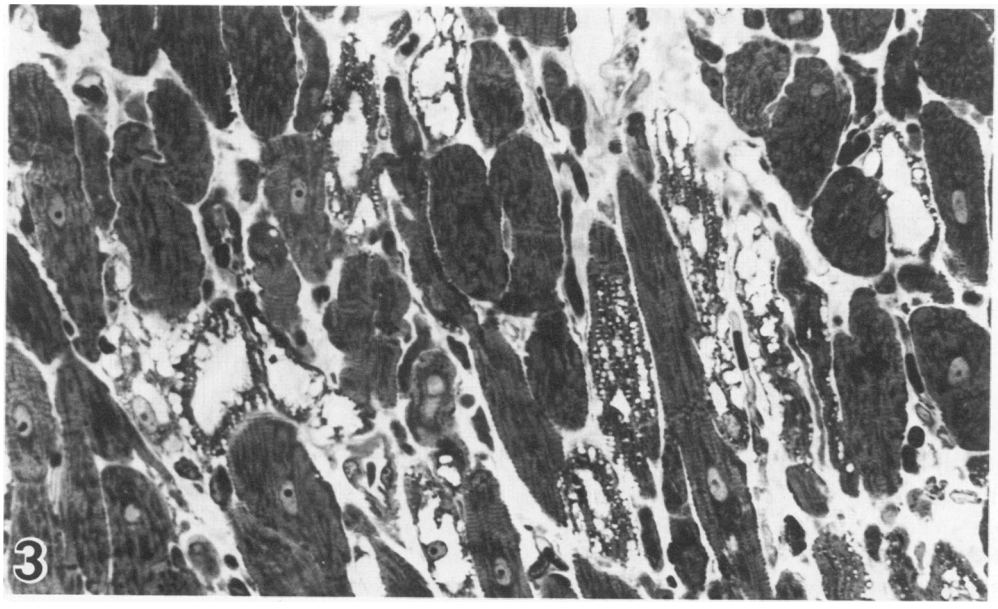


Figure 1—Biopsy sample from a female dog in Group 3 that received four doses of doxorubicin (cumulative dose of 7 mg/kg) illustrating a grade of 1, with a single vacuolated cell. (Glutaraldehyde-fixed, osmium tetroxide-postfixed, stained with toluidine blue and basic fuchsin, ×470) **Figure 2**—Heart sample from a male dog in Group 3 that received four doses of doxorubicin (cumulative dose of 7 mg/kg) and three doses of mitoxantrone (cumulative dose of 0.75 mg/kg) illustrating a grade of 2. (Glutaraldehyde-fixed, osmium tetroxide-postfixed, stained with toluidine blue and basic fuchsin, ×470) **Figure 3**—Heart sample from a female dog in Group 4 that received seven doses of doxorubicin (cumulative dose of 11.5 mg/kg) illustrating a grade of 3. (Glutaraldehyde-fixed, osmium tetroxide-postfixed, stained with toluidine blue and basic fuchsin, ×470)

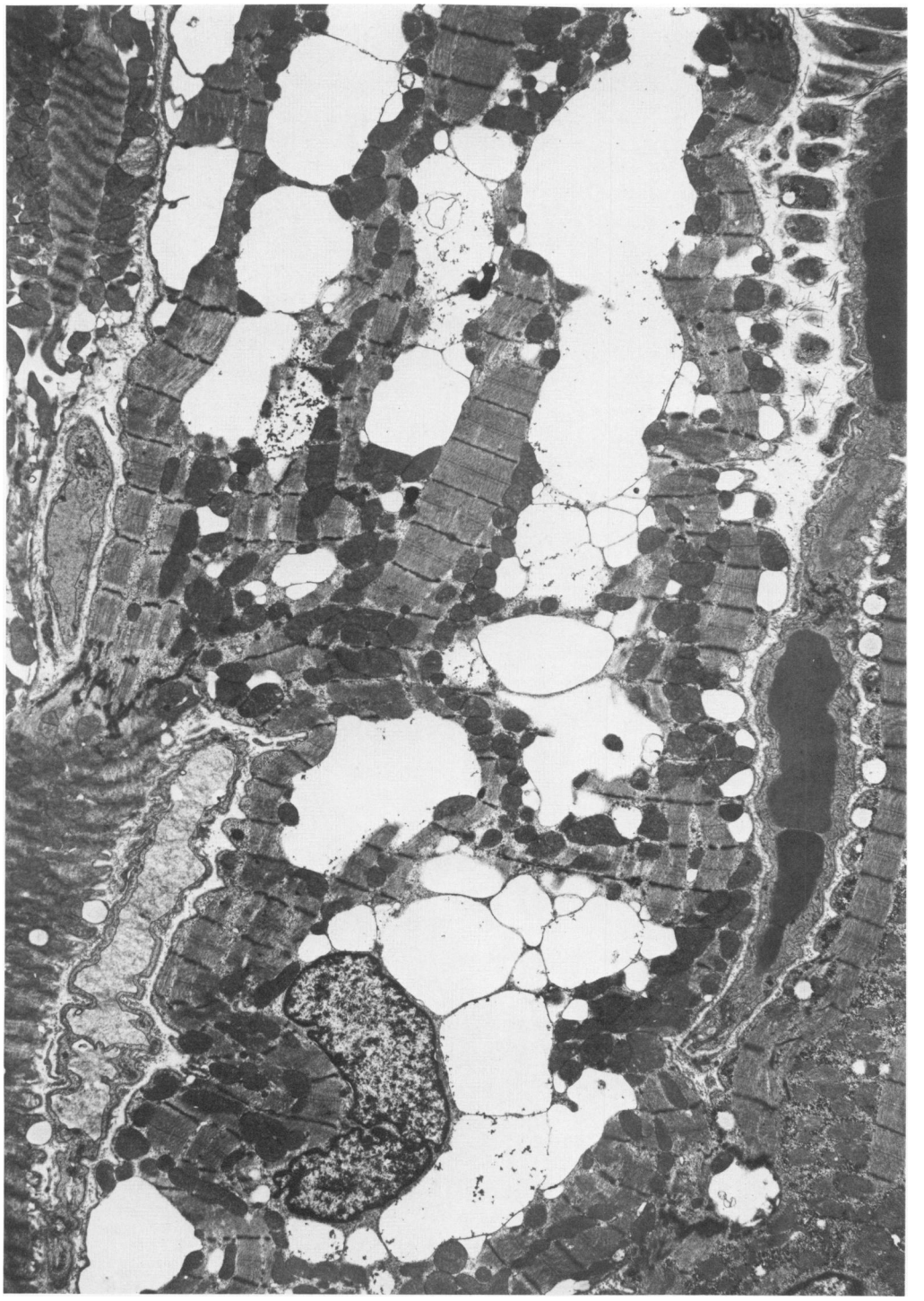


Figure 4—Heart sample from a female dog in Group 4 that received seven doses of doxorubicin (cumulative dose of 11.5 mg/kg) and was sacrificed moribund, showing a myocyte with aggregates of vacuoles derived from the sarcotubular system. (Glytaraldehyde-fixed, osmium tetroxide-postfixed, stained with uranyl acetate and lead citrate, ×4000)

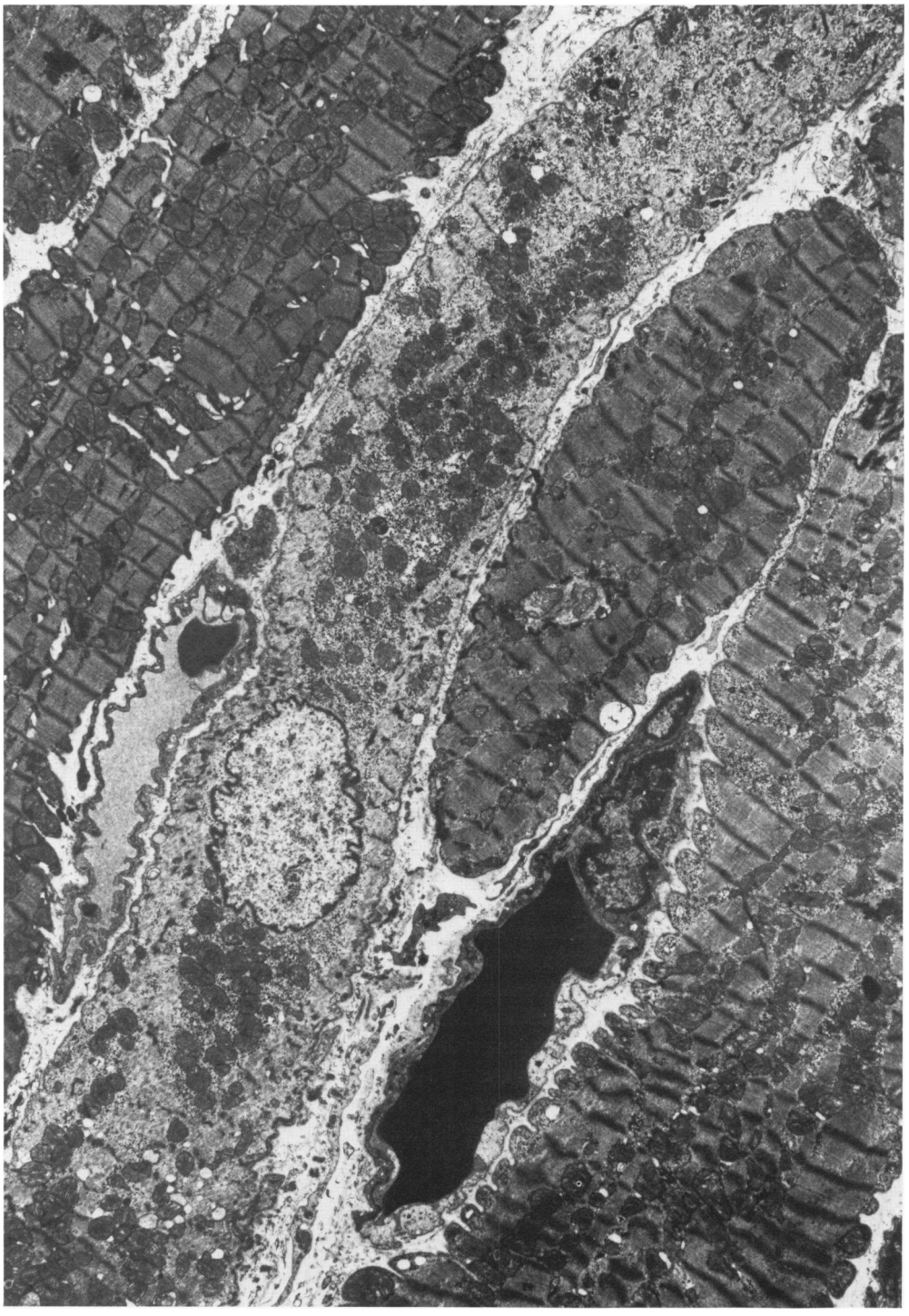


Figure 5—Heart sample from a male dog in Group 3 that received four doses of doxorubicin (cumulative dose of 7 mg/kg) and three doses of mitoxantrone (cumulative dose of 0.75 mg/kg) showing a ribosome-rich pale myocyte with remnants of Z bands. (Glutaraldehyde-fixed, osmium tetroxide-postfixed, stained with uranyl acetate and lead citrate, ×4000)

exhibit minor distortions and artifacts that resemble vacuolation of myocytes and widening of the intercellular space.

The myocardial changes seen in 1-μ sections that are characteristic of anthracycline cardiotoxicity are vacuolated myocytes or myocytes with homogeneous pale cytoplasam. In the dog, myocytic vacuolation is the predominent morphologic alteration. The modified grading system used in our laboratory is based upon the prevalence of morphologically altered cells with secondary emphasis on severity of histologic alteration of individual cells. Use of this system provides a good correlation between morphologic findings and clinical signs. Grade 1 is not associated with any clinical symptoms, and is the earliest indicator of cardiac involvement. Although Grade 2 represents a progression from Grade 1, clinical symptoms are also absent from animals at this grade. However, clinical symptoms may be present with a grade of 2+. Grade 3 is associated with clinical symptoms of cardiomyopathy and is seen almost exclusively in moribund animals which demonstrated irreversible cardiomyopathy with ECG changes, hypotension, and CPK-MB isoenzyme bands.

In this study, only two biopsy specimens were not graded, because of paucity of myocytes. Usually two to three samples of approximately 1 cu mm were obtained from each biopsy specimen. Forty-six percent of the dogs with a score of 1 had Grade 1 lesions in only 1 of 2 or more samples examined, whereas 43% of the dogs had gradable lesions in all samples. In contrast, 88% of the dogs with scores of 2 had gradable lesions (1, 1+, or 2) in all samples. Eight animals had Grade 1 at terminal sacrifice, and gradable scores were seen in four to eight of ten samples examined. In almost all cases, gradable lesions were seen in all ten samples when a grade of 2 or 3 was scored at sacrifice.

A mean grade of 0.4 (in 2 of 5 animals) was seen after four doses of doxorubicin and three doses of mitoxantrone in the group with 19-week treatment-free periods. At final sacrifice, the mean score was 1.0 (in 4 of 4 animals). This does not represent a change in severity, rather than an apparent increase in incidence in dogs with Grade 1 lesions, probably because more samples were available for evaluation at terminal sacrifice.

In a previous dog study with uninterrupted dosing once every 3 weeks, myocardial lesions that progressed with time and dose were observed with prolonged doxorubicin treatment. In contrast, no evidence of cardiotoxicity, either microscopic or clinical, was seen in dogs that received the maximum tolerated dose of mitoxantrone during the course of treatment.[8,9] In the present study, the data showed that four courses of doxorubicin (7 mg/kg, 136 mg/sq m) caused low-grade cardiotoxic change. In order to enable dogs to tolerate more courses of doxorubicin, the treatment-free period after threshold cardiotoxicity was induced had to be prolonged to allow time for intracellular repair. Clinical signs of doxorubicin-associated cardiotoxicity were seen after six courses (10 mg/kg, 204 mg/sq m) in the previous study when doxorubicin was administered at 3-week intervals without interruption.[8] In this study, with a 7-week treatment-free period after a low-grade cardiotoxicity was induced (before resuming doxorubicin treatment), clinical signs were observed after seven courses (12 mg/kg, 238 mg/sq m). Furthermore, 3 of the 5 dogs that received only doxorubicin in this study were given ten courses prior to death, which showed a longer survival period than in the previous study, where all doxorubicin-treated dogs were sacrificed in poor condition after nine courses.

The low-grade myocardial changes induced by doxorubicin progressed with time up to a period of 2–3 months, even in the absence of additional doxorubicin treatment, before they became stabilized. In humans, the cardiotoxic event can occur months after the initial dose of doxorubicin is given.[12]

The cause of death in 1 male dog that received three courses of mitoxantrone 7 weeks after four courses of doxorubicin was attributed to the residual effect of doxorubicin because 1) the cardiac lesion of this dog was not stabilized prior to the administration of mitoxantrone, 2) other animals in the same group did not demonstrate any signs of deterioration of physical or cardiac condition after a total of six courses of mitoxantrone, 3) none of the animals in the group that received mitoxantrone after the doxorubicin-induced cardiac lesion was stabalized showed any evidence of impairment of physical or cardiac condition, and 4) mitoxantrone is not cardiotoxic in dogs, as was demonstrated in our previous study.[8]

Endomyocardial biopsies provide a useful and reliable means of monitoring cardiotoxicity in humans[10,13] and in dogs.[8,9] The dogs in the present study tolerated up to 7 biopsies; on a few occasions, the biopsies were taken only 3 weeks apart. A possible residual effect of the biopsy procedure was the increased incidence of endocardial fibrosis within the right ventricle at the completion of the study. Also, in 1 dog the cause of death was attributed to complications of the biopsy procedure itself and was not related to drug-induced changes in the heart. Therefore, the frequency of endomyocardial biopsies during a study should be limited so that unnecessary stress is avoided.

The treatment-free interval after the induction of

low-grade cardiotoxicity by doxorubicin is the most crucial factor affecting the outcome of the study. As indicated by sequential biopsy and mortality data, or even by comparison with a previous study,[6] the severity of myocardial changes induced by doxorubicin progressed for up to 3 months with no additional treatment. These changes became stabilized or reversible at the end of this period, and treatment with mitoxantrone after stabilization of the doxorubicin-induced myocardial changes did not result in any clinical symptoms of cardiotoxicity or mortality. On the other hand, dogs in the group that received additional doxorubicin after the 7-week treatment-free period demonstrated progressive myocardial lesions, associated with clinical signs of cardiotoxicity, which resulted in death in all animals after a total of seven to ten courses.

The results of the present study indicates that four courses of doxorubicin (cumulative dose of 7 mg/kg, or 136 mg/sq m) is the threshold dose in the dog at which cardiotoxicity occurs; the severity of these changes progressed for up to 2–3 months in the absence of additional treatment. Therefore, residual cardiotoxic effects of doxorubicin should be considered before any other agents are given during this susceptible period. Mitoxantrone did not have an additive or synergistic effect in dogs that were pretreated with a threshold cardiotoxic dose of doxorubicin because an adequate treatment-free period was allowed prior to initiating mitoxantrone administration.

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