INVESTIGATIVE NUCLEAR MEDICINE

The Predictive Value of Myocardial Radioisotope Scanning in Animals Treated with Doxorubicin

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Thirty-four New Zealand white rabbits were treated with doxorubicin and imaged weekly with Tc-99m pyrophosphate to define the value of abnormal myocardial images in predicting doxorubicin-induced cardiac toxicity. Increased myocardial uptake was detected in most animals on sustained treatment with doxorubicin. A greater proportion of the heart was involved with doxorubicin-related histologic changes in animals with strongly positive myocardial images than in treated animals with moderately positive or normal scans. The myocardial images returned to normal levels 2–6 wk after doxorubicin was discontinued. Five of seven rabbits that received doxorubicin after they had three moderately positive myocardial scans, died from congestive heart failure. Three rabbits whose doxorubicin was discontinued because of scan findings, survived for 6 wk or more before dying from renal failure. The three rabbits who received the highest total dose of doxorubicin died of renal failure without developing abnormal myocardial scans.

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Doxorubicin is an antitumor anthracycline antibiotic of substantial importance in cancer chemotherapy. The major limiting factor in long-term administration of this agent is its dose-related cardiac toxicity. The high mortality (50%) of doxorubicin-related cardiac toxicity and the increasing incidence of toxicity above a cumulative dose of 500 mg/m^2, has been the basis for empiric limitation of the total doxorubicin dosage to 550 mg/m^2 (*1*). Such a limit might lead to premature cessation of drug administration in responsive patients, or could place a small but significant portion of more sensitive patients at risk of congestive heart failure (CHF) (*2,3*). The reliable prediction of clinically significant doxorubicin-induced cardiac toxicity has not been achieved. Electrocardiograms, phonocardiograms, and echocardiograms have been used to evaluate the toxic changes, but have proved unreliable and have detected changes too early to be useful in maximizing the dose (*4–11*). Promising results have been reported for a combination of endomyocardial biopsy, cardiac catheterization, and phonocardiogram techniques (*8*), but these are too difficult for widespread use. More recently, Alexander et al. (*12*) have used changes in ejection fraction, as determined by radionuclide angiography, to predict moderate myocardial toxicity, but the technique has not reliably predicted mild toxicity.

Cardiac imaging using Tc-99m pyrophosphate (PPi) has proven useful for the detection of myocardial injury. Previous observations at our institution indicate that some patients on doxorubicin treatment develop positive myocardial images after injection with Tc-99m PPi (*13*). The most prominent images were seen in patients with CHF. We report here a study, using the rabbit model (*14*), of the correlation of the Tc-99m PPi cardiac scan with doxorubicin-related histologic changes and its use in predicting and preventing CHF.

MATERIALS AND METHODS

Thirty-one mature New Zealand white rabbits were treated weekly with 2.4 mg/kg of doxorubicin by i.v.

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injection. This dose permits myocardial changes to occur slowly enough for careful study. Two animals received 3.5 mg/kg weekly and one received 4 mg/kg weekly, to determine whether these dose schedules would be tolerated, which they were for at least 4 wk. Sixteen animals (including the three receiving higher doses) were killed selectively at various durations of doxorubicin therapy to compare scans with histology. To evaluate the predictive value of the scan, 15 animals were used. Ten of these were treated until death occurred or they had received 31.2 mg/kg of doxorubicin; five animals had treatment stopped based on the scan findings. To simulate the empiric discontinuation of doxorubicin in patients, three animals were treated to a total dose of 14.4 mg/kg. Fresh doxorubicin solution was used on each injection date and administration was accomplished as soon as possible after the solution was prepared. Ten animals served as controls, receiving only saline injections.

All animals were injected with 1-1.5 mCi of Tc-99m PPi on the day before scheduled doxorubicin or saline administration. Myocardial imaging was performed 1-2 hr after tracer injection, with a gamma camera, using a high-sensitivity collimator and collecting 200,000 counts for each image. The animals were restrained without sedation and scanned in the AP position. Analog images were recorded on film and digitized images were generated with a computer. The heart shadow was graded "negative" if it were invisible, or a faint image was present; "moderately positive" if the myocardial image were clearly apparent but were no more intense than the ribs; it was "markedly positive" if it approximated the intensity of the sternum (Fig. 1). All myocardial scans were read without knowledge of treatment.

Animals dying spontaneously were autopsied to determine the cause of death. Death was classified as due to CHF if the typical changes (*15*) were present, pneumonia if there were evidence of acute bronchopneumonia without CHF, or renal failure if kidneys were small and the typical histologic changes of end-stage renal disease were found.

All animals who died or were killed had heart, kidney, and other organs evaluated histologically. Myocardial samples were graded without knowledge of treatment. Equivocal changes were defined as minor degrees of vacuolization, difficult to separate from artifact. If the heart had definite doxorubicin-related vacuolization or fibrosis, the percentage of the myocardium involved was estimated visually as greater than or less than 10% involvement in the sections examined. For brevity we will use the terms ">10% involvement" or "<10% involvement."

Animals killed *in extremis* or electively were injected with 1.5 μCi of Tc-99m PPi, 1 hr before death. Weighed fragments of heart, liver, and spleen were counted in a scintillator counter. The ratios of cps/g heart to cps/g liver, or cps/g spleen, were calculated and used in data evaluation. Ratios of the activity in the myocardial area, compared with the adjacent rib area, were generated from the digitized images using a light pen (Fig. 2).

RESULTS

Twenty-four animals receiving standard doses of doxorubicin developed the first positive scan after 4-12 doses of doxorubicin (average 6.9 doses). The three animals who received higher weekly doses developed positive scans earlier than the 24 receiving the standard dose. Seven animals received 2-13 doses and did not develop positive scans.

Correlation of scan with pathology findings. The pathologic changes in the hearts of 34 treated animals were correlated with scan results (Table 1). Animals developing a positive scan before sacrifice or death had a higher incidence of unequivocally doxorubicin-related histologic changes. Of the 13 rabbits that developed

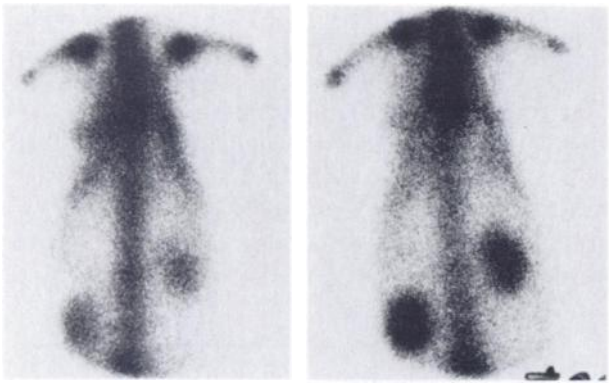

FIG. 1. Left: image of rabbit before receiving doxorubicin. Right: markedly positive image of same animal after doxorubicin administration.

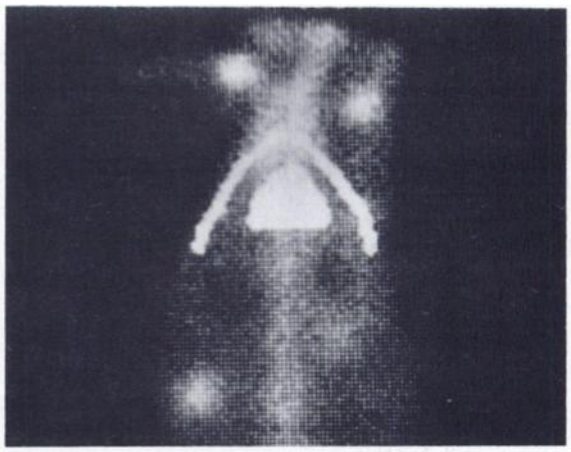

FIG. 2. Computer-stored image of rabbit with rib and myocardial areas flagged by light pen.

TABLE 1. COMPARISON OF IMAGE FINDINGS WITH DEGREE OF MYOCARDIAL ABNORMALITY

Image findings	Normal	Equivocal	<10% of heart involved	>10% of heart involved
Negative or normal	3	1	2	1
Positive	3	1	5	5
Positive at least 3 wk, or markedly positive	0	1	0	12

positive images for 3 or more wk, or a markedly positive image, 12 had >10% damage. One animal with a markedly positive image and equivocal myocardial findings had a concurrent pneumonia. The two rabbits with negative images had <10% involvement. One had received eight doses before sacrifice and the other had received 13 doses and was not killed until 3 wk after the drug was stopped. The rabbit with >10% involvement and negative images had received ten doses and was not killed until 3 wk after the last dose of doxorubicin.

Ten rabbits were not killed until 2 or more wk after doxorubicin was discontinued; nine of them had >10% involvement. These observations are similar to those of Jaenke (*16*), who noted progressive changes in the hearts of rabbits after doxorubicin was discontinued.

In the eight rabbits with positive images who survived 3 or more wk after doxorubicin was discontinued, the myocardial images returned to normal within 2–6 wk. In four rabbits, the images increased in intensity for 1–2 wk before returning to normal, whereas in the other four, the images returned rapidly to normal. Histologic changes persisted after the images had returned to normal.

Ten concurrent control animals were studied. Nine of them had persistently normal scans, but one developed a moderately positive scan that persisted for several weeks. A mediastinal abscess was discovered and the image returned to normal following penicillin treatment.

Predictive values. Twelve of the 15 animals in this group developed positive scans.

Seven animals received further doxorubicin after having had three moderately positive scans or one markedly positive scan. Five of these seven rabbits died from congestive heart failure (Table 2). One died from pneumonia and another from respiratory distress with mucous plugs and hyperinflated lungs.

Five animals had treatment discontinued because they had moderately positive scans for 3 wk; all had >10% involvement. Three of these rabbits survived for 6–10 wk after stopping doxorubicin, and all three died from renal failure without any evidence of CHF. Two rabbits were electively killed and showed no evidence of CHF.

TABLE 2. PREDICTIVE VALUE OF THE MYOCARDIAL SCAN RELATIVE TO CARDIAC TOXICITY AND ABILITY TO TOLERATE FURTHER DOXORUBICIN

Scan	Dose	Doxorubicin	Clinical result
Markedly positive, or positive for 3 or more weeks	194–315 mg/m^2.	Continued	5 died in congestive heart failure; 2 died from infection
		Stopped	3 survived 6 wk or more; 2 killed for study
Negative or positive only on final scan	343 mg/m^2	Continued	3 died in renal failure, without CHF; 1 showed >10% involvement

In three of the treated animals the scintigrams remained normal or were only transiently abnormal, and treatment was continued up to 13 doses totalling 31.2 mg/kg. This is the highest dose in our study, equivalent to 343 mg/m^2, assuming that 11 kg ≈ 1 m^2 (*14*). All three died from renal failure; none from CHF. At autopsy, two showed <10% myocardial involvement, one >10%.

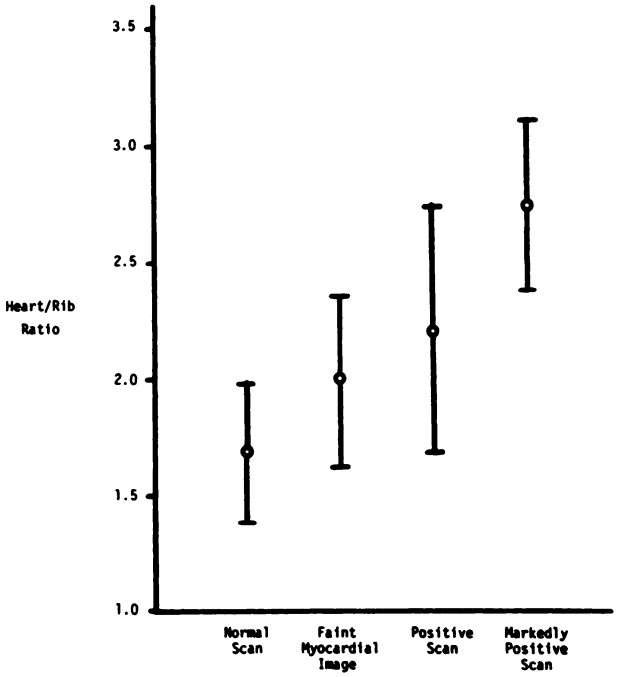

FIG. 3. Mean and standard deviation of computer-generated heart-to-rib ratios for different image classifications.

TABLE 3.

Organ activity (cps/g) ratio	Scan			Histology			
	Normal	Moderately positive	Markedly positive	Normal	Equivocal	<10% of heart involved	>10% of heart involved
Heart/spleen and heart/liver >2.0	0	2	3	0	1	0	4
Heart/spleen or heart/liver <2.0	4	4	0	2	2	2	2

Empiric discontinuation. Three animals had dosage limited to 14.4 mg/kg (158 mg/m^2). Each of these developed only moderately positive scintigrams. One died from renal failure and the other two were killed. One rabbit had evidence of moderately severe renal dysfunction at the time of sacrifice. One animal had no myocardial changes; the other two had >10% involvement.

Quantitative data. The rabbit heart is located high in the thorax in the midline (Fig. 1), thus the increase in counts from myocardial uptake was small relative to a background that included the sternum. In order to confirm our visual interpretations of the scans objectively, the heart-to-rib ratios derived from the digital images were evaluated for each category of scan. As shown in Fig. 3, the heart-to-rib ratio increased as the myocardial image appeared more intense. The ratios obtained for each group were subjected to the chi-squared test and each group was significantly different from all other groups, with $P < 0.005$.

Ratios of radioactivity per gram of heart to radioactivity per gram of liver and of spleen were obtained on 13 animals dying or killed while actively on treatment. These ratios were compared with visual interpretations of the most recent myocardial scan and with histologic findings. If both ratios were greater than two, the images were rated moderately or markedly positive. If one or both ratios were less than two, the scans were normal or moderately positive (Table 3). Those animals in whom both ratios were greater than two also demonstrated a higher probability of having >10% involvement.

DISCUSSION

Using the rabbit model and Tc-99m pyrophosphate, we were able to detect myocardial damage before its clinical appearance. The changes in the image occurred early, in that a minimum of three more doses of doxorubicin were required after the first positive myocardial scan before death from congestive heart failure occurred. In three animals, death from CHF was prevented by discontinuation of the doxorubicin on the basis of a positive cardiac image. These animals had been treated well above a total dosage of 14.4 mg/kg (158 mg/m^2), a level that would be expected to cause 1-5% deaths from CHF (*15,16*). We felt that this was analogous to levels above the empiric human safe-treatment level of 550 mg/m^2 dose. Five of the seven animals treated beyond the safe image limits—which we defined as three moderately positive and one strongly positive image—died from CHF. The three animals given the highest cumulative dose of doxorubicin (343 mg/m^2) because of persistently normal images, did not develop CHF. In spite of the high dose of doxorubicin, two of these three rabbits showed <10% involvement. This strongly suggests a predictive value for the myocardial image.

Abnormal myocardial images reverted to normal after doxorubicin was discontinued, although there was progressive histologic change. Myocardial images become positive early in myocardial infarction and revert to normal 1-2 wk after infarction, before the fibrotic reaction is complete (*17*). We speculate that a return to normal of the myocardial image during progressive histologic change, indicates that the scan detects an earlier, possible biochemical, change in the heart. There is obvious importance in this observation in terms of the need for appropriate and consistent timing in designing future studies.

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