Myocardial Uptake of Tc-99m Skeletal Agents in the Rat after Experimental Induction of Microscopic Foci of Injury

D. G. Miller, R. F. Gilmour, Z. D. Grossman, S. Mallov, B. W. Wistow, and R. F. Rohner

State University of New York, Syracuse, New York

The cardiac uptake of Tc-99m tagged skeletal agents was studied after myocardial injury produced by subcutaneous catecholamine injection and random foot-shock stress. Rats stressed for 2 hr developed microfocal myocardial injury, without gross change, whereas those stressed for 12 hr sustained more confluent and sometimes grossly visible damage. Tc-99m MDP and Tc-99m PP_i concentrations in these hearts were significantly above control (undamaged) heart levels, producing positive gamma-camera images. Subcutaneous epinephrine injections resulted in grossly visible lesions, with tracer concentrations higher than those previously reported in vaso-occlusive infarcts.

We postulate that the stress-induced scattered microfocal lesions may accumulate radiopharmaceutical on a per-gram basis in the same way as the larger catecholamine-induced lesions, since tracer delivery to the injured areas in each case is probably less impeded than in frankly vaso-occlusive models. Such microfoci, then, could provide an explanation for some of the "false positive" myocardial scans observed clinically.

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Technetium-99m stannous pyrophosphate has achieved rapid and widespread acceptance for the imaging of myocardial infarcts in humans (*1–3*). Studies of dogs (*4–6*), rabbits (*7,8*), and rats (*9,10*) have established that Tc-99m bone-seeking agents, such as pyrophosphate, are sequestered in injured myocardium, with infarct-normal ratios averaging 20–40. Increased myocardial tracer concentration has also been observed in patients with stable and unstable angina (*11,12*), ventricular aneurysm (*13*), amyloidosis (*14*), and valvular calcification (*15*). In asymptomatic subjects Prasquier et al. (*16*) and Berman et al. (*17*) reported diffusely positive scans, some probably the result of persisting blood-pool activity, but others unexplained. On the other hand, Perez et al. (*18*) reported positive scans only in persons with clinical evidence of myocardial damage.

Prior experimental studies have used procedures that produce a large focus of damage: ligation or catheterization of a coronary artery (*4,5,10*), blunt chest trauma (*19*), direct thermal injury (*9*), or the percutaneous intramyocardial injection of vasopressin in oil (*8*). No study has reported the uptake of Tc-99m bone-seekers in hearts with scattered, microscopically visible damage but without grossly visible, large foci of necrosis. Thus there has been no experimental simulation of the scattered damage postulated by Willerson et al. (*20*), which could result in a diffuse pattern of radiopharmaceutical uptake. We report now the results of subcutaneous catecholamine injection and intermittent electrical foot-shock —procedures that can produce diffuse, microscopically visible myocardial injury without, or in addition to, grossly visible focal necrosis. We postulate that multiple microscopic foci of myocardial ischemia may provide an explanation for some of the "false-positive" myocardial images observed clinically.

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For reprints contact: Dean G. Miller, Dept. of Pharmacology, State University of New York, Upstate Medical Center, 766 Irving Ave., Syracuse, NY 13210.

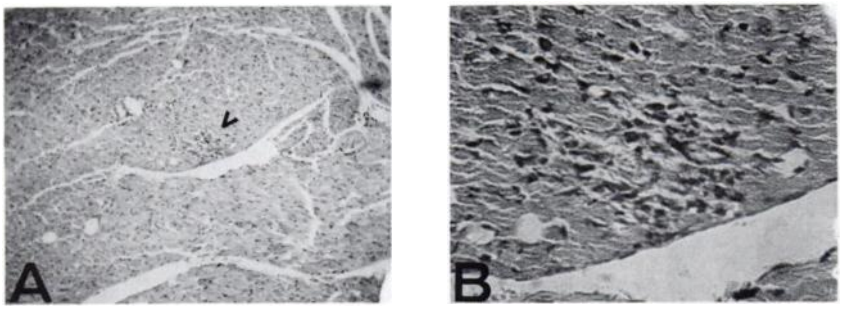

FIG. 1. Photomicrographs of a stained section (H. & E.) of a typical 2-hr-stressed heart, fixed 72 hr after stress. No grossly visible damage. Note microfocus of necrosis surrounded by normal-looking tissue. (A = 100 ×, and B = 450 × before photographic reduction.)

METHODS

Subjects were male Sprague-Dawley rats, weighing 300–500 g.

Catecholamine injection. 0.1 cc of 1% lidocaine was injected into the shaved dorsal skin of the rat. Through a small incision, subcutaneous tissue was exposed by forceps, and epinephrine bitartrate in saline injected (3 mg free base per kg). The incision was closed with wound clips and covered with collodion. Eighteen hours then elapsed before the intravenous injection of the Tc-99m agent.

Stress. Subjects were stressed individually in galvanized steel cages measuring 30 × 23 × 16 cm, with an electrically isolated floor of 6-mm stainless steel rods spaced 2.25 cm apart. At random intervals having a 24-sec mean, 1-sec shocks were delivered to the rods. Constant-current scrambled shocks of 1-mA intensity were presented. The shock current was considerably less than that used in conditioned avoidance experiments (*21,22*). The stimulus was administered for either 2 hr or 12 hr. An 8-hr interval was used between termination of both 2- and 12-hr stress periods and traced injection, so that uptakes of 12-hr-stressed subjects were comparable to those of catecholamine-injected subjects. That is, 20 hr elapsed between the beginning of the 12-hr stress period and tracer injection.

Radiobioassay studies. Twenty-five μCi of Tc-99m pyrophosphate (PP_i), or stannous methylene diphosphonate (MDP), in 0.1 ml saline were injected into the left femoral vein of pentobarbital-anesthetized rats. After 100 or 300 min the animals were killed, and immediately 1 ml of blood was drawn from the inferior vena cava. Hearts were removed, washed, blotted and weighed. Whole hearts and blood samples were counted in a scintillation counter. In an additional group of stressed rats following removal of the hearts, the coronary arteries were perfused with 10 ml ice-cold Krebs Ringer bicarbonate solution before radioassay. Ratios for injured myocardium-to-normal myocardium were calculated for the stress experiments by comparison of whole-heart activity to that of control animals; these received no stress but were injected with the same dose of radiopharmaceutical the same number of hours before sacrifice. For the epinephrine study, two injured-to-normal ratios were calculated, comparing injured myocardium with myocardium of control rats, and with "normal" areas of epinephrine-damaged hearts.

Histology. Hearts of 2- and 12-hr-stressed rats were fixed 72 hr after the beginning of stress. Catecholamine-injured hearts were fixed 18 hr after epinephrine injection. All specimens were stained with hematoxylin and eosin for light microscopy. Control hearts were prepared similarly.

Radionuclide imaging. Excised, whole, unfixed hearts of control and 2-hr-stressed rats were washed, perfused with 10 ml Ringer bicarbonate solution, and imaged simultaneously, side by side, with a gamma camera using a pinhole collimator.

RESULTS

Gross and microscopic pathology. Initially, conventional subcutaneous epinephrine injection proved unsatisfactory because the effects varied from no myocardial damage to massive fatal damage. Catecholamine injections through a cutaneous incision, however, produced large and relatively uniform, grossly visible foci of damage along the posterior interventricular septum and base of the left ventricle. No animal mortality resulted from this type of injection, or from the subsequent cardiac muscle damage,

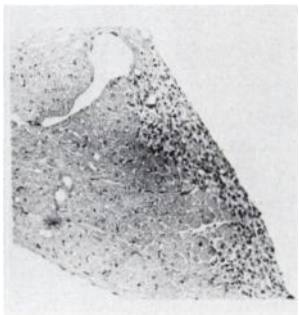

FIG. 2. Photomicrograph of a stained section (H. & E.) of a typical 12-hr-stressed heart, fixed 72 hr following termination of stress. Note focus of damage larger than in Figure 1A. (× 100 before photographic reduction.)

TABLE 1. ORGAN DISTRIBUTION OF Tc-99m SKELETAL AGENTS IN 12-HR-STRESSED EPINEPHRINE-INJECTED AND CONTROL RATS*

	PP_i			MDP		
	100 min					
	No. of animals	Whole heart	Blood	No. of animals	Whole heart	Blood
Controls	19	0.109 ±0.018	0.268 ±0.010	16	0.054 ±0.003	0.113 ±0.008
Stressed	23	0.335 ±0.035	0.286 ±0.014	10	0.287 ±0.044	0.126 ±0.008
Epinephrine-injected	29	3.612 ±0.332	—	20	2.056 ±0.263	—
	300 min					
Controls	4	0.088 ±0.003	0.330 ±0.017	9	0.038 ±0.004	0.068 ±0.003
Stressed	5	0.287 ±0.073	0.273 ±0.019	10	0.248 ±0.048	0.061 ±0.004
Epinephrine-injected	9	3.242 ±0.395	—	14	1.825 ±0.336	—

* % dose/1% body weight; mean values ± 1 s.e.m.

within the first 18 hr. Intermittent foot-shock stress resulted in a continuum of damage, ranging from no gross evidence of injury following 2 hr of stress, to widely scattered small foci of grossly visible injury following 12 hr of stress. Histologically the differences appeared to be quantitative rather than qualitative (Figs. 1 and 2). No thrombosed vessels were observed.

Radiopharmaceutical distribution. The concentration of both MDP and PP_i at 100 and 300 min is significantly greater in the damaged myocardium of both stressed and epinephrine-injected rats than in that of control rats (Table 1). At 300 min, the ratios for injured-to-normal myocardium, and for injured myocardium-to-blood, were greater with MDP than with PP_i; this presumably results from the former's lower blood and control heart levels (Table 2). The whole-heart concentration of MDP even after coronary perfusion in 2-hr-stressed rats is significantly increased at 100 min compared with control levels (Table 3). The whole-heart concentration for epinephrine-injected rats is consistently an order of magnitude greater than that of 12-hr-stressed rats, resulting in extremely high injured-to-control ratios (Table 4).

Coronary-artery perfusion before radioassay decreased the mean radiopharmaceutical concentration of control hearts by an amount equivalent to the activity in 0.12 ml of blood. The change in activity due to perfusion was not statistically detectable in stress-injured hearts.

When injury-to-normal ratios were calculated for epinephrine-injured hearts, by comparing the tracer concentration of a heart's damage with its own "normal" left-ventricular tissue, the ratios were much lower than when infarcted tissue was compared with tissue from undamaged control hearts. In the latter case, ratios for catecholamine-induced damage to control myocardium reached 600 (Table 4). This ratio is far higher than any previously reported.

TABLE 2. EXPERIMENTAL-TO-CONTROL RATIOS FOR TRACER CONCENTRATIONS IN WHOLE, UNPERFUSED HEARTS OF 12-HR-STRESSED AND EPINEPHRINE-INJECTED RATS

	100 min	300 min
PP_i: Stressed/control	3.07	3.26
MDP: Stressed/control	5.31	6.52
PP_i: Epinephrine-injected/control	33.14	36.84
MDP: Epinephrine-injected/control	38.07	48.03

TABLE 3. PERFUSED WHOLE-HEART CONCENTRATION OF MDP: 2-HR-STRESSED VS. CONTROL RATS*

No. of animals	Stressed	No. of animals	Control	Stressed/control ratio
12	0.122 ±0.011	18	0.034 ±0.002	3.58

* % dose/1% body weight.

TABLE 4. CONCENTRATION IN DAMAGED MYOCARDIUM OF Tc-99m MDP IN THE EPINEPHRINE-INJECTED RAT

Rat No.	Damaged concentration*	Damaged/ "normal" ratio	Damaged/ undamaged control ratio
1	31.36	80.41	627.00
2	5.60	23.0	116
3	3.45	15.0	71

* % dose/1% body weight.

DISCUSSION

Random foot-shock stress produces myocardial injury in conscious rats independent of exercise or direct electrical effects. Identical foot shock in anesthetized rats produces no gross myocardial injury and no increased MDP accumulation (*23*). In addition, curarized but conscious rats identically stimulated do develop myocardial lesions with increased MDP accumulation (*23*).

The myocardial damage observed following random foot shock is histologically similar to that of human ischemic lesions. As expected, serum-enzyme elevations similar to the human post-infarct pattern are observed (*23*).

The biodistributions of MDP and PP_i were similar to those previously reported for rabbits (*8*). The blood clearance of MDP was more rapid than that of PP_i in both species, and the clearance rates in the rat were more rapid than in the rabbit, as expected. This faster blood clearance of MDP, and the resulting higher injury-to-normal ratios for it than for PP_i, suggest that MDP should be superior for clinical infarct imaging and for experimental evaluation of extremely low levels of myocardial damage.

Perfusion of damaged hearts with Ringer bicarbonate solution before radioassay effectively excludes the possibility of generalized hyperemia (increased blood volume per gram of tissue) as the cause of the increased radiopharmaceutical concentration.

The 2-hr-stress technique has two features that make it distinctly different from other experimental models of myocardial necrosis. First, the procedure produces small foci of isolated necrosis scattered throughout the heart, with no large confluent infarct. Second, the severity of myocardial damage by the random foot-shock technique can be controlled, precisely regulated, or "titrated" to very low levels. The procedure may be interrupted for the administration of myocardial protective agents. On the other hand, most mechanical, thermal, or arterial-ligation methods depend upon a single insult and produce a more variable degree of myocardial injury.

If the microfoci of injury, as produced by 2-hr of random foot-shock stress, accumulate Tc-99m MDP on a per-gram basis to the same degree as do the confluent lesions produced by subcutaneous catecholamine injection, then the threefold whole-heart uptake (compared with controls) found in the 2-hr-stressed animals could result from the scattered necrosis. Such myocardial injury in humans would very likely result in a diffusely positive scan (Fig. 3).

The injury-to-normal ratios for epinephrine-injured hearts approximate ratios reported previously (*4,9,22*) when the "normal" tissue is obtained from grossly unremarkable areas of epinephrine-injured hearts. When the normal tissue is obtained from undamaged control hearts, however, the ratios are greater (Table 4), because the grossly "normal" tissue of epinephrine-injured hearts is actually damaged to some extent. The extremely high ratios result from the high tracer concentration within the damaged muscle, since the activity levels of undamaged control hearts are not unusually low (*8*). Several mechanisms have been proposed to explain the infarct production by subcutaneous catecholamine injection, and one of these—increased myocardial work with resultant hypoxia—would be consistent with the extremely high infarct tracer concentration observed. Since no permanent vascular occlusion is postulated in this mechanism, tracer delivery to the damaged areas would be unimpeded.

Various vaso-occlusive models, including those using coronary ligation, produce ischemic necrosis by a mechanism that also inhibits tracer delivery to the damaged tissue. Isolated patches of necrosis surrounded by normal myocardium, as observed histologically in 2-hr-stressed animals, would receive an unimpeded tracer supply, since no vascular occlusion is involved in the lesion. We suggest that these isolated, scattered microfoci of damage might well accumulate tracer as does catecholamine-induced necrosis, rather than as vaso-occlusive lesions. This type of scattered intense activity, then, could produce diffusely positive gamma-camera images (Fig. 3).

FIG. 3. Typical anterior, simultaneous, side-by-side excised, perfused, 2-hr-stressed (A) and control (B) hearts, 100 min following intravenous injection of 1 mCi Tc-99m stannous MDP into each animal. Note much higher activity in the stressed heart, which is grossly unremarkable.

CONCLUSION

Both subcutaneous catecholamine injection and random foot-shock stress provide simple, reliable models for the study of radiopharmaceutical accumulation in the injured myocardium of rats. The increased tracer concentration in hearts with tiny scattered foci of injury suggests a possible explanation for the diffusely positive myocardial images occasionally observed clinically.

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BOOKS RECEIVED

The receipt of the following books is acknowledged:

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