Subcutaneous Isoproterenol: A Convenient Rat Model for Early Detection of Myocardial Necrosis

Hank F. Kung and Monte Blau

State University of New York at Buffalo, New York

The uptake of Tc-99m pyrophosphate was studied in rat myocardial lesions produced by a single subcutaneous injection of isoproterenol (10–50 mg/kg of body weight). The uptake in the whole heart of treated rats is directly proportional to the isoproterenol dose. The Tc-99m PPi uptake measured at various times after lesion initiation parallels the myocardial calcium concentration changes. This model is useful for screening radiopharmaceuticals, and may also be suitable for studying early uptake in myocardial infarcts.

J Nucl Med 19: 948–951, 1978

Many radiopharmaceuticals have been shown to localize in human myocardial infarcts (*1*); among them Tc-99m pyrophosphate (PPi) has achieved widespread acceptance (*1–3*). However, in the majority of patients with acute myocardial infarcts, there is little uptake for the first 24 hr. This increases and is maximum at 48–72 hr after the onset of symptoms. It would be useful to develop radiopharmaceuticals that can demonstrate myocardial infarcts at an early stage.

There are several animal models that have been used for studies of myocardial infarct localization: coronary-artery ligation in rats (*4*) and dogs (*1*), local heat-induced necrosis in rats (*5,6*), and vasopressin-induced local necrosis in rabbits (*7*). However, these models are impractical for screening large numbers of agents or for studying myocardial infarct localization at different time intervals.

Isoproterenol-induced myocardial necrosis in animals (either by subcutaneous or intraperitoneal injection) was first reported by Rona (*8,9*). The biochemical and histologic changes occurring after administration of this agent in rats have been well documented (*10–17*). The pharmacologic effect of isoproterenol is believed to be associated with its β-adrenergic effect, which increases heart rate, decreases blood pressure, and diminishes the oxygen supply to the myocardium. As early as 6 min after intraperitoneal isoproterenol injection, histologic changes occur, including myofilament fragmentation, contraction-band formation, and hyalinization associated with dilation of the sarcoplasmic reticulum (*15*). Focal necrosis, extensive inflammation, and infiltration by polymorphonuclear leucocytes were found in the damaged heart at 24 hr after the injection. These changes resemble those observed in human myocardial infarction. Comparable biochemical alterations such as shifting electrolytes, serum enzyme elevation, etc., have also been reported (*10,12,13,16*). All of these studies suggest that this experimental lesion is comparable to the infarct found in humans. We have investigated the cardiac uptake of Tc-99m PPi at various isoproterenol dose levels and at different time intervals after initiation of the lesion.

MATERIALS AND METHODS

Isoproterenol hydrochloride (dissolved in 0.2–0.5 ml of saline) was injected subcutaneously into

Received Nov. 15, 1977; revision accepted Jan. 30, 1978.

For reprints contact: Hank Kung, Dept. of Nuclear Medicine, Veterans' Administration Hospital, 3495 Bailey Ave., Buffalo, NY 14215.

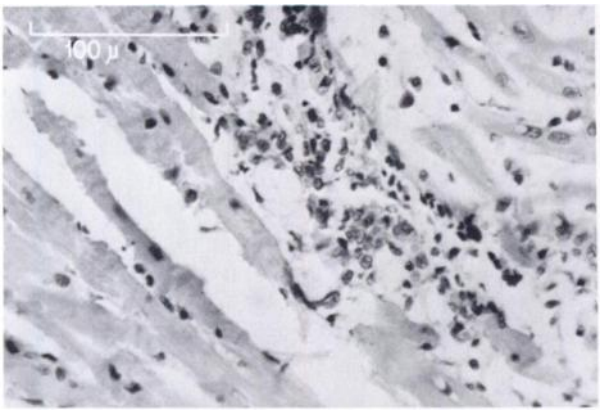

FIG. 1. Photomicrograph of section of damaged heart 24 hr after single subcutaneous injection of 50 mg/kg of isoproterenol. A large necrotic area near center shows various changes described in text.

Sprague-Dawley rats (220–280 g) at a dose of 10, 20, 30 or 50 mg/kg of body weight. After various time intervals, Tc-99m PPi was injected intravenously, and 1 hr after the tracer injection the rats were killed. Control rats received the i.v. Tc-99m PPi injection only. The whole heart was removed, squeezed, and blotted. Other organs of interest (blood, liver, and femur) were also excised and counted in a well counter. The % dose per organ was calculated by comparing the radioactivity in the organ with a standard prepared from the initial dose. DH/N heart ratios were calculated by comparing the % dose in the whole damaged heart to that in the normal heart.

The PPi kit was prepared by lyophilyzing a solution containing 10 mg of tetrasodium pyrophosphate, 100 μg of stannous chloride, and 2 ml of saline. Tc-99m PPi was prepared by adding the desired amount of [^{99m}Tc] pertechnetate to a PPi kit. In each injected dose, there are 10–100 μCi of Tc-99m PPi and 400–1000 μg of PPi. Little difference in distribution was found in repeated experiments using as little as 50 μg of PPi in each dose. More than 95% labeling efficiency was found by ascending paper chromatography using 85% methanol as the solvent.

Twenty-four hours after isoproterenol injection (50 mg/kg of body weight), hearts were removed, fixed, and stained with hematoxylin and eosin. Sections of control hearts were prepared similarly.

RESULTS AND DISCUSSION

Myocardial lesions were produced in rats by a single subcutaneous injection of isoproterenol (50 mg/kg of body weight). The damage produced after 24 hr was confirmed by microscopic study (Fig. 1). The same pattern of histologic changes reported in the literature (*8,9,15–17*) was observed.

To provide a standard for normal cardiac uptake, we determined the average whole-heart uptake of Tc-99m PPi in 20 untreated rats: % dose/organ ± S.E.M. = 0.045 ± 0.005. The damaged heart-to-normal heart (DH/N) ratios for rats treated with different isoproterenol doses were then calculated (Table 1). The increase in Tc-99m PPi uptake is directly proportional to the isoproterenol dose (Fig. 2). When the highest dose (50 mg/kg) was used, a dramatic increase in liver uptake (~20% of injected dose was noted, indicating a drastic change in liver metabolism. The weight of the heart in the high-dose rats was also increased (from ~0.7 g to ~1.1 g). Therefore, a lower dose, 30 mg/kg of body weight, was chosen for studies of the time course of myocardial lesion development.

The changes in DH/N ratio during the development of the lesion were studied by varying the waiting period after isoproterenol injection (from 0 to 24 hr) while keeping constant the time between Tc-99m PPi injection and excision (1 hr). The car-

TABLE 1. UPTAKE OF Tc-99m PPi IN ISOPROTERENOL-TREATED RATS*

Isoproterenol dose (mg/kg)	0	10	20	30	50
n	20	4	5	6	5
% dose/organ ± s.e.m.					
Heart	0.045 ± 0.005	0.133 ± 0.002	0.230 ± 0.014	0.316 ± 0.008	0.438 ± 0.017
Blood	2.91 ± 0.04	3.20 ± 0.13	3.51 ± 0.09	3.49 ± 0.05	2.53 ± 0.14
Femur	1.52 ± 0.01	1.77 ± 0.03	1.50 ± 0.03	1.72 ± 0.03	1.43 ± 0.02
Liver	4.41 ± 0.16	3.92 ± 0.10	4.46 ± 0.14	5.66 ± 0.39	20.14 ± 0.37
DH/N heart	1.00	2.94 ± 0.06	5.07 ± 0.32	6.96 ± 0.18	9.74 ± 0.37
% dose/gram ± s.e.m.					
Heart	0.054 ± 0.001	0.135 ± 0.012	0.227 ± 0.078	0.340 ± 0.08	0.380 ± 0.013

* Rats were injected subcutaneously with a single dose of isoproterenol; 24 hours later Tc-99m PPi was injected intravenously. Rats were killed 1 hour later.

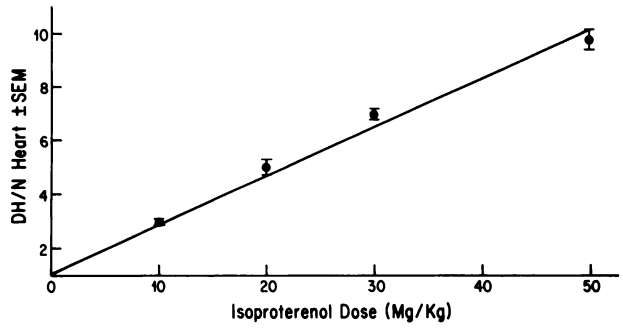

FIG. 2. Effect of isoproterenol dose on DH/N ratio. Rats were injected subcutaneously with isoproterenol (10–50 mg/kg). After 24 hr, they were injected intravenously with Tc-99m PPi and killed 1 hr later. DH/N ratios were calculated by comparing % dose in whole damaged heart with that in normal heart. Average values for 4–6 rats.

diac uptake of Tc-99m PPi is maximum at 6 hr after the isoproterenol injection (Fig. 3). Currently we are studying the significance of the small but early peak at 1 hr after isoproterenol injection. It will be compared with the uptake curve for Tc-99m glucoheptonate, which is known to concentrate in early infarcts (*18*). This simple time-course study could be used to evaluate various radiopharmaceuticals, especially for their uptake in early myocardial lesion.

Bloom and Davis (*12*) have also studied the variation in myocardial calcium concentration with time in the isoproterenol-induced myocardial injury using Sprague-Dawley rats. They found that the calcium concentration is maximum at 4–8 hr after a 10-mg/kg isoproterenol injection. Apparently there is a parallel relationship between the change in myocardial calcium concentration and Tc-99m PPi uptake in the damaged hearts. The significance of this observation with respect to early infarct is being explored.

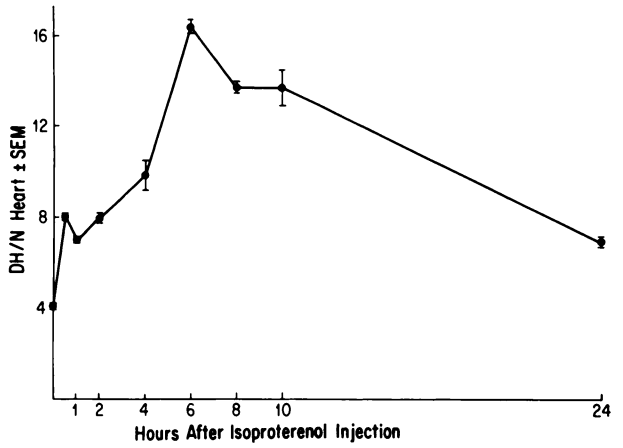

FIG. 3. DH/N ratio at different times after myocardial lesion initiation. Rats were injected subcutaneously with isoproterenol (30 mg/kg). After different time intervals, they were injected intravenously with Tc-99m PPi and killed 1 hr later. DH/N ratios were calculated by comparing % dose in whole damaged heart with that in normal heart. Average values for four to ten rats.

Recently, Miller et al. reported a study of the heart uptake of Tc-99m PPi and methylene diphosphonate after myocardial injury produced by subcutaneous epinephrine injection, and also after random foot-shock stress (*19*). These models also seem to be preferable to those in common use for radiopharmaceutical development work, but they are not as convenient as the isoproterenol model described here.

In summary, a simple myocardial lesion model produced by a single subcutaneous injection of isoproterenol in rats was chosen to study Tc-99m PPi uptake. The uptake is dependent on dose and time. This model is useful not only in screening large numbers of radiopharmaceuticals, but is also suitable for the evaluation of early myocardial infarct localization.

REFERENCES

1. PARKEY RW, BONTE FJ, BUJA M, et al: Myocardial infarct imaging with Tc-99m phosphates. *Seminar in Nucl Med* 7: 15–28, 1977

2. PRASQUIER R, TARADASH MR, BOTVINICK EH, et al: The specificity of the diffuse pattern of cardiac uptake in myocardial infarction imaging with technetium-99m stannous pyrophosphate. *Circulation* 55: 61–66, 1977

3. LOWENTHAL IS, PARISI AF, TOW DE, et al: Diagnosis of acute myocardial infarct in patients undergoing open heart surgery: A Comparison of serial myocardial imaging with cardiac enzymes, electrocardiography and vectocardiographly. *J Nucl Med* 18: 770–775, 1977

4. LESSEM J, POLIMENI P, PAGE E, et al: Tc-99m pyrophosphate image of rat ventricular infarct: correlation of time course with microscopic pathology. *Am J Card* 39: 279, 1977

5. ADLER N, CAMIN LL, SHULKIN P: Rat model for acute myocardial infarction: application to technetium-labeled glucoheptonate, tetracycline and polyphosphate. *J Nucl Med* 17: 203–207, 1976

6. DAVIS MA, HOLMAN BL, CARMEL AN: Evaluation of radiopharmaceuticals sequestered by acutely damaged myocardium. *J Nucl Med* 17: 911–917, 1976

7. GROSSMAN ZD, FOSTER AB, MCAFEE JG, et al: Myocardial uptake (rabbit) of six ^{99m}Tc-tagged pharmaceuticals and ^{85}Sr after vasopressin-induced necrosis. *J Nucl Med* 18: 51–56, 1977

8. RONA G, CHAPPEL CI, BALAZS T, et al: An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. *Arch Path* 67: 443–455, 1959

9. KAHN DS, RONA G, CHAPPEL CI: Isoproterenol-induced cardiac necrosis. *Ann NY Acad Sci* 156: 285–293, 1969

10. LEHR D: Tissue electrolyte alteration in disseminated myocardial necrosis. *Ann NY Acad Sci* 156: 344–378, 1969

11. ZBINDEN G, MOE RA: Pharmacological studies on heart muscle lesions induced by isoproterenol. *Ann NY Acad Sci* 156: 294–308, 1969

12. BLOOM S, DAVIS DL: Calcium as mediator of isoproterenol-induced myocardial necrosis. *Am J Path* 69: 459–470, 1972

13. YAROM R, BEN-ISHAY D, ZINDER O: Myocardial cationic shifts induced by isoproterenol: Electron microscopic and electron probe studies. *J Mol Cell Card* 4: 559–570, 1972

14. HANDFORTH CP: Isoproterenol-induced myocardial infarction in animals. *Arch Path* 73: 161–165, 1962

15. CSAPÓ Z, DUSEK J, RONA G: Early alterations of the cardiac muscle cells in isoproterenol-induced necrosis. *Arch Path* 93: 356–365, 1972

16. WEXLER BC, KITTINGER GW: Myocardial necrosis in rats: Serum enzymes, adrenal steroid and histopathological alterations. *Circulation Res* 13: 159–171, 1963

17. FERRANS VJ, HIBBS RG, WALSH JJ, et al: Histochemical and electron microscopical studies on the cardiac necrosis produced by sympathomimetic agents. *Ann NY Acad Sci* 156: 309–332, 1969

18. JACOBSTEIN AG, ALONSO DR, ROBERTS AJ, et al: Early diagnosis of myocardial infarction in the dog with ^{99m}Tc-glucoheptonate. *J Nucl Med* 18: 413–418, 1977

19. MILLER DG, GILMOUR RF, GROSSMAN ZD, et al: Myocardial uptake of Tc-99m skeletal agents in the rat after experimental induction of microscopic foci of injury. *J Nucl Med* 18: 1005–1009, 1977