DISTRIBUTIONS OF SEVERAL AGENTS USEFUL IN IMAGING MYOCARDIAL INFARCTS

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Myocardial cell death due to infarction is accompanied by an influx of calcium ion. The calcium ion seems to localize in crystalline structures that form within mitochondria and resemble hydroxyapatite. Based on this phenomenon ^{99m}Tc-stannous pyrophosphate has been successfully used to image myocardial infarcts within 24 hr of infarction and within 1 hr following tracer administration both in dogs and patient volunteers. In this report, canine distribution studies of ^{99m}Tc-pyrophosphate are compared with similar studies with ^{99m}Tc-stannous polyphosphate, ^{99m}Tc-stannous 1, hydroxy-ethylidene-1, 1-disodium phosphonate diphosphonate), and ^{18}F as sodium fluoride. Pyrophosphate, polyphosphate, and diphosphonate are each potentially useful in myocardial infarct imaging but bone uptake of ^{18}F occurs sufficiently early to prevent the use of this radionuclide in infarct scintigraphy.

In 1964 D'Agostino (*1*) described the localization of calcium within the mitochondria of rat myocardial cells in steroid-induced focal necrosis. Turning to human ischemic myocardial infarcts, D'Agostino and Chiga (*2*) later observed that mitochondrial calcium seemed to be incorporated within a crystalline structure that they believed to be hydroxyapatite. Further, Shen and Jennings (*3,4*) have described calcium accumulation of this sort as an index of irreversible myocardial cell damage resulting from ischemia. These observations suggested to us a means of identifying myocardial infarcts by radionuclide imaging in which we might employ ^{99m}Tc-stannous pyrophosphate or related compounds as apatite-labeling tracers (*5*).

MATERIALS AND METHODS

The subjects of the following experiments were mongrel dogs weighing from 15 to 30 kg. All experiments were conducted under intravenous nembutal anesthesia. A Searle Radiographics HP scintillation camera with 16,000-hole "resolution" collimator was used for imaging.

Each of a group of four dogs was given 3.0 mCi ^{99m}Tc-stannous pyrophosphate containing 5.0 mg pyrophosphate. Control images of 200,000 counts each were made of the thorax in anterior, left anterior-oblique, and left lateral projections at intervals during a period of 1–2 hr after injection. On the following day, we catheterized the left coronary arterial system of each animal and created 0.1 ml metallic Hg emboli in branches of either the left anterior descending or circumflex arteries. Roentgenograms were made to show the distribution of the emboli. Each animal was returned to the laboratory 24 hr after embolization and once again was given a tracer dose of 3.0 mCi ^{99m}Tc-pyrophosphate. Imaging was begun immediately and carried out as already described. Two of the animals were returned for followup imaging studies 2, 4, 8, 12, and 16 days after embolization. On each occasion, the subject animal received a tracer dose of ^{99m}Tc-pyrophosphate and the usual imaging regimen was followed.

Technetium-99m-pyrophosphate distribution studies were carried out employing 17 dogs each of which had a control image and received on the following day a Hg coronary embolus to create a myocardial infarct. Thereafter three subjects were sacrificed from 1 to 2 hr after infarction, three at 5–8 hr, and three at 12–16 hr. Five subjects were sacrificed at 24 hr after infarction, and one each at 84 hr, and 12 and 38 weeks. Each subject received 3.0 mCi ^{99m}Tc-pyrophosphate 1 hr prior to sacrifice.

Tissue samples were obtained from several areas within the normal and infarcted myocardium of each

Received July 15, 1974; revision accepted Sept. 19, 1974.

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subject. Samples of blood, liver, and lung were also obtained, as well as bone samples from those sacrificed in the period from 12 to 16 hr and at 24 hr. All samples were assayed for radioactivity content and distribution results were expressed in terms of percent of the injected dose per gram of tissue ($\times 10^{-3}$).

Since pyrophosphate is known to chelate calcium ion and since arrhythmias have been observed in hypocalcemia, electrocardiograms were obtained in the control state and before, during, and after the injection of ^{99m}Tc-pyrophosphate, both before and following myocardial infarction.

A distribution study was then undertaken with respect to three additional agents that are known to label hydroxyapatite: ^{99m}Tc-stannous polyphosphate; ^{99m}Tc stannous 1, hydroxy-ethylidene-1, 1-disodium phosphonate; and ^{18}F as sodium fluoride. The numbers of dogs employed for each agent were as follows: ^{99m}Tc-polyphosphate, three dogs; ^{99m}Tc-diphosphonate, seven; Na^{18}F, two. Control imaging studies were performed in the usual manner, and Hg emboli were deposited. Twenty-four hours after infarction, each animal was given 3.0 mCi of the appropriate agent and the usual scintillation camera images were obtained at intervals up to 1 hr after injection. At this time, each animal was sacrificed and tissue samples of normal and infarcted myocardium, blood, liver, lung, and bone were obtained. The samples were assayed for radioactivity in terms of percent of injected dose per gram of tissue ($\times 10^{-3}$). The means of all tissue values in the distribution were then normalized to the values for uninfarcted myocardium, which were set at 1.0.

RESULTS

Results of the preliminary imaging studies are illustrated by Fig. 1. Figure 1A shows a left lateral control image of the thorax made 1 hr after administration of 3.0 mCi ^{99m}Tc-pyrophosphate with the expected visualization of skeletal structures. Figure 1B is a lateral chest roentgenogram made to record the distribution of the Hg embolus. In general, infarcts are coextant with radiographically demonstrable Hg deposition. Figure 1C is a lateral thoracic scintigram made 24 hr after infarction and 1 hr after the administration of another tracer dose of ^{99m}Tc-pyrophosphate. Note that, in addition to the expected skeletal visualization, there is clearly demonstrable localization of radioactivity in the general area of the Hg embolus. Subsequently, images were made almost daily up to 2 weeks after infarction. Localization remained intense for a period of 4–6 days and then subsided until at 2 weeks (Fig. 1D) there was virtually no localization of tracer within the infarcted area.

The distribution of ^{99m}Tc-pyrophosphate is summarized in Table 1. Note that at from 1 to 2 hr after infarction, blood level of tracer is relatively high and there is little distinction in tracer content between infarct and normal myocardium. Although a relatively significant difference seems to be appearing by 5–8 hr, the infarct cannot be resolved on scintigrams. Considerable differential between infarct and normal myocardium is observed at 12–16 hr (9.7/1.0), and infarct localizations are sometimes visible on scintigrams made at this time. Imaging of the infarct can always be performed at 20–24 hr and favorable imaging concentrations persist for several days thereafter. Distribution studies confirm disappearance of the infarct localization phenomenon with time and with healing of the infarct.

The electrocardiographic study showed no evidence of the production of arrhythmias by the doses of pyrophosphate employed and none would be expected since there is a safety factor of at least 300 with respect to this possible effect according to the work of Stevenson and Dunson (*6*). It is especially noteworthy that arrhythmias that had been produced by infarction were not accentuated by the minimal calcium ion chelation produced by the tracer amount of pyrophosphate employed.

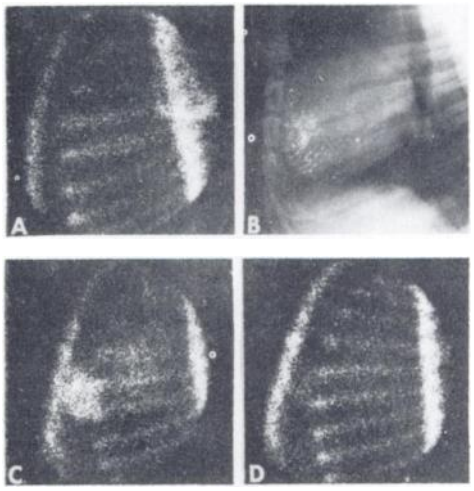

FIG. 1. (A) Lateral scintigram of chest of 20-kg dog 1 hr after administration of 3.0 mCi ^{99m}Tc-pyrophosphate. Note expected deposition of tracer in sternum, spine, and ribs. There is no localization in region of heart. (B) Lateral chest roentgenogram of dog in Fig. 1A. An embolus (0.1 ml Hg) has been introduced into branches of left anterior descending coronary artery. Infarction occurs in pattern of mercury distribution. (C) Lateral chest scintigram of same dog as in Fig. 1A, 24 hr after Hg embolization and 1 hr after administration of 3.0 mCi ^{99m}Tc-pyrophosphate. Note localization of tracer in pattern corresponding to that of visible mercury embolus, and identifying myocardial infarct. (D) Lateral chest scintigram of same dog, made 2 weeks after infarction and 1 hr after administration of 3.0 mCi ^{99m}Tc-pyrophosphate. Note virtual disappearance of localization in region of healing infarct and return of appearance to that seen in control scintigram in Fig. 1A. (From Bonte, et al (5). Reprinted by permission of *Radiology*.)

TABLE 1. DISTRIBUTION OF ^{99m}Tc-PYROPHOSPHATE [% INJECTED DOSE/GM ($\times 10^{-3}$)]

Time postinfarction	Subjects (No.)	Myocardium		Blood	Liver	Lung	Bone
		Normal	Infarct				
1–2 hr	3	2.8	3.2	5.7	3.2	4.9	—
5–8 hr	3	0.9	6.5	2.6	1.6	2.2	—
12–16 hr	3	1.0	9.7	3.4	2.1	2.5	25.0
24 hr	5	1.1	12.5	2.5	2.0	2.2	19.5
84 hr	1	1.7	16.4	8.4	2.4	5.4	—
12 weeks	1	1.5	1.7	3.1	1.1	2.1	—
38 weeks	1	1.4	1.6	4.6	1.8	3.8	—

When ^{99m}Tc-pyrophosphate was compared with other hydroxyapatite-labeling tracers such as ^{99m}Tc-polyphosphate, ^{99m}Tc-diphosphonate, and ^{18}F at an optimum imaging time, i.e., 24 hr after infarction and 1 hr after administration of tracer radiopharmaceutical, the results seen in Table 2 were obtained. Note that pyrophosphate provides the best ratio of infarct to normal myocardium but shows a relatively high bone content. The most favorable bone content is exhibited by polyphosphate which also shows a favorable infarct/normal myocardium ratio. Although diphosphonate does not have as high an infarct/normal myocardium ratio as do the other agents, it has an intermediate bone value favorable for imaging. Figures 2A, B, and C show that diagnostic images can be obtained with each of these three agents. It is interesting to note that ^{18}F gives a favorable infarct/normal myocardium ratio (7.9/1.0), but the bone content of this agent is so high as to preclude successful scintigraphy with an instrument of the camera type. Even so, there is a faint suggestion of localization of tracer at the infarct site in Fig. 2D.

TABLE 2. RATIOS OF TISSUE TRACER CONCENTRATION (% INJECTED DOSE/GM TISSUE) TO CONCENTRATION IN NORMAL MYOCARDIUM*

Tissue	Pyrophosphate†	Polyphosphate‡	Diphosphonate‖	^{18}F
Myocardium, normal	1.0	1.0	1.0	1.0
Myocardium, infarct	10.9	10.0	7.1	7.9
Blood	2.0	3.0	3.7	4.4
Liver	1.7	2.1	1.4	3.1
Lung	2.0	2.3	2.7	5.7
Bone	15.5	5.3	8.8	132.0

* Twenty-four hours after infarction, 1 hr after tracer administration.
† ^{99m}Tc-stannous pyrophosphate.
‡ ^{99m}Tc-stannous polyphosphate.
‖ ^{99m}Tc-stannous diphosphonate.

DISCUSSION

For many years, cardiologists have been intrigued by the phenomenon of rapid influx of calcium ion into myocardial cells undergoing ischemic infarction. D'Agostino (*1*) observed electron-dense crystals forming within the mitochondria of these cells as the probable focus of attraction for calcium ion. He identified the crystals as hydroxyapatite.

Later Shen and Jennings (*3,4*) approached this problem with $^{45}CaCl_2$ tracer studies, giving the agent at selected intervals before and after occlusion of major branches of the left coronary arteries in dogs. They made the interesting observation that, following permanent occlusion of a coronary artery, there was no significant uptake of ^{45}Ca in the infarcted tissue. However, after 40 min of ischemia followed by 10 min of arterial flow, there was a highly significant uptake of ^{45}Ca. Shen and Jennings further found that myocardium, which has been reversibly injured by 10 min of ischemia followed by 20 min of unobstructed arterial flow, did not accumulate significant levels of ^{45}Ca. Shen and Jennings drew two conclusions: (A) calcium uptake is a feature of irreversible cellular injury that occurs only when arterial blood flow is present, and (B) calcium uptake behaves as if it were an active process associated with mitochondrial accumulation of calcium into what they believe to be intramitochondrial granules of calcium phosphate. However, whether the structures formed within the mitochondria represent hydroxyapatite or some other molecule containing calcium and phosphate, they are very likely the site of localization not only of calcium ion but of the bone-imaging tracers used in the present study. Our laboratory is carrying out further studies on this point.

Although selective tracer localization is demonstrable in damaged myocardium within 5 hr of infarction (Table 1), images of diagnostic quality are not reliably obtainable until from 16 to 24 hr have

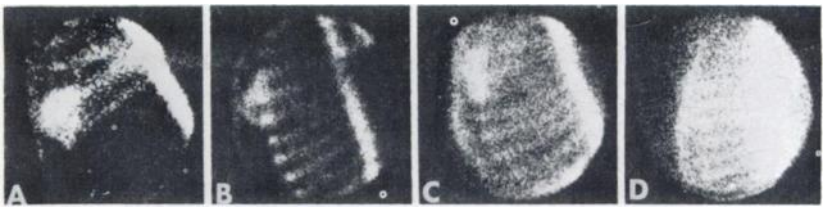

FIG. 2. Lateral chest scintigrams of four 15–30-kg dogs made 24 hr after Hg embolization of branches of left anterior descending coronary artery, and 1 hr following administration of 3.0 mCi of indicated tracer: ^{99m}Tc-pyrophosphate (A), ^{99m}Tc-polyphosphate (B), ^{99m}Tc-diphosphonate (C), or Na^{18}F (D). Comparable results are obtained with Tc-phosphate products but localization is not seen with ^{18}F.

elapsed. This observation is also borne out by our experience in a series of volunteer patients thought to have myocardial infarcts and reported elsewhere (7). Deposition of radiopharmaceutical in the infarct reaches and sustains a maximum from about 1 to 6 days after infarction and then slowly disappears, probably as a function of the healing of the infarct, until, in dogs, localization disappears approximately 2 weeks after the acute incident. This property of the test makes it useful in evaluating the satisfactory resolution of an infarct and in the detection of extensions.

Each of the three ^{99m}Tc-labeled agents, pyrophosphate, polyphosphate, and diphosphonate, yield distribution values supporting its usefulness as an imaging agent in human myocardial infarcts. In our animal experience, we have formed the subjective opinion that perhaps our best results have been obtained with ^{99m}Tc-pyrophosphate. Although electrocardiographic studies were carried out only with pyrophosphate, it can be assumed that, if any of these three agents is used at a high specific activity, such that 5.0 mg or less of the pharmaceutical is used, a complete margin of safety with respect to such changes will obtain.

Our preliminary studies indicate that ^{18}F will have no value as a tracer for imaging of myocardial infarcts because of its high bone content soon after administration. Indeed, bone tracer content is the only apparent disadvantage inherent in the ^{99m}Tc-phosphate group of agents, which permit imaging of an infarct within 24 hr of its occurrence and within 1 hr of administration of radiopharmaceutical. This test appears to be of sufficient merit so much so that an intensive search for an agent of the ^{99m}Tc-phosphate family possessing the property of labeling mitochondrial crystals, while being less avid for bone, seems to be strongly indicated.

ACKNOWLEDGMENTS

The authors wish to acknowledge the support of Mallinckrodt Chemical Company (pyrophosphate), Procter & Gamble (diphosphonate), and Union Carbide Company (diphosphonate), who contributed the indicated agents to the study. This work was supported in part by USPHS Grant HL 13625 and in part by a grant from the Southwestern Medical Foundation.

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