Evaluation of Radiopharmaceuticals Sequestered by Acutely Damaged Myocardium

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Eighteen radiopharmaceuticals were screened in a small-animal model as potential infarct-localizing agents. Twelve of the 18 compounds were labeled with $^{99m}$Tc, four with $^{203}$Hg, one with $^{131}$I, and one with either $^{205}$Hg or $^{125}$I. The model used heat-induced myocardial lesions in the rat. The absolute concentration within the lesion and also the activity ratios of injured myocardium to normal heart tissue, blood, and muscle were determined for all compounds. Among the $^{99m}$Tc-labeled agents, two bone-seekers [pyrophosphate (PP) and 1-hydroxyethylidene-1,1-diphosphonate (HEDP)] showed the most promise; these were followed by $^{99m}$Tc-tetracycline analogs and $^{99m}$Tc-glucoheptonate. The tracer showing the most favorable concentration in the lesion and the best target-to-nontarget ratios was an iodinated derivative of hydroxymercurifluorescein labeled with either $^{125}$I or $^{203}$Hg. Consideration of the structure of these compounds suggests that the presence of mercury or of a polycyclic aromatic structure, such as that found in tetracycline and fluorescein, was associated with localization in damaged myocardial tissue. Mercury bound to such an aromatic moiety may produce an additive or even a synergistic effect.


A number of radiopharmaceuticals localize in acutely infarcted myocardium in animals (1—8) and in man (9—11). By externally imaging the focal myocardial uptake, acute infarction can be detected and its extent determined (12). In addition, repeated imaging may be useful in evaluating therapeutic regimens aimed at limiting or diminishing infarct size (13).

All of the currently available agents have biologic or physical limitations that hamper their usefulness. Mercury-labeled fluorescein is difficult to prepare in pure form, and both $^{197}$Hg and $^{202}$Hg have poor physical decay characteristics. Technetium-$^{99m}$-labeled tetracycline clears slowly from the blood and requires delayed imaging (3), whereas $^{99m}$Tc-labeled pyrophosphate accumulates in bone and may localize in ischemic as well as infarcted myocardium (14).

Using heat-induced lesions of the rat myocardium as a model for acute myocardial infarction, we studied a large number of radiolabeled compounds with wide variations in chemical structure in order to determine their biologic distribution and to ascertain those structural properties associated with affinity for damaged myocardium.

MATERIALS AND METHODS

Agent preparation. The following radiopharmaceuticals, obtained in nonradioactive kit form from commercial sources, were labeled with $^{99m}$Tc in accordance with the manufacturers' instructions: diethylenetriamine pentaacetic acid (DTPA, CIS Radiopharmaceuticals, Bedford, Mass.); glucoheptonate (GH, New England Nuclear Corp., North Billerica, Mass.); pyrophosphate (PP, Mallinckrodt/Nuclear, St. Louis, Mo.); 1-hydroxyethylidene-1,1-diphospho-
nate (HEDP, Procter & Gamble, Cincinnati, Ohio); 2,3-dimercapto-succinic acid (DMSA, Medi-Physics, Emeryville, Calif.); 2-mercapto-isobutyric acid (MIBA, Medi-Physics); and dihydrothioctic acid (DHT, 3M Co., St. Paul, Minn.). The technique for preparing the $^{99m}$Tc-labeled tetracycline analogs has been described elsewhere (15). The thioglycerol (TG) and penicillamine (Pen) kits were graciously supplied to us by M. K. Dewanjee of Tufts Medical School, Boston, Mass. Diiodohydroxymercurifluorescein (I$_2$HMF), labeled with $^{203}$Hg, was synthesized substituting 4',5'-diiodofluorescein (Eastman) for mercuric nitrate (HEDP, Procter & Gamble, Cincinnati, Ohio); 2-mercapto-isobutyric acid (MIBA, Medi-Physics) and a butanol—acetic acid—water (4:1:1) solvent system for the other mercuric-containing compounds. Mercury-203-l-bromomercurocuri-2-hydroxypropane (BMHP), $^{203}$Hg-chlormerodrin, $^{203}$Hg-mercuric nitrate, $^{203}$Hg-phenylmercuric acetate, and $^{131}$I-rose bengal were obtained as radio-pharmaceuticals or radiochemicals from CIS, Malinkrodt, New England Nuclear, International Chemical and Nuclear (Burbank, Calif.), and Squibb, respectively. With the exception of the mercuric nitrate, which was diluted 1:375 with isotonic saline before injection, all these agents were administered as supplied by the manufacturer.

Chromatographic analysis. Ascending paper chromatography was performed on Whatman 3MM strips to determine the labeling efficiency and radiochemical purity of each agent. Methanol (85%) was used as the solvent for $^{99m}$Tc-labeled DTPA, GH, DMSA, MIBA, MDP, HEDP, and PP; anhydrous acetone for $^{99m}$Tc-labeled TG, Pen, MIBA, DMSA, DHT, tetracycline, and oxytetracycline; saline for $^{99m}$Tc-MIBA; methanol—ammonium hydroxide (1:1) for $^{203}$Hg-I$_2$HMF; and a butanol—acetic acid—water (4:1:1) solvent system for the other mercury-containing compounds.

Chromatography and Millipore filtration (0.22 $\mu$m) were performed on aliquots of all samples before injection to determine whether free pertechnetate or colloid was present. Since neither was found, it was assumed that the technetium was reduced and bound to the carrier species. In all cases in which the radiopharmaceutical was not prepared from a commercial kit, the carrier species had a purity of at least 85% and generally greater than 95%. The exact structure of the radiopharmaceutical after labeling with $^{99m}$Tc was not known, and no attempt was made to determine it either in vitro or in vivo since the intent of this investigation was to determine the avidity of the labeled tracer for damaged myocardium irrespective of changes in chemical structure subsequent to labeling or administration.

In the case of I$_2$HMF labeled with either $^{203}$Hg or $^{125}$I, elemental analyses of C, H, Hg, and I were within $\pm 0.4\%$ of the theoretical values. Thus, in this particular instance, the chemical structure and stability of these compounds in vitro was known.

**Damaged heart model.** A modification of the technique developed by Adler et al. (17) was used in this study. Albino outbred Wistar or Sprague—Dawley rats of either sex, weighing 150—200 gm (Charles River Breeding Labs), were used. The rats were anesthetized with ether, placed in the supine position, and taped by their hind legs to the dissecting board. An ether cone was placed over the face so that additional anesthesia could be administered when necessary. The chest area was swabbed with alcohol and a longitudinal incision made approximately 1 cm to the left of the midline. The ribs were exposed and an incision made between the seventh and eighth ribs to allow access to the ventricle. The ventricle was touched for 1 sec with a hot soldering iron (Wahl Iso-Tip, regular size). This maneuver was repeated four or five times in adjacent areas to produce a circular pattern. The muscle and skin were then clipped together.

Evaluation of radiopharmaceuticals. Three hours after the lesion was produced, the radiopharmaceutical of interest was injected into the saphenous vein. The injected volume ranged from 0.1 to 0.3 ml. The specific activity of each radiopharmaceutical was calculated from the amount of radioactivity and purity of at least 85% and generally greater than 95%. The exact structure of the radiopharmaceutical after labeling with $^{99m}$Tc was not known, and no attempt was made to determine it either in vitro or in vivo since the intent of this investigation was to determine the avidity of the labeled tracer for damaged myocardium irrespective of changes in chemical structure subsequent to labeling or administration.

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TABLE 2. REPRODUCIBILITY STUDY OF RAT MYOCARDIAL INFARCT MODEL

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of animals</th>
<th>MI-to-normal ratio</th>
<th>% ID/gm MI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean ± s.d.</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>18.7–30.6</td>
<td>24.1 ± 6.4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>25.5–47.2</td>
<td>37.8 ± 11.1</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>22.4–27.3</td>
<td>25.1 ± 2.5</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>23.3–37.4</td>
<td>29.6 ± 7.2</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>18.7–47.2</td>
<td>28.8 ± 8.5</td>
</tr>
</tbody>
</table>

* Percent of injected dose per gram of damaged myocardium.

on surface; (4) distinct lesion (hole) in the heart. This grading was performed visually after dissection of the damaged area from the normal myocardial tissue. Only visibly damaged myocardium of Grade 2 or 3 was assayed for radioactivity. In addition, tissue from the uninvolved posterior portion of the heart was excised for assay. Blood samples were obtained from a vein in the thoracic cavity. Samples of skeletal muscle, abdominal muscle, and small intestine (with contents) were excised from the carcass. The liver, kidneys, and stomach were removed intact. In cases where a bone-scanning agent had been used, the tibia and fibula from one leg were also collected. Care was taken to prevent cross-contamination of samples. All dissecting instruments were cleaned well or replaced between each sample collection. Tissue samples were weighed, and the radioactivity was measured in a NaI(Tl) well scintillation counter.

The count rates per gram of damaged myocardium and normal tissue were obtained without including counts from the tissue adjacent to the damaged myocardium. The percent of the injected dose in both of these areas was determined and the ratio of the percentage activity per gram of damaged myocardium to the percentage activity per gram of normal heart was calculated. Analogous ratios comparing the activity in the infarct with that in the blood and skeletal muscle were also obtained.

In five rats, the damaged myocardium was excised 3–4 hr after induction of the lesion. Normal myocardium was obtained as above. The tissue was fixed in formalin and stained with hematoxylin and eosin. Histopathologic examination was then performed to determine the characteristics of the lesions produced in this model.

The day-to-day variation in the infarct-to-normal concentration ratios was determined in 16 rats. Four rats were studied each day for 4 days using different batches of $^{99m}$Tc-pyrophosphate each day. Damage to the myocardium was induced as described above and $^{99m}$Tc-pyrophosphate was injected 3 hr after induction of the lesion. One hour later, the damaged and normal samples were excised, the radioactivity was determined, and the concentration ratio between infarcted and normal myocardium and the percent injected dose per gram were determined.

RESULTS

Labeling efficiency. Chromatographic analyses showed that all the compounds tested, with the exceptions of thioglycerol and 2-mercaptoisobutyric acid, contained less than 5% free pertechnetate. In the two studies with thioglycerol, the labeling efficiency was 98% and 83%. The additional 15% free pertechnetate in the second study did not appear to have a significant effect on any of the values or ratios obtained. Whereas chromatographic analysis did not identify the structure of the labeled compound, it did indicate the absence of free pertechnetate or colloidal particles.

Histopathologic examination. The severity of the myocardial damage in the five rats was either grade 2 or 3 by gross inspection. Macroscopic and microscopic examination revealed an area of injury in the left ventricle that was sharply demarcated from the surrounding myocardium. In all cases, the tissue was severely congested and contained foci of cellular necrosis. The cellular changes were peracute and characterized by nuclear pyknosis and karyolysis. The injured cells had an eosinophilic granular cytoplasm that contrasted with the basophilia and cross-striation seen in the normal myocardial cells. The lesions were characterized by severe congestion, hemorrhage, and necrosis of individual cardiac cells. These changes appeared to be confined to the epicardial surface and involved approximately the outer third of the myocardial wall. No inflammatory changes were associated with the damaged focus. In a few areas polymorphonuclear cells were found either marginalized within vessels or as a mild focal perivascular infiltrate.

The cellular changes are similar to those observed in cases of myocardial infarction in man occurring
within 12 hr of histologic examination. Tissues taken from grossly normal areas of myocardium and from sham-operated animals not subjected to the heat-induced lesion showed no histologic evidence of injury.

Reproducibility of myocardial infarct model. Over 90% of the rats subjected to the procedure survived. Only Grade 2 and 3 lesions were used for this study. A comparison of these two grades showed no significant differences in uptake for any of the agents tested.

The day-to-day variability in the technique is shown in Table 2. There was no significant difference in the mean concentration ratios between damaged and normal myocardium for each of the 4 days. However, a wide variation in the individual concentration ratios was seen within each of the groups. The range for all 16 rats was 18.7–47.2.

Evaluation of radiopharmaceuticals. The concentrations of several common radiopharmaceuticals in the damaged rat myocardium, compared to those found in normal heart, muscle, and blood, are listed in Table 3. The highest ratios of damaged to normal myocardium in this group were found with the bone-seeking radiopharmaceuticals (99mTc-PP, 99mTc-HEDP, and 99mTc-MDP). The highest percent injected dose per gram in damaged myocardium was found with 99mTc-PP (2.2%). The tetracycline analogs and 99mTc-glucoheptonate had concentration ratios 50–80% of those found with the bone-seeking pharmaceuticals. The concentration ratios for 111In-rose bengal and 99mTc-DTPA were somewhat less than for the tetracycline analogs, as were their infarct-to-blood concentration ratios. Within the range of specific activities examined, there was no indication of a shift in the biologic distribution of any agent.

Table 4 lists those pharmaceuticals investigated containing one or more sulfhydryl groups. The concentration ratios of all of these pharmaceuticals tested (except for 99mTc-dihydrothioctic acid) had damaged-to-normal myocardial concentration ratios greater than 6. None of these ratios was as high as those found with the tetracycline analogs and the bone-seeking radiopharmaceuticals. From an examination of the structures of the compounds in Table 4 (Fig. 1), the major difference between 99mTc-dihydrothioctic acid and the other sulfhydryl-containing compounds is the absence of a functional group (amino, hydroxyl, or carboxyl) on the carbon atom adjacent to the one containing the sulfhydryl moiety.

Table 5 lists the mercury-containing compounds investigated. Radiolabeled diiodohydroxymercurifluorescein showed the highest concentration ratios for damaged to normal myocardium and damaged heart to blood and also the greatest percent injected dose per gram. The percent injected dose was six times as high as that for any of the compounds in the other
two groups. Both $^{203}$Hg-chlormerodrin and $^{203}$Hg-mercuric nitrate had high damaged-to-normal myocardial concentration ratios; in fact, the percent injected dose per gram for mercuric nitrate was higher than that for any other compounds except diiodohydroxymercurifluorescein. The $^{203}$Hg-phenylmercuric acetate and $^{203}$Hg-1-bromomercuri-2-hydroxypropane (BMHP) showed damaged-to-normal myocardial ratios of less than 6. Examination of the structures of these compounds (Fig. 2) shows that, in addition to the presence of mercury, the labeled diiodohydroxymercurifluorescein has a polycyclic aromatic structure similar to that of the tetracycline analogs.

**DISCUSSION**

Technetium-99m-labeled tetracycline, pyrophosphate, and glucoheptonate have been used to detect acute myocardial infarction in man. While $^{99m}$Tc-pyrophosphate is the most satisfactory of the currently available tracers for external detection of acute myocardial infarction, each of these agents has both physical and biologic limitations (14). Technetium-99m-tetracycline requires 24 hr after intravenous administration for effective detection of the infarct, which limits its clinical utility and markedly reduces the photon flux available for imaging (8). Glucoheptonate gives poor target-to-background ratios with a corresponding low detection rate for acute infarcts (18,19).

Imaging with $^{99m}$Tc-pyrophosphate, while effective in detecting acute myocardial infarction soon after intravenous injection (11), is hampered by uptake in bone, which may limit its utility in the estimation of infarct size (19). Furthermore, significant numbers of abnormal scans are obtained with this agent in patients with unstable angina but without other evidence of acute infarction (19,20). This observation has been attributed to nests of necrotic cells in patients with unstable angina (21). Other investigators, however, have found increased uptake of these bone-seeking tracers in reversibly damaged myocardium as well as in infarcted tissue (14,22). These findings would limit the specificity and thereby the utility of $^{99m}$Tc-pyrophosphate in the diagnosis of acute myocardial infarction.

Besides the biologic limitations of these tracers, it appears that they differ in their biologic distribution. For instance, $^{99m}$Tc-tetracycline primarily limits its distribution to irreversibly damaged myocardium, whereas $^{99m}$Tc-pyrophosphate defines the tissue at risk as well (14). The radiomercurifluorescein compounds are hampered by their poor physical decay characteristics, which result in a high patient radiation dose and, in the case of $^{197}$Hg, poor compatibility with current scintillation imaging devices. Thus, it would be advantageous to identify those chemical structures that facilitate localization in acutely infarcted myocardium. This would permit the synthesis of compounds more ideally suited for infarct detection and particularly for serial estimation of infarct size. The latter looms as an important diagnostic requirement with the advent of therapies aimed at limiting and reducing the size of acute infarctions (23).

Currently available canine models of acute myocardial infarction are unsatisfactory for the large-scale screening necessary to determine the relationship between chemical structure and biologic ac-

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**TABLE 5. CONCENTRATION OF MERCURY-CONTAINING RADIOPHARMACEUTICALS IN DAMAGED RAT MYOCARDIUM**

<table>
<thead>
<tr>
<th>Agent</th>
<th>% ID/gm M</th>
<th>MI-to-normal ratio</th>
<th>MI-to-muscle ratio</th>
<th>MI-to-blood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{197}$diiodomercurihydroxyfluorescein</td>
<td>12.4 ± 3.4</td>
<td>31.7 ± 9.7</td>
<td>133.1 ± 43.4</td>
<td>22.9 ± 7.6</td>
</tr>
<tr>
<td>$^{203}$Hg-diiodomercurihydroxyfluorescein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{203}$Hg-chlormerodrin</td>
<td>1.5 ± 0.5</td>
<td>26.3 ± 6.6</td>
<td>55.7 ± 26.0</td>
<td>8.0 ± 1.7</td>
</tr>
<tr>
<td>$^{203}$Hg-mercuric nitrate</td>
<td>7.1 ± 3.4</td>
<td>15.5 ± 4.6</td>
<td>87.1 ± 33.5</td>
<td>5.8 ± 2.0</td>
</tr>
<tr>
<td>$^{203}$Hg-phenylmercuric acetate</td>
<td>3.7 ± 1.8</td>
<td>5.4 ± 2.3</td>
<td>19.2 ± 10.8</td>
<td>3.2 ± 3.2</td>
</tr>
<tr>
<td>$^{203}$Hg-bromomercuri-2-hydroxypropane</td>
<td>1.2 ± 0.2</td>
<td>2.5 ± 0.5</td>
<td>17.8 ± 3.8</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>
mal. This is in excellent agreement with the value of 22–41% reported by Adler et al. (17) using $^{201}$TI or $^{99m}$Tc-macroaggregated albumin.

With our model, it has been possible to show that a large number of radiopharmaceuticals have the property of being sequestered by acutely damaged myocardium. Of the commonly available radiopharmaceuticals, the bone agents, including $^{99m}$Tc-pyrophosphate, $^{99m}$Tc-diphosphonate, and $^{99m}$Tc-methylene diphosphonate, have the highest infarct-to-normal and infarct-to-blood concentration ratios. Of these, the percent injected dose per gram of damaged tissue is highest with $^{99m}$Tc-pyrophosphate; $^{99m}$Tc-glucoheptonate and $^{99m}$Tc-tetracycline have concentration ratios approximately one-half to two-thirds that of the bone-seeking tracers. The presence of a sulfhydryl group does not appear to be a major factor associated with specificity. Nor is the presence of a carboxyl group essential to the binding of the tracer in damaged muscle, as shown by the affinity of most of the compounds tested in this group. It does raise the question whether technetium binds between a hydroxyl or carboxyl and a sulfhydryl group or whether technetium forms a bridge between two sulfhydryl groups on two molecules (R—S—Tc—S—R).

In addition, the presence of mercury in the radiocompounds correlates with infarct concentration. Tissue binding, in the case of sulfhydryl groups and that of mercury, maybe associated with the release of lysosomal enzymes since lysosomes are released from injured or dying cells (24). These lysosomes contain pockets of protective enzymes, and their release leads to a local increase in the available sulfhydryl groups which may form disulfides in the presence of sulfhydryl-containing compounds and $\text{R—S—Hg—R'}$ bonds in the presence of mercury-containing compounds.

One of these mercury-containing compounds, diiodohydroxymercurifluorescein, yielded a higher concentration ratio between damaged and normal myocardium than any other radiopharmaceutical tested. In addition, the percent injected dose per gram was almost six times that of the bone-seeking radio tracers. It is of great interest that structurally this radiotracer contains, in addition to mercury, a polycyclic aromatic ring structure. The general configuration is similar to that found with the tetracycline analogs. Thus, the coincidental presence of mercury and a polycyclic aromatic ring may have resulted in a substantial increase in the avidity of the compound for acutely damaged tissue.

This study suggests the predictability of the structural relationships that augment the specificity of a radiopharmaceutical for acutely infarcted myocardium. Given such structure–activity relationships as
the possible presence of sulfhydryl groups, the presence of mercury, and the presence of polycyclic aromatic ring compounds, it should be possible to synthesize compounds with ideal biologic and physical properties for the estimation of acute myocardial infarct size in animal models and in man.

Note that all agents were evaluated 1 hr after injection. This time was selected on the basis of the model as established by Adler et al. (17) and with the clinical need for very early scanning in mind. A pilot time-course study with GH, tetracycline, and PPi indicated that at later times (6 and 24 hr) tetracycline actually attained infarct-to-normal ratios of 3.5 greater than those of either of the other agents. An in-depth study of the optimum time for each agent was clearly beyond the scope and initial intent of this investigation.

ACKNOWLEDGMENTS

The authors wish to dedicate this work to the memory of the late William Brady for his efforts in initiating this study. His untimely death saddened us all. The editorial assistance of Rebekah Taube in the preparation of this manuscript is deeply appreciated.

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REFERENCES
