Experimental Basis for Myocardial Imaging with ^{123}I-Labeled Hexadecenoic Acid

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Progress in myocardial perfusion imaging has been slowed by the lack of radiopharmaceuticals with suitable physical and biologic characteristics. Hexadecenoic acid, terminally labeled with ^{123}I, partially overcomes these limitations by providing a compound that concentrates in the myocardium in proportion to relative regional blood flow and carries a gamma-emitter with desirable detection and imaging qualities. After intravenous injection in experimental animals, the clearance half-times of hexadecenoic acid for blood and myocardium are 1.7 and 20 min, respectively. These values compare favorably with 18-carbon fatty-acid analogs labeled with ^{11}C. In acute and chronic infarction, similar distribution patterns are found for hexadecenoic acid and ^{43}K, which indicates that hexadecenoic acid is a suitable substitute for the potassium analogs now in use for myocardial imaging. Because of the high count rates obtainable with ^{123}I-hexadecenoic acid, good-quality images can be acquired in as little as 2–3 min per view. Iodine-123-hexadecenoic acid is potentially a useful radiopharmaceutical for clinical application.

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The feasibility of using intravenously administered gamma-emitting radiopharmaceuticals and scintigraphic imaging for identification of deficits in regional myocardial perfusion has been well established both experimentally and clinically (*1–5*). However, attempts to quantify regional blood flow have not proven satisfactory, due largely to the unfavorable physical characteristics of available radionuclide labels. Most of these radionuclides possess energies unsuitable for optimum resolution with present commercially available imaging systems. In addition, the detectable photon yield per rad of absorbed dose is too low to provide valid counting statistics for small regions of interest. Iodine-123 may provide a solution to this problem; it emits an almost pure 159-keV gamma photon with high abundance. Photons with this energy are efficiently detected by scintillation cameras and can be used with high-resolution collimators. Relatively large doses of ^{123}I can be given because the short half-life (13 hr) and lack of beta emission combine to reduce the absorbed radiation dose. The problem has been to find a carrier molecule for ^{123}I that will be distributed in the myocardium in proportion to blood flow and remain there long enough to provide statistically significant quantifiable data.

Fatty acids are an important energy source for the heart and are efficiently extracted from the blood by myocardial cells. In 1964 Evans prepared ^{131}I-labeled oleic acid by saturation of the double bond with iodine monochloride (*6*). Although a satisfactory experimental heart-scanning agent was produced, labeling by double-bond saturation appears to alter the biologic behavior of the parent compound and reduce myocardial extraction efficiency. In contrast, labeling of the 16-carbon fatty acid, 16-iodo-9-hexadecenoic acid, by radioiodine exchange with bromine produces a compound that retains the myocardial extraction characteristics of noniodinated long-chain fatty acids (*7*). The purpose of this investigation was to define the biologic characteristics

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of intravenously administered iodinated hexadecenoic acid and to evaluate its potential as a quantitative indicator of relative regional myocardial blood flow.

MATERIALS AND METHODS

Mongrel dogs, weighing 14–22 kg, were used as the experimental model. After an overnight fast, anesthesia was attained with intravenous pentobarbital (25 mg/kg), supplemented as needed. During open-chest procedures, a cuffed endotracheal tube was used to maintain respiration by positive-pressure breathing of 100% O_2. Myocardial ischemia and/or infarction were produced under aseptic conditions by ligating the anterior descending coronary artery just below its first major branch. If visual or electrocardiographic changes of ischemia were not immediately noted, up to six collateral vessels were also ligated. Intravenous propranolol (1–4 mg) was given to prevent fatal arrhythmias. Postoperatively, pharmacologic doses of meperidine were administered for pain.

Except for commercially obtained carrier-free ^{131}I and ^{125}I, all radionuclides used in this study were produced on site in a biomedical cyclotron using standard production methods. The procedures for preparing the ^{11}C fatty acids and the terminally iodinated hexadecenoic acid have been described previously (*7,8*). Briefly, the ^{11}C compounds are made by Grignard synthesis using $^{11}CO_2$ and appropriate alkyl magnesium bromides. Radioiodinated hexadecenoic acid is made by refluxing carrier-free ^{131}I, ^{125}I, or ^{123}I with 16-bromo-9-hexadecenoic acid in methyl ethyl ketone solution.

The final ^{11}C compounds exist in true trace quantities and contain little excess unlabeled fatty acid. In this form they could be injected intravenously without first being dissolved in an albumin solution. However, for standardization, both the ^{11}C-fatty acids and the radioiodinated hexadecenoic acid were dissolved in a 6% human or canine serum albumin solution for these studies. For the studies of blood and precordial clearance, ^{131}I-hexadecenoic acid was used. The ^{11}C reference compounds and the iodinated fatty acid were given in amounts of approximately 150 μCi. Test agents were administered intravenously and blood samples were withdrawn through separate indwelling venous catheters.

Blood-clearance rates for the labeled fatty acids were obtained by counting serial samples of venous blood in a well counter. Separation of the relative percentages of "free" and "bound" radioiodine in blood was accomplished by passing serum samples through an anion exchange resin column. Myocardial clearance rates were estimated by external counting using a heavily collimated probe with a 2 × 2-in. sodium iodide crystal placed over the heart and angled slightly away from the liver. After pulse height analysis of the signals a scintigraphic data analyzer* was used for data storage, decay correction, and computation of clearance rates.

The uptake characteristics of iodinated hexadecenoic acid in ischemic myocardium were determined by direct tissue counting using ^{43}K as the reference. Infarction or ischemia was produced as described above. Seven to ten minutes after the simultaneous intravenous injection of 100 μCi each of ^{43}K and ^{125}I-hexadecenoic acid, the animals were killed by intravenous injection of a saturated solution of potassium chloride. Immediately, 8–10 samples (0.6–2 gm) of the anterior left-ventricular wall were removed from the excised heart. Tissue samples were systematically selected to ensure representation of normal, ischemic, and infarcted tissue. (Samples taken from the high lateral portion of the left ventricle supplied by the circumflex artery were assumed to be "normally" perfused. Usually the infarcted or most ischemic area was apparent by inspection. Otherwise, a section of ventricle thought to represent the central region of myocardium supplied by the anterior descending coronary artery was selected.) The samples were then counted by differential spectrometry in a well counter. The results, expressed in counts per gram, were then normalized to the normal sample containing the highest concentration of ^{43}K.

Myocardial imaging was performed in 15 animals: 5 normal controls and 10 with ligated anterior coro-

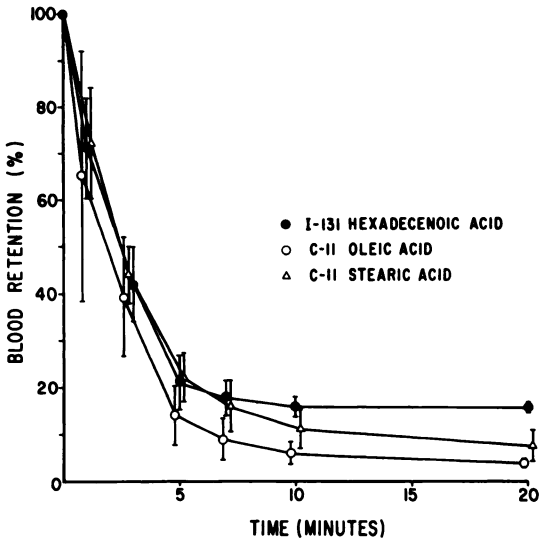

FIG. 1. Blood clearance characteristics of ^{131}I-hexadecenoic acid, ^{11}C-stearic acid, and ^{11}C-oleic acid are compared after intravenous injections. Data were obtained by direct blood sampling except for the 100% value calculated from quantified injected dose and estimated blood volume. Points represent mean ± s.d.

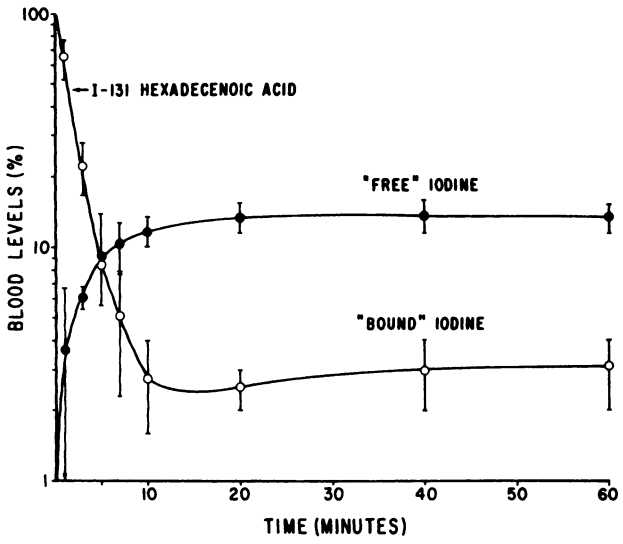

FIG. 2. Relative concentrations of "bound" and "free" radioiodine were determined by separation on anion exchange column.

nary arteries. Imaging was begun approximately 3 min following the intravenous administration of 1–5 mCi of ^{123}I-hexadecenoic acid. A Pho/Gamma HP scintillation camera with a pinhole collimator (0.9-cm aperture) was used. The collimator was placed 6–10 cm from the chest wall, a distance estimated to provide a magnification factor of 2. The acquired data were stored in the scintigraphic data analyzer for subsequent background correction and an additional twofold magnification. The average background counts were determined from a region circumscribed around the perimeter of the myocardial image. This background value was uniformly subtracted from the final image.

For analysis of the clearance data, Student's t-test was used to determine significant differences between mean measurements. Half-times were derived from semilog plots of the clearance data. The relative uptakes of potassium and hexadecenoic acid in normal and ischemic tissue are displayed in a scattergram with the association between the two expressed by the linear regression line ($y = ax + b$), the standard error of the estimate of the regression line (s.e.e.), and the correlation coefficient (r). The MIRD system was used for dosimetry calculation.

RESULTS

Blood clearance. Linear plots of the blood-clearance data from 19 animals are presented in Fig. 1. A biphasic clearance pattern is evident, with maximal tracer disappearance occurring during a rapid first phase. Half-time values for the first phase are 1.7 ± 1.0 min for the hexadecenoic acid ($n = 9$), 1.6 ± 2.2 min for the stearic acid ($n = 5$), and 2.2 ± 0.8 min for oleic acid ($n = 5$). The break in the curves comes at about 5 min after injection, and a trend toward greater blood retention of the radioiodine label follows. At 10 min the radioiodine blood level is significantly higher than the stearic acid ($p < 0.001$). By 20 min the iodine level also becomes significantly greater than that for the oleic acid ($p < 0.001$) and amounts to 18% of the injected radioactivity.

The temporal change in character of the radioiodinated material is further clarified in Fig. 2. The radioiodine in the serum of four animals was separated into "free" and "bound" components. The results at 10 min show that over 75% of the activity in the blood is attributable to free iodide, which presumably results from complete metabolism of the original fatty-acid molecule with hydrolysis of the terminal iodine atom. The bound component represents a mixture of residual labeled hexadecenoic acid, iodinated breakdown products, and released iodine bound to plasma proteins.

Precordial clearance. Tracer clearance from the myocardium was measured in 16 dogs (Fig. 3). The data are normalized to the maximum count rates reached in the first minute after injection and are expressed as percentages. Although the disappearance of the ^{131}I label approximates a monoexponential function, a slightly slower clearance rate develops after 10 min. The slower phase is more pronounced for stearic and oleic acids. Clearance half-times were determined only for the 3–10-min segment of the curve. Hexadecenoic acid ($n = 5$) has the most rapid clearance half-time (20.0 ± 2.3 min) followed by oleic acid ($n = 6$; 24 ± 7 min) and stearic

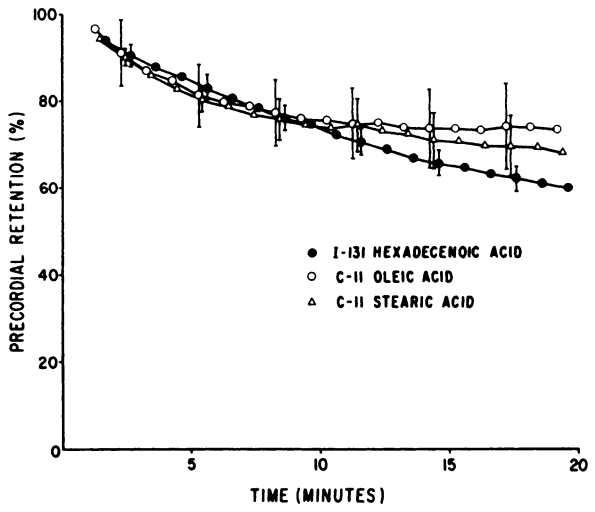

FIG. 3. Relative precordial clearance characteristics of ^{131}I-hexadecenoic acid, ^{11}C-stearic acid, and ^{11}C-oleic acid are compared following intravenous administration.

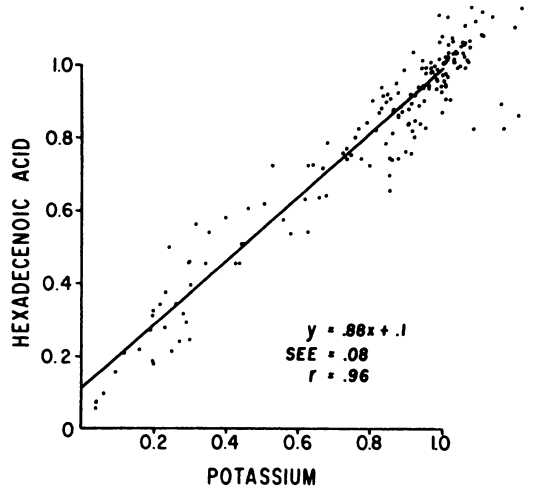

FIG. 4. Comparative tissue concentrations (1.0 = 100%) of ^{131}I-hexadecenoic acid and ^{42}K in series of 20 dogs with experimental myocardial ischemia. Data are pooled from animals followed for 4 hr, 24 hr, 1 week, and 1 month after coronary-arterial ligation.

acid (n = 5; 25.5 ± 6.7 min). During the first 10 min after injection no significant differences are seen in the clearance of the three tracers. By 15 min a borderline-significant ($p < 0.05$) slowing develops in stearic acid clearance compared to hexadecenoic acid. This difference becomes clearly significant ($p < 0.005$) 3 min later. Oleic acid follows a pattern similar to stearic acid.

Uptake in ischemic tissue. Comparative uptakes of potassium and hexadecenoic acid were determined in five animals each at 4 hr, 24 hr, 1 week, and 1 month after coronary artery occlusion. As a high degree of correlation is observed at all stages (r = 0.94–0.98), the data are pooled and displayed in the scattergram in Fig. 4. The relation between the two tracers, expressed by the least-squares regression line, approaches unity and has a correlation coefficient of 0.96.

Imaging. Using the pinhole camera system described above, precordial images can be acquired 3–5 min following intravenous administration, with an average of 50,000–75,000 cpm/mCi of ^{123}I-hexadecenoic acid injected. Approximately 40–50% of the counts actually emanate from the myocardium. An illustrative serial study is shown in Fig. 5. This is a left lateral projection of an animal with an anterior infarct following administration of 1.4 mCi of ^{123}I-hexadecenoic acid. All four images contain 200,000 total counts. The ischemic defect (arrow) is clearly visualized, as is the hepatic uptake caudal to the heart (Fig. 5A). Although minor quantitative changes can be observed in the second image (Fig. 5B), the diagnostic quality of the image persists through the first 10 min. Beyond 20 min, however, the regional bloodflow pattern is practically lost (Figs. 5C and 5D). Although there is some clearance from the liver, the hepatic concentration remains visually constant.

Dosimetry calculations. Assumptions used in calculating the absorbed radiation dose to the myocardium from ^{123}I-hexadecenoic acid include the following: a 300-gm heart (for nonpenetrating radiation only), a myocardial biologic $T_{1/2}$ of 30 min, and 5% of the injected ^{123}I taken up by the myocardium, with the other 95% uniformly distributed in the body as iodide. The biologic half-time in the body is taken as 15.1 hr. The total-body dose is calculated assuming uniform distribution of 100% of the administered ^{123}I as iodide with a half-time for biologic clearance equal to 15.1 hr. The dose to the heart becomes 0.04 rad/mCi and the dose to the total body 0.03 rad. The actual absorbed doses for commercially available ^{123}I preparations are about 50% greater than estimated because of the presence of other radioisotopes of iodine.

DISCUSSION

The similarities in blood clearance and precordial clearance between hexadecenoic acid and the reference ^{11}C-labeled stearic and oleic acids support an earlier hypothesis that the radioiodinated 16-iodo-9-hexadecenoic acid is an adequate long-chain fatty-acid analog (7). However, this characteristic holds only during the uptake and initial clearance phases, since significant differences in relative radioactive clearance patterns in the heart and blood can be demonstrated beyond 10 min. The observed differences may be due to rapid metabolism of the

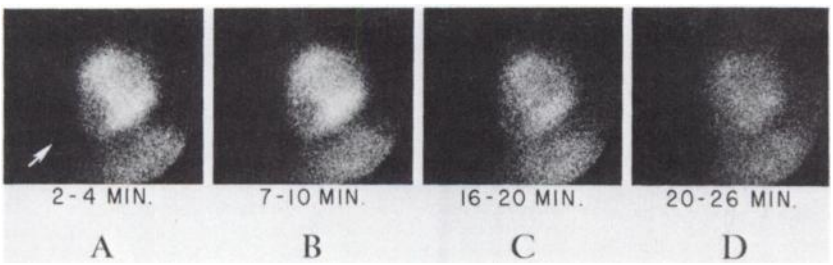

FIG. 5. Sequential left lateral images after intravenous administration of 1.4 mCi ^{123}I-hexadecenoic acid in dog with occlusion of left anterior descending coronary artery. Each image contains 200,000 total counts. Note initial high quality of original image and good resolution of perfusion defect (arrow).

original compounds and subsequent redistribution of metabolic products. In the case of the ^{11}C compounds, the radioactive label is located in the carboxyl group which, on metabolic degradation, can be incorporated into other organic molecules in the muscle substance or further degraded to CO_2 and exhaled. Release of radioiodine label from hexadecenoic acid is probably the result of beta oxidation of the fatty acid with hydrolysis of the final radioiodinated metabolic product, iodoacetate, rather than of the parent hexadecenoic acid (*8*). In any event, the radioiodine is rapidly freed to enter the circulating iodine pool.

No proven technique is available for absolute determination of flows for small regions within an organ where the influx and efflux of blood cannot be sampled. The distribution of minute amounts of radioactive microspheres probably provides the most reliable experimental reference standard (*9*). This standard has been employed by different investigators to determine the reliability of radioactive potassium, rubidium, and cesium as regional myocardial bloodflow indicators in normal, ischemic, and infarcted tissue (*10–12*). In normal and ischemic myocardium, good correlation was found with all three nuclides. In hyperemia, however, potassium uptake does not increase linearly and will give falsely low flow estimates (*10*). Presumably, most other soluble tracers suitable for intravenous administration will suffer a similar deficiency. In this study relative uptakes of potassium and fatty acid were evaluated only in conditions of normal flow and ischemia. The results indicate that ^{123}I-tagged hexadecenoic acid should be as reliable an indicator for qualitative regional myocardial blood flow as are the available potassium analogs.

For quantitative measurements of regional myocardial perfusion, the blood should be totally devoid of the radioactive label. At the optimum time for imaging with iodinated hexadecenoic acid, the blood does retain appreciable amounts of radioiodine (Fig. 2). The contribution of this radioactivity in the blood does not visually degrade the image, although greater contrast would be obtained if this background contribution were not present. With the short imaging periods required with ^{123}I-hexadecenoic acid, the slight temporal changes in the blood levels are not expected to invalidate quantitative regional measurements. But if quantitative imaging with this radiopharmaceutical does prove to be clinically useful, and if the radioiodine in the blood does reduce the reliability and reproducibility of the test, then further attempts will be made to inhibit the process of beta oxidation by chemically modifying the molecular structure of the fatty acid.

The high count rates possible with dosimetrically reasonable amounts of ^{123}I-hexadecenoic acid (e.g., 5 mCi) permit three approaches to clinical application. First, multiview qualitative images could be rapidly acquired at the rate of 2–4 min per view, and a four-projection study could be completed in less than 15 min after tracer injection. Second, because of the rapid myocardial clearance, repeat studies could be performed within a few hours. Third, a selective very-high-count single-projection image could be acquired. This approach would be particularly suited to image quantification where sequential studies would be desirable to detect small changes in regional myocardial perfusion or function. A variant of this approach would be to measure regional clearance rates, since metabolism of the labeled fatty acid would presumably differ between normal and injured myocardium.

The final breakdown products of 16-iodo- and 16-bromo-9-hexadecenoic acid (namely, iodoacetate and bromoacetate) are toxic. As only trace amounts of iodoacetate are produced from the administered material, no problems would arise, but in the labeled hexadecenoic acid preparation used in this study, up to 10 mg of the parent bromo compound was given along with the radioiodinated material. From a 10-mg quantity of 16-bromo-9-hexadecenoic acid, 2 mg of bromoacetate might be produced. Although this amount is well under the toxic level of 20–50 mg/kg, the desirability of producing very-high-specific-activity preparations of iodohexadecenoic acid is obvious, particularly when administration of multiple doses is anticipated (*13*). The preparation of a radiopharmaceutical using only 1 mg of bromohexadecenoic acid seems feasible.

The physical characteristics of ^{123}I make it a highly desirable radionuclide for scintigraphic imaging. The nearly monoenergetic intermediate-energy gamma emissions can be effectively collimated and are efficiently absorbed by the thin sodium iodide crystals used in scintillation cameras. The short half-life and absence of beta radiation minimize the radiation dose delivered to patients. Iodine chemistry is well understood and, in contrast to many other labels, the iodine atom can be incorporated into many molecules by a covalent bond. Unfortunately, long-chain fatty acids are not the ideal carrier for ^{123}I. They are rapidly metabolized and the iodine label is subsequently liberated to circulate in the iodine pool, which in turn raises the nonmyocardial background and reduces the reliability of quantitative measurements. At the present time, however, ^{123}I-hexadecenoic acid appears to be an excellent indicator for the scintigraphic determination of relative regional myocardial perfusion.

ACKNOWLEDGMENTS

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FOOTNOTE

* Hewlett-Packard Model 5407-A (Palo Alto, Calif.).

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