MYOCARDIAL UPTAKE OF LABELED OLEIC AND LINOLEIC ACIDS

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Oleic acid labeled with ^{14}C, (^{14}C-OA) or ^{131}I (^{131}I-OA) and ^{131}I-labeled linoleic acid (^{131}I-LOA) were administered intravenously to rats and tissue distribution studies completed at various time intervals from 5 to 60 min. Tissue distribution of ^{131}I-labeled oleic acid or linoleic acid was also studied in dogs at 5- and 30-min time intervals after intravenous administration of the tracer dose. There were distinct differences in the patterns of tissue distribution between ^{14}C-OA, ^{131}I-OA, and ^{131}I-LOA.

Radioactivity concentration in the myocardium was the highest at all time intervals in the rats given ^{131}I-OA only. In dogs, the myocardial uptake of ^{131}I-OA was significantly higher than the radioactivity in the blood or other tissues at 30 min after injection. The disappearance rates of ^{131}I-OA and ^{131}I-LOA were almost identical but myocardial concentration of ^{131}I-LOA at 30 min after the dose in the dog was half that of ^{131}I-OA whereas ^{131}I-LOA liver concentration was higher than that of ^{131}I-OA. Since the concentrations of our formulated ^{131}I-OA in the blood and in the myocardium are both highest at the earlier intervals, it should be difficult to detect myocardial ischemia or infarction with ^{131}I-OA scanning.

The heart shadow as visualized roentgenographically is the result of superimposition of several tissues including the blood within the heart. Other roentgenographic and radionuclide techniques used to visualize the heart show only heart cavities. The studies of Burch and his associates (*1,2*) which indicated rapid uptake of ^{86}Rb by the myocardium, suggested the possibility of demonstrating the myocardium by photoscanning after administration of this radioisotope. Our previous work (*3*) confirmed the possibility of demonstrating the myocardium of the healing heart of living dogs by this technique. This work was followed in the dog with ^{203}Hg-chlormerodrin (*4*) and with ^{131}Cs in man by Carr, et al (*5*) and with ^{43}K by Zaret, et al (*6*).

Evans and Gunton produced marginal visualization of myocardial infarcts in dogs (*7*) and man (*8*) using ^{131}I-oleic acid (^{131}I-OA) with the formulation of ^{131}I-OA mixed and bound to human serum albumin in a relatively large volume. Since Bing, et al (*9*) demonstrated that the rate of fatty acid extraction by the myocardium depends upon the concentration of the fatty acids in the blood, we have tried to increase the uptake of ^{131}I fatty acids in the myocardium using a different formulation that allows the intravenous injection of a smaller volume. We were interested in ^{131}I-OA because the fatty acids, as the principal nutrient of the myocardium, offer the opportunity to detect biochemical as well as metabolic defects in the myocardium in addition to coronary insufficiency.

MATERIALS AND METHODS

Radiopharmaceuticals. Oleic and linoleic acids labeled with ^{131}I were obtained from Abbott Laboratories. The products were obtained with specific activities of 2–3 mCi/m*M*. Oleic acid labeled with ^{14}C was obtained from New England Nuclear with a specific activity of 40–60 mCi/m*M*. Radiochemical purity of the products was ascertained with thin-layer chromatography using silica gel with fluorescent indicator as the adsorbent and a solvent system of petroleum ether:diethyl ether:acetic acid (90:10:0.5). The labeled products gave Rr = 1 with gas chromatographic nonlabeled standards. Oleic acid has an $R_f = 0.44$ and linoleic acid, $R_f = 0.36$, in the solvent system.

The radiopharmaceuticals were formulated by adding in sequence the following: 1% fatty acid, 10% polysorbate 80, and q.s. dextrose 5% in water containing 10% propylene glycol.

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TABLE 1. RELATIVE TISSUE DISTRIBUTION IN RATS OF ^{14}C-OA, ^{131}I-OA, AND ^{131}I-LOA AFTER INTRAVENOUS ADMINISTRATION*

	Minutes														
	5			10			20			30			60		
	Compounds														
Tissues	^{14}C-OA	^{131}I-OA	^{131}I-LOA	^{14}C-OA	^{131}I-OA	^{131}I-LOA	^{14}C-OA	^{131}I-OA	^{131}I-LOA	^{14}C-OA	^{131}I-OA	^{131}I-LOA	^{14}C-OA	^{131}I-OA	^{131}I-LOA
Liver	5.92 ±0.36	1.33 ±0.04	1.69 ±0.04	2.99 ±0.37	0.78 ±0.04	1.06 ±0.06	4.19 ±0.27	0.60 ±0.03	0.83 ±0.04	3.40 ±0.52	0.77 ±0.20	0.51 ±0.03	2.85 ±0.23	0.53 ±0.01	1.00 ±0.36
Heart	3.42 ±0.60	1.78 ±0.30	1.34 ±0.05	0.94 ±0.09	1.52 ±0.22	1.02 ±0.05	2.86 ±0.22	1.12 ±0.09	0.85 ±0.02	2.01 ±0.55	0.82 ±0.04	0.97 ±0.04	3.12 ±0.86	0.84 ±0.11	0.80 ±0.13
Blood	0.72 ±0.04	1.15 ±0.07	1.08 ±0.05	1.07 ±0.05	1.37 ±0.54	0.40 ±0.06	0.48 ±0.08	1.03 ±0.25	0.64 ±0.04	0.20 ±0.01	0.59 ±0.04	0.39 ±0.08	0.41 ±0.04	0.71 ±0.07	0.61 ±0.15
Lung	0.98 ±0.13	1.11 ±0.08	0.85 ±0.16	0.49 ±0.08	0.72 ±0.02	0.52 ±0.07	0.70 ±0.07	0.69 ±0.05	0.49 ±0.11	0.94 ±0.18	0.58 ±0.03	0.44 ±0.02	0.74 ±0.09	0.52 ±0.05	0.55 ±0.15
Spleen	0.83 ±0.05	0.86 ±0.05	0.48 ±0.02	0.16 ±0.07	0.58 ±0.02	0.36 ±0.02	0.64 ±0.02	0.55 ±0.04	0.38 ±0.09	0.60 ±0.01	0.61 ±0.03	0.35 ±0.02	0.79 ±0.03	0.58 ±0.09	0.38 ±0.08
Kidney	1.45 ±0.14	0.77 ±0.03	0.87 ±0.02	0.73 ±0.03	0.35 ±0.11	0.50 ±0.03	1.05 ±0.06	0.40 ±0.02	0.62 ±0.09	1.11 ±0.13	0.43 ±0.03	0.42 ±0.08	1.23 ±0.04	0.55 ±0.10	0.55 ±0.10
Adrenal	1.06 ±0.09	1.18 ±0.06	1.21 ±0.07	1.68 ±0.04	1.06 ±0.07	0.79 ±0.03	2.00 ±0.16	1.06 ±0.11	0.77 ±0.07	1.64 ±0.28	0.90 ±0.07	0.81 ±0.12	1.90 ±0.42	1.24 ±0.11	1.08 ±0.05
Intestine	0.60 ±0.09	0.38 ±0.02	0.36 ±0.06	0.55 ±0.02	0.37 ±0.07	0.39 ±0.06	0.68 ±0.04	0.19 ±0.04	0.39 ±0.07	0.65 ±0.03	0.35 ±0.08	0.24 ±0.04	0.67 ±0.05	0.17 ±0.05	0.27 ±0.04
Fat	0.46 ±0.03	0.23 ±0.02	0.24 ±0.02	0.33 ±0.01	0.20 ±0.02	0.18 ±0.02	0.50 ±0.09	0.13 ±0.01	0.31 ±0.07	0.41 ±0.01	0.29 ±0.11	0.12 ±0.00	0.35 ±0.01	0.19 ±0.02	0.17 ±0.04
Skeletal muscle	0.93 ±0.09	0.32 ±0.02	0.25 ±0.02	0.42 ±0.02	0.25 ±0.02	0.25 ±0.02	0.80 ±0.06	0.27 ±0.02	0.36 ±0.03	0.72 ±0.04	0.21 ±0.02	0.24 ±0.04	1.18 ±0.05	0.18 ±0.07	0.32 ±0.02

* Data from three rats at each time interval for each compound expressed as percent dose per gram fresh tissue ± standard error of mean.

Animals. Forty-five adult Sprague-Dawley rats of either sex were given ^{14}C-oleic acid (^{14}C-OA), ^{131}I-oleic acid (^{131}I-OA), or ^{131}I-linoleic acid (^{131}I-LOA) intravenously through the femoral vein. Three rats in each of these three groups were sacrificed at 5-, 10-, 20-, 30-, and 60-min intervals after dosing. Three aliquots of each tissue, i.e., liver, heart, blood, lung, spleen, kidney, adrenal, intestine, fat, and skeletal muscle were removed for radioactivity measurements.

The iodinated fatty acids were also given intravenously to 12 adult mongrel dogs of either sex weighing 8–12 kg. Three dogs were sacrificed at 5 min and 3 at 30 min after intravenous administration of ^{131}I-OA and ^{131}I-LOA. Dogs sacrificed 30 min after the tracer had blood samples withdrawn at 1-, 3-, 5-, 10-, 20-, and 30-min for radioactivity assay. Three aliquots were taken from liver, ventricular myocardium, atrial myocardium, blood, lung, spleen, kidney, adrenal, intestine, fat, muscle, and thyroid for radioactivity measurements.

Radioactivity measurements. For the measurement of ^{14}C radioactivity, all specimens were placed in liquid scintillation counting vials, digested overnight in 0.3 ml of 10% NaOH, dissolved by warming to 70° for 20–30 sec, and after cooling, three drops of 30% H_2O_2 were added. Then 10 ml of phase combining system (Amersham/Searle) solubilizer and liquid scintillation cocktail mixture was added. After 12 hr of dark adaptation and cooling in the counter, all samples were counted for 10 min each in a liquid scintillation counter (Searle Radiographics Unilux IIA). The channels ratio method was used for quench correction. Radioactivity of ^{131}I was measured with a NaI crystal scintillation well counter (Searle Radiographics). The data were expressed as percent administered dose per gram of fresh tissue.

RESULTS

Radioactivity concentration from ^{14}C-OA, ^{131}I-OA, or ^{131}I-LOA in various rat tissues are shown in Table 1. Peak concentration of radioactivity in the blood from ^{14}C-OA and ^{131}I-OA was observed at 10 min. The radioactivity concentration of ^{14}C-OA in the myocardium was significantly higher than that of the blood or other background tissues at all time intervals (except in blood at 10 min) but was always lower than that in liver except 60 min after the dose when radioactivity in the myocardium was slightly higher. The target-to-nontarget (heart-to-blood) ratio for ^{14}C-OA at 5 and 20 min was about 5. The data at 10 min showing most of the tissue values to be half the concentrations at 5 or 20 min are unexplainable except perhaps on the basis of poor formulation of the ^{14}C-OA used at the 10-min time interval.

The relative tissue distribution of ^{131}I-OA or ^{131}I-LOA in various tissues of the rat exhibited significant differences from the distribution with ^{14}C-OA. Radioactivity concentration from ^{131}I-OA was highest in the myocardium at all time intervals rather than that observed in liver after ^{14}C-OA. However,

TABLE 2. RELATIVE TISSUE DISTRIBUTION IN DOGS OF ^{131}I-OA AND ^{131}I-LOA AFTER INTRAVENOUS ADMINISTRATION*

	Minutes			
	5	5	30	30
	Compounds			
Tissue	^{131}I-OA	^{131}I-LOA	^{131}I-OA	^{131}I-LOA
Liver	0.0286 ± 0.001	0.044 ± 0.002	0.0226 ± 0.009	0.030 ± 0.002
Left ventricle	0.035 ± 0.001	0.034 ± 0.001	0.060 ± 0.025	0.026 ± 0.002
Right ventricle	0.038 ± 0.006	0.031 ± 0.001	0.044 ± 0.008	0.023 ± 0.001
Left atrium	0.027 ± 0.003	0.027 ± 0.003	0.027 ± 0.006	0.018 ± 0.001
Right atrium	0.028 ± 0.005	0.032 ± 0.003	0.022 ± 0.007	0.019 ± 0.001
Adrenal	0.022 ± 0.001	0.024 ± 0.002	0.026 ± 0.003	0.011 ± 0.001
Spleen	0.017 ± 0.002	0.014 ± 0.001	0.016 ± 0.001	0.012 ± 0.001
Pancreas	0.005 ± 0.002	0.012 ± 0.002	0.009 ± 0.003	0.010 ± 0.001
Fat	0.004 ± 0.001	0.006 ± 0.001	0.007 ± 0.003	0.007 ± 0.001
Muscle	0.005 ± 0.001	0.007 ± 0.001	0.005 ± 0.001	0.007 ± 0.001
Lung	0.024 ± 0.003	0.027 ± 0.002	0.013 ± 0.001	0.012 ± 0.001
Stomach	0.009 ± 0.001	0.008 ± 0.002		0.014 ± 0.004
Intestine	0.010 ± 0.001	0.014 ± 0.001	0.008 ± 0.001	0.012 ± 0.001
Renal cortex	0.018 ± 0.002	0.032 ± 0.001	0.0113 ± 0.002	0.019 ± 0.001
Aorta	0.004 ± 0.001	0.004 ± 0.001	0.008 ± 0.002	0.007 ± 0.001
Thyroid	0.031 ± 0.005	0.055 ± 0.011	0.888 ± 0.168	0.150 ± 0.02
Testes	0.002 ± 0.000	0.003 ± 0.001	0.004 ± 0.001	0.004 ± 0.001
Blood	0.037 ± 0.007	0.038 ± 0.004	0.0145 ± 0.003	0.015 ± 0.001

* Data averaged from three dogs and expressed as percent dose per gram. Fresh tissue ± the standard error of mean.

the difference in radioactivity concentration between the myocardium and blood or liver was not marked. The target-to-nontarget ratio (heart-to-blood) for ^{131}I-LOA and ^{131}I-OA was less than 2.

Iodine-131-LOA radioactivity concentration, on the other hand, was highest in the liver at 5 and 10 min. Radioactivity concentration in the blood from ^{131}I-LOA was always lower than that of the myocardium or liver.

Table 2 shows the relative tissue distribution of ^{131}I-OA or ^{131}I-LOA in various dog tissues at 5 or 30 min after the tracer dose was administered.

At 5 min, the radioactivity concentration from ^{131}I-OA was highest in the blood and paralleled the ventricular myocardial radioactivity. Thirty minutes after intravenous dosing of ^{131}I-OA, the radioactivity concentration was highest in the ventricular myocardium (except for that of the thyroid gland). The radioactivity from ^{131}I-OA in the ventricle at 30 min was four times higher than the radioactivity in the blood and almost three times higher than that in liver. The combination of ^{131}I radioactivity concentration in myocardium and in blood was sufficient to obtain distinct images of the heart in dogs with the Anger camera after intravenous administration of 0.5–1.0 mCi of ^{131}I-OA (Fig. 1). The high concentration of radioactivity in the thyroid gland at 30 min suggests that a significant amount of deiodination from oleic acid had occurred.

Radioactivity concentration from ^{131}I-LOA was highest in the liver (except for that in the thyroid gland) at both time intervals.

The blood disappearance rates of radioactivity from ^{131}I-OA or from ^{131}I-LOA in dogs are illustrated in Fig. 2.

Disappearance rates of radioactivity from the circulating blood were exponential and no significant difference was apparent between the two compounds. The blood biologic half-life from ^{131}I-OA in dogs obtained from extrapolation of the initial fast component of the blood disappearance curve was 5 min.

DISCUSSION

Along with carbohydrates, fatty acids are a major source of energy for the myocardium (*9,10*). Gordon and Cherkes have reported that the source of fatty acids for myocardial metabolism is the nonesterified fatty acid and from 25 to 75% of the myocardial oxygen usage could be accounted for by the uptake of nonesterified fatty acid (*11*). In circulating blood, oleic acid is the largest component of the free fatty acids and myocardial uptake rate was the highest with oleic acid in both man and dog (*12*).

Recently Bonte, et al reported the "cold spot" visualization of myocardial infarctions in photoscanning of the heart in dogs with ^{99m}Tc-labeled oleic acid as a scanning agent (*13*). The ^{99m}Tc-oleic acid results were not routinely uniform due to variations in ra-

FIG. 1. Image of dog heart, using Anger camera, 30 minutes after 0.5 mCi ^{131}I-OA (left anterior oblique view, arrows indicate level of diaphragm).

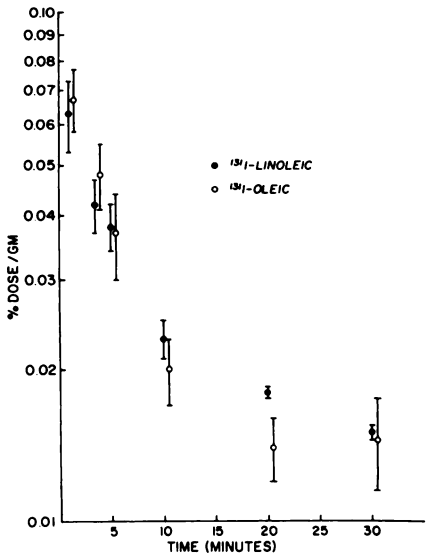

FIG. 2. Blood disappearance rates of radioactivity from ^{131}I-OA and ^{131}I-LOA in dogs.

diolabeling. Our data exhibit significant differences in the distribution (and metabolism) between ^{14}C-OA and ^{131}I-OA. These differences suggest that the mechanism of tissue "uptake" of iodinated oleic acid may be different from unlabeled oleic acid.

The relative tissue distribution of ^{131}I-OA radioactivity concentration in the dog at 30 min after the intravenous dose demonstrated radioactivity concentration in the ventricular myocardium about three times higher than radioactivity in the liver and four times higher than the activity in the blood. This radioactivity concentration in the myocardium and blood was sufficient to obtain distinct images of the heart in a series of images obtained in dogs with the Anger camera after intravenous administration of 0.5–1 mCi of ^{131}I-OA. We believe, however, that the amount of blood in the ventricular chamber contains sufficient radioactivity to prevent separate imaging of ventricular myocardial ischemia. It would also make it impossible to produce accurate separate curves of uptake and disappearance of radioactivity from the myocardium in the exploration of possible abnormalities in metabolism of these fatty acids by the myocardium.

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