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Myocardial Extraction of Labeled Long-Chain Fatty Acid Analogs[1] (38509)

NORMAN D. POE, GERALD D. ROBINSON, JR., AND NORMAN S. MACDONALD
(Introduced by George V. Taplin)

Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, California 90024

Investigations of long-chain fatty acid metabolism and distribution with radioactive labeled compounds have been conducted primarily with the carbon-14 label. Because carbon-14 emits beta particles, which require a liquid scintillation counter for measurement of the radioactivity, studies have been limited to *in vitro* tissue counting or analyses of excretion products of the organs of interest. If the desired fatty acid could be labeled with a gamma emitting radionuclide, regional function measurements *in vivo* could be achieved using external detection systems. The short lived carbon-11 radionuclide yields high energy photons by positron annihilation. However, the 20 min half life, the lack of suitable instrumentation for precise localization of the annihilation photons (0.511 Mev) and the requirement of an on-site cyclotron combine to limit the usefulness of this physiologic label.

The potential application of long-chain fatty acids for measuring regional coronary blood flow stimulated an investigation of iodinated compounds as analogs for natural biologic fatty acids. Oleic acid, iodinated by saturation at a double bond, has previously been studied by Evans (1). An alternative labeling method which yields a product that is structurally more similar to the natural molecule is terminal iodination achieved by a replacement labeling type process (2). Comparison of long-chain fatty acids labeled by carbon-11 inclusion in the carboxyl position, iodine-131 addition to a double bond and iodine-131 substitution in the terminal position were made by determining direct coronary artery extraction of each compound in dog hearts.

Materials and Methods. The carbon-11 fatty acids are prepared by the Grignard reaction. Carbon-11 CO_2, produced by proton bombardment of nitrogen, is bubbled through the appropriate Grignard reagent for about 20 min until radioactive equilibrium is achieved. The final result is a carrier free product containing 2–7 mCi in a 5–10 ml vol with the label in the carboxyl group. The existence of a long-chain labeled fatty acid is confirmed by thin layer radiochromatography. Occasional samples are more precisely identified by gas radiochromatography of the methyl esters. The Grignard precursor of stearic (octadecanoic) acid is available in pure form commercially, but the oleic (*cis*-9-octadecenoic) acid precursor (1-bromo-8-heptadecene) has to be synthesized (3). This complex procedure is carried out with difficulty and preparation of a pure end product is inconsistent.

Iodinated oleic (the labeled product is assumed to be an equal mixture of 9-chloro-10-iodo-octadecanoic acid and 10-chloro-9-iodo-octadecanoic acid), linoleic, and linolenic acids are prepared in a special high specific activity form using a microscale labeling system (2). Briefly, after oxidation of radioiodide in aqueous solution, radioiodine is extracted into diethyl ether and converted to radioiodine monochloride by addition of chlorine in ether. Ten milligrams of unsaturated fatty acid are then added to this labeling reagent. The labeling reaction is terminated by shaking the reaction mixture with an aqueous solution containing bisodium sulphite and a small quantity of iodide carrier.

The terminally iodinated hexadecenoic acid is prepared from 16-bromo-9-hexadecenoic acid (Aldrich Chemical Co., San Leandro, CA) by interhalogen replacement of bromine by radioiodine (2). The desired

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amount of carrier free radioiodine is added to 10 ml acetone containing 10 mg of the bromine precursor. The mixture is refluxed to effect the interchange. The result is an unsaturated molecule with the iodine atom in the terminal position.

As both groups of radioiodine compounds contain substantial amounts of unlabeled fatty acid, the material for injection is prepared by dissolving the labeled and unlabeled fatty acid mixture in a 6% aqueous solution of human serum albumin. The end product contains approximately 1 mCi per ml of solution. Although the carbon-11 preparations are carrier-free, they also are administered in a solution of human serum albumin.

Under pentobarbital anesthesia (25–30 mg/kg), medium to large size mongrel dogs are intubated intratracheally and maintained on positive pressure respiration. The heart is surgically exposed and the anterior descending coronary artery isolated. A 27 gauge needle, bent to 90°, is inserted into the artery using the technique of Ross (4). The needle is connected to a polyethylene tubing with a volume of 0.2 ml. A 2 x 2 inch sodium iodide gamma scintillation detector with a flat-field lead collimator is positioned over the heart. The detector output is directed to a Hewlett–Packard 2100A computer. The labeled test material is preloaded into the 0.2 ml tubing which is maintained outside the field of view of the detector. At the appropriate time the tubing is flushed with a 1 ml dose of normal saline over a 5 sec period. The computer stores the raw data and corrects for residual background and radionuclide decay. The peak count rate, attained at 5–6 sec, is assumed to represent the entire injected dose, i.e. 100%. Over the next 2 min a biphasic curve is inscribed. The following additional assumptions are made: (a) The first phase of the curve represents wash out of nonextracted fatty acid; (b) the second phase represents fatty acid retained in the heart (plus a small amount, considered insignificant, in the blood pool and surrounding tissues) and metabolized; (c) extrapolation of the second phase back to zero time with the intercept expressed as a percent of the maximum count rate gives an approximation of the extraction efficiency.

Several different test agents were investigated sequentially in each animal preparation. The data from individual animals were normalized to the highest count rate. For each group of compounds the mean and standard deviations were determined for every second of the 2-min study, and a "best fit" curve was inscribed through these points. The linear segment of the second phase of the resultant curve was extended to time zero. The *t* test was employed to determine existence of significant differences in extraction efficiencies.

Results. The results obtained with the four major test agents are presented in Fig. 1.

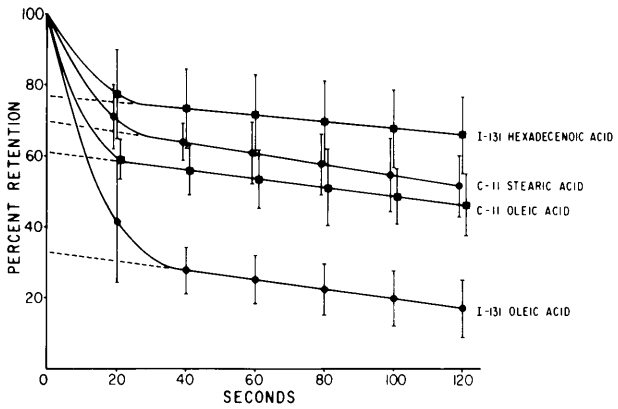

FIG. 1. Biphasic myocardial clearance curves of labeled fatty acids injected directly into the coronary artery of dogs. Data have been normalized to maximum count rates (i.e. 100%) with individual points representing mean values (± SD). Flat phase of curves are extrapolated (broken lines) to zero time for estimation of extraction efficiencies.

Carbon-11 oleic acid was the intended reference, but inconsistency in production of unsaturated fatty acids labeled with carbon-11, in comparison with the ease of preparation of carbon-11 saturated fatty acids, led to the use of stearic acid as the natural reference. No significant difference was found between the stearic (extraction efficiencies 70 ± 7.5% $n = 5$) and hexadecenoic acids (77 ± 11.0%, $n = 7$), and between stearic acid and carbon-11 oleic acid (61 ± 7.8% $n = 3$). A questionable difference was seen between the hexadecenoic acid and carbon-11 oleic acid ($0.05 > P > 0.01$). However, all these molecules showed approximately two times greater extraction efficiency than the iodinated saturated oleic acid (33 ± 5.2%, $n = 6$). The difference is significant at $P < 0.001$. The iodinated linoleic ($n = 5$) and linolenic acids ($n = 6$) showed results indistinguishable from the oleic acid.

The myocardial clearance half times calculated from the extraction curves for the hexadecenoic acid and the carbon-11 stearic and oleic acids were 8.9 min, 4.4 min, and 5.0 min respectively.

Discussion. The myocardial extraction efficiencies for the 18 carbon fatty acids labeled with carbon-11 in this investigation are consistently higher by nearly a factor of two than the values reported in the earlier investigations with carbon-14 or unlabeled compounds (5, 6). However, distinctly different methods for determination of extraction efficiency were used. Most previous investigators measured differences in arterial and venous blood levels across the myocardium. In contrast a direct coronary arterial injection, single circulation method was utilized in this study. This technique has been validated previously using radioactive potassium and cesium (7). Respective extraction values of 71% and 22% were obtained. These results are identical to values for potassium and cesium obtained by Love using arteriovenous differences and constant infusion methods (8).

On the assumption that the carbon-11 fatty acids prepared for this project are true substitutes for the natural compounds, it is evident that the labeled analogs prepared by iodine monochloride saturation do not retain the extraction characteristics of the parent compound. This observation holds true for the three derivatives resulting from iodination of oleic, linoleic and linolenic acids. Loss or retention of double bonds also has no effect on myocardial extraction. The conclusion to be drawn is that the large iodine atom protruding from the side of the fatty acid molecule sterically alters the molecule in such a way as to interfere with normal metabolic transfer mechanisms. In contrast, addition of the iodine atom in the terminal position yields an end product which possesses extraction characteristics indistinguishable from the carbon labeled analog. In the terminal position, it is postulated that the iodine atom maintains a configuration similar to a methyl group and the resultant molecule behaves like the next higher carbon containing fatty acid. In this context 16-iodo-hexadecenoic acid would behave like heptadecenoic acid.

The terminally iodinated fatty acid can serve as a substitute for the natural analog of similar carbon number only during initial uptake and distribution. Subsequent myocardial clearance is about two times as long as for the carbon-11 compounds, and thereafter, the distribution of the radioactivity will be influenced by the iodine atom freed by breakdown of the fatty acid molecule. The iodine will have a short term tendency to remain in the blood while the radioactive carbon atom will be rapidly exhaled as CO_2 or incorporated into other organic molecules.

Blood levels of individual fatty acids have also been shown variably to affect myocardial extraction. The carbon-11 fatty acids were usually administered carrier free, i.e. the amount of substrate injected was measured in nanogram quantities. The addition of small amounts of unlabeled CO_2 did not alter the extraction efficiencies in a limited number of studies. The concentration of iodinated fatty acids, labeled either by terminal substitution or double bond saturation, were relatively high compared to normal blood levels and averaged a mg/ml of solution. While it is possible that the low extraction of the iodine saturated compounds could be related to the concentration of the injected solution, comparable amounts of

terminally iodinated material were highly extracted. The results suggest that within the ranges used in this investigation, concentration of the fatty acid is not a factor in myocardial extraction.

For measurements of the radioactivity in the heart after the rapid initial uptake phase, the relatively slow tissue clearance of the radioiodine label compared to the radiocarbon label is a distinct advantage. Longer counting intervals leading to better statistics can be obtained. This advantage is of particular importance if scintigraphic imaging is employed to determine and quantitate inter- or intraorgan distribution of specific fatty acids. At the same time the relatively rapid clearance of the label permits sequential imaging if instrumentation is available for subtraction of retained background and correction of radioactive decay. Iodine is a particularly useful label for fatty acids because iodine chemistry is well understood and iodine forms a covalent bond with carbon atoms. Furthermore, the availability of at least three iodine radioisotopes (I-123, I-125, and I-131) with distinctly different half-lives and radiation emission characteristics, provide the investigator with a variety of approaches for *in vivo* studies of the distribution and metabolism of fatty acids. More specifically the high myocardial extraction of terminally iodinated 16-hexadecenoic acid, comparable to that obtained with radiopotassium (7), suggests that this fatty acid would be useful as a regional myocardial blood flow indicator.

Summary. The single circulation myocardial extraction of terminally iodinated hexadecenoic acid (77 ± 11.0%) is approximately two times the extraction of 18 carbon fatty acids (33 ± 5.2%) prepared by iodination of double bonds. The results compare favorably with natural 18 carbon stearic (70 ± 7.5%) and oleic (61 ± 7.8%) acids labeled with carbon-11 in the carboxyl group. It is concluded that terminally radioiodinated long chain fatty acids can be used as substitutes for in vivo investigations of fatty acid distribution and in particular may be useful as a regional myocardial blood flow indicator.

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