RADIOIODINATED FATTY ACIDS FOR HEART IMAGING: IODINE MONOCHLORIDE ADDITION COMPARED WITH IODIDE REPLACEMENT LABELING

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Radioiodinated fatty acids have been proposed as agents for use in heart imaging. Previous studies in experimental animals and humans using ^{131}I-oleic acid of low specific activity were marginally successful. Higher specific activity compounds offer potential improvement for use as imaging agents for normal myocardium.

Methods for preparation of high specific activity, radioiodinated fatty acids by iodine monochloride addition to oleic, linoleic, and linolenic acids, and iodide replacement of terminal bromine in 6-bromohexanoic, 11-bromoundecanoic, and 16-bromo-9-hexadecenoic acids are presented and compared.

Although both labeling procedures are suitable for use with ^{123}I, the latter synthetic route gives labeled fatty-acid analog molecules and 16-iodo-9-hexadecenoic acid appears to show improved myocardial specificity in preliminary animal studies.

Medium- and long-chain carboxylic (fatty) acids, which are found in blood plasma in concentrations between 2 and 4 mg/ml (*1*), represent a relatively large, dynamic metabolite pool and their biologic fates are an important aspect of overall metabolism. It is surprising that little has been achieved with regard to applying gamma-emitting fatty acids to studies in clinical nuclear medicine following intravenous administration. Since these carbohydrate compounds serve as important sources of fuel for myocardial tissue (*2,3*) and are, therefore, taken up by the heart, the labeled molecules have been proposed as potential agents for myocardial imaging.

Iodine-131-oleic acid has been repeatedly studied as an experimental agent for heart scanning (*4,5*). Although preliminary investigations were encouraging, early evaluations of this agent were limited by the low specific activity of labeled acid used in the studies and the relatively poor physical characteristics of ^{131}I. Recently, ^{11}C, ^{18}F, ^{123}I, and even ^{99m}Tc have been proposed as labels for myocardium-specific fatty acids (*6–8*). Significantly, Poe, et al (*6*) have shown that myocardial extractions of many labeled fatty acids are somewhat lower than those of the more "physiologic" (i.e., ^{11}C-labeled) molecules. This was tentatively attributed to alteration of molecular structure by the labeling process, the result being labeled molecules which are biologically distinguishable from the parent compounds.

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One possible technique for obtaining greater heart specificity is to use gamma-emitting molecular analogs of the natural fatty acids. Although 2-^{18}F-fluorotetradecanoic acid (an F for H substituted analog) has shown rather poor myocardial extraction (*6*), an alternative is the use of ω-iodo- or ω-bromocarboxylic acids in which the I or Br atom occupies a volume and position similar to that of a terminal methyl group. In addition to offering retention of higher extraction efficiencies, the use of this type of analog molecule might also make it possible to utilize the desirable physical characteristics of ^{123}I as the isotopic label.

As an extension of the work of Evans, et al (*4*), we have radioiodinated oleic, linoleic, and linolenic acids using both ^{131}I and ^{123}I, at high specific activities (>2mCi/mg). 16-Iodo-9-hexadeconoic, 11-iodoundecanoic, and 6-iodohexanoic acids were labeled for studies of the effects of molecular modification upon myocardial uptake. In this latter group of fatty-acid analog molecules, the iodine atom is in the ω-position, where its steric effect was expected to be minimal (Fig. 1). Preliminary studies have shown that the analogs may, indeed, retain the higher myocardial specificities of the "physiologic" molecules, and serve as improved heart-imaging agents.

EXPERIMENTAL

Oleic, linoleic, and linolenic acid were radioiodinated by addition of iodine monochloride across a single unsaturated position in a diethyl ether solution. In order to achieve rapidly the relatively high specific activities required for convenient intravenous administration, the miniaturized system which is shown in Fig. 2 was devised.

Elemental radioiodine was generated by addition of 40 μl of 2 *N* H_2SO_4 to a 0.2-ml solution containing 0.1 mg of KI and 0.2 mg of KIO_3 plus the desired quantity of carrier-free radioiodide in a 2.5-ml multidose vial. The radioiodine was extracted into 0.5 ml of diethyl ether by vigorous stirring until no iodine color remained in the aqueous phase. The ether layer and a small quantity of the aqueous phase were removed under pressure by means of a 20-gage syringe needle extending to just below the ether–water interface. The removed liquids were passed through a "dehydrating cartridge" where water is retained and the dry ether phase, containing radioiodine, was collected in a 5-ml pear-shaped flask (14/20 ⧫ fitting). The "dehydrating cartridge", which consists of a 20-cm length of 5 mm i.d. glass tubing filled with a 3:7 mixture of Sephadex and glass beads sealed by small rubber stoppers, was devised to solve the difficult problem of easily and efficiently separating the small volumes of water and ether involved. The system was rinsed with two 0.5-ml portions of ether (also collected in the receiving flask), the radioiodine was converted to ICl by dropwise addition of Cl_2 saturated ether until the I_2 color disappeared, 10 mg of the unsaturated fatty acid was added, and the labeling reaction was allowed to proceed in the stoppered flask. The radioiodination was terminated by addition, with stirring, of a 1.0-ml aqueous solution containing 50 mg each of KI and $Na_2S_2O_3$ and the ether phase, containing the labeled fatty acid, was isolated as before. When labeled in this manner, the oleic, linoleic, and linolenic acid molecules retain zero, one, and two double bonds, respectively.

Radioiodinated 16-iodo-9-hexadecenoic, 11-iodoundecanoic, and 6-iodohexanoic acid were prepared by interhalogen replacement of bromine in the cor-

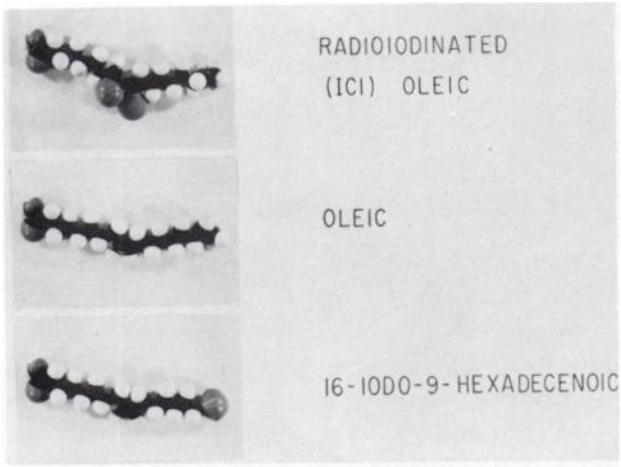


FIG. 1. Radioiodine labeled fatty acids: ICl addition compared with bromine replacement.

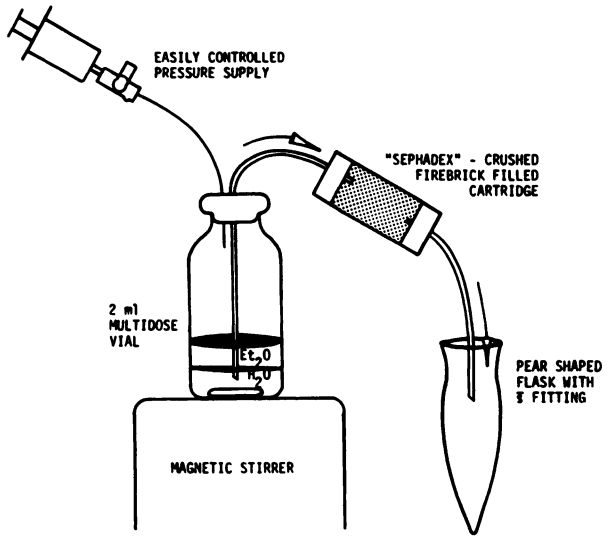


FIG. 2. System for microscale isolation of dry ether phase from ether–water mixture.

responding bromocarboxylic acids under reflux with radioiodide in acetone solution. Ten milligrams of the appropriate bromocarboxylic acide was dissolved in 10 ml of acetone in a 25-ml round-bottom flask. The desired quantity of radioiodide in a small aqueous volume ($\leq$ 20 μl) was added and the reaction proceeded in the boiling acetone solution. With the small volume of water added, hydrolysis of the halocarboxylic molecules was found to be negligible and the labeling reaction went smoothly in the essentially organic medium. The resulting 16-iodo-9-hexadecenoic acid prepared as described above is analogous to 9-heptadecenoic acid in which the radioiodine atom is found in the position normally occupied by the terminal methyl group in the "natural" molecule. Similarly, 11-iodoundecanoic and 6-iodohexanoic acids correspond to dodecanoic and heptanoic acids.

Subsequent processing of the radioiodinated fatty acids for intravenous administration was similar for both the ICl addition and bromine replacement labeled compounds. Solvent was removed by evaporation under the influence of a stream of dry air directed over the surface of the organic solution. The residual labeled fatty acid was then taken up in 2 ml of 25% human serum albumin. The hydrophobic acids are readily soluble in this solution since they can bind to the protein molecules up to a limit of 1:30 *M* ratio of HSA to fatty acid. Free iodide was removed by passing the HSA-fatty-acid solution through a 3-cm $\times$ 1-cm diam anion-exchange column (Dowex 1-8X) eluted with 2 ml of physiologic saline. Following dilution to 6% HSA, the solution was sterilized by passage through a 0.22-micron Millipore filter. Analysis by gel permeation chromotography through a 10-cm $\times$ 1-cm diam column of Bio-Gel P-10 with normal saline elution was used to verify the absence of free radioiodine in the final solution.

Stability of the terminally labeled fatty acids with respect to hydrolysis in 6% HSA solution was measured by incubation of solutions at 37°C for up to 48 hr. Liberation of free iodide was determined both by anion exchange and gel permeation chromatography as described above.

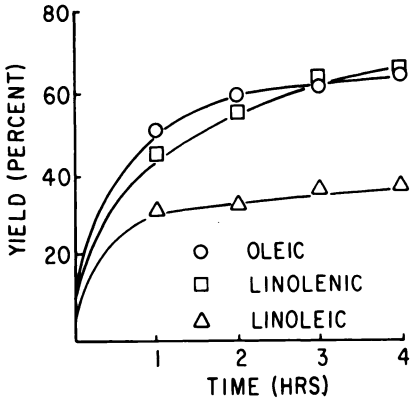
RESULTS AND DISCUSSION

Yields of the fatty acids labeled by ICl addition are shown as a function of time in Fig. 3. For each of the three molecules, labeling kinetics (determined by $S_2O_3^{-2}$ extraction) were found to be qualitatively similar with initially rapidly increasing yields reaching maximum values within 2–4 hr. Yields at 24 hr were found to be indistinguishable from those at 4 hr.

It was surprising to note that the "plateau" values for oleic and linolenic acids which contain one and three double bonds are essentially identical (62 and 64%, respectively) whereas yields for linoleic acid which contains two double bonds reach only half of that value (34%). The molar ratio of ICl to fatty acid is identical in each case, and thus the ratio of ICl molecules to double bonds is a factor of 2 and 3 greater for linoleic and linolenic acids than for oleic acids; however, since the fatty acids are in approximately 100-fold excess, ICl is expected to be the limiting reagent in the labeling reaction. With the ICl concentration being identical in each case, labeling kinetics were expected to be nearly identical also. The markedly reduced labeling yield for linoleic acid remains unexplained.

For routine preparations, using 10 mg of fatty acid and 20 mCi of ^{131}I, specific activities of 0.6–1.2 mCi/mg with concentrations ranging between 0.75 and 1.5 mCi/ml in 6% HSA solution were easily obtained. Since the total time required for production of the labeled compounds is approximately 5 hr (with a 3-hr reaction time), the method is suitable for use with ^{123}I.

The radioiodinated fatty-acid analogs, produced by iodide replacement of terminal bromine, have the labeling reaction kinetics shown in Fig. 4. Free iodide compared with radioiodine-labeled fatty acid was determined by TLC on cellulose acetate using 320:80:1 heptane:diethyl ether:acetic acid as solvent. With this system, free iodide remains near the origin while the fatty acids migrate with R_f values of 0.8–0.9. For each compound, yields initially increase rapidly with subsequently decreasing rates of incorporation of radioiodide with time. Maximum radiochemical yields of 89, 82, and 74% are achieved for 6-iodohexanoic, 11-iodoundecanoic, and 16-iodo-9-hexadecenoic acids, respectively, with 4 hr of refluxing. As



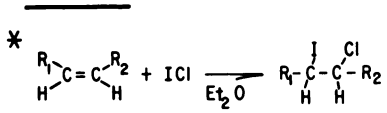


FIG. 3. Radiochemical yields of radioiodinated fatty acids labeled by ICl addition.

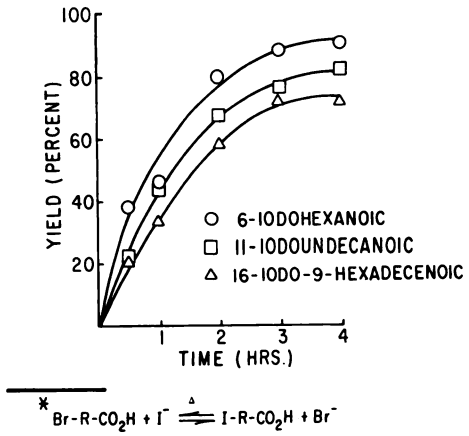


FIG. 4. Radiochemical yields of radioiodinated "analog" fatty acids labeled by bromine replacement.

with the unsaturated fatty acids labeled by ICl addition, yields at 24 hr were found to be indistinguishable from those at 4 hr.

The relative radiochemical yield "plateau" values are consistent with the impression that increasing carbon-chain length gives rise to more stereochemically complex molecules, the result being greater difficulty for bromine atom replacement by radioiodide. The net effect is that decreasing labeling rates and radiochemical yields occur with increasing molecular weight.

Using the standard labeling procedure described previously, starting with 10 mg of ω-bromofatty acid and 20 mCi of radioiodide, specific activities of 1.5–1.9 mCi/mg were obtained with final concentrations in 6% HSA solution ranging from 2.0 to 2.5 mCi/ml. Total preparation time is approximately 4 hr (with 3 hr refluxing) and the process is, therefore, compatible with the use of ^{123}I.

Hydrolysis of the terminally labeled iodofatty acids was investigated because of concern with respect to liberation of free iodide in vitro (after preparation but before administration) and also in vivo (after administration) with resultant degradation of myocardial images. As is shown in Fig. 5, 16-iodo-9-hexadecenoic and 11-iodoundecanoic acids are relatively stable with 93 and 87% retention of the label after 48 hr incubation in 6% HSA solution. The 6-Iodohexanoic acid was rapidly hydrolized under these conditions, however, showing 80% free iodide immediately after being taken up in the HSA solution. This again correlates with changing molecular complexity as a result of increasing molecular weight.

In preliminary experiments on the myocardial extraction of a variety of labeled fatty acids, Poe, et al (*6*) found little difference between oleic, linoleic, and linolenic acids labeled by ICl addition (≅35% extraction following intracoronary administration). In contrast, the terminally labeled 16-iodo-9-hexadecenoic acid had twice the uptake (≅70%) of any of the ICl addition labeled molecules. This higher uptake value was essentially identical to that found for ^{11}C-labeled oleic acid and also, significantly, for ^{43}K. Willebrands has reported (*9*) that, in the human heart, unsaturated fatty acids are extracted by a factor of 2 more than are the saturated molecules (21–26% compared with 12–13%). Since both linoleic and linolenic acids remain unsaturated after labeling by the method described above, the low uptakes (and lack of significant differences in uptake) of these compounds has been tentatively attributed to the extent of molecular modification of these small molecules when labeled by addition of the relatively bulky iodine and chlorine atoms (*6,10,11*). The higher extraction efficiency of 16-iodo-9-hexadecenoic acid was ascribed to its behavior as a true analog of a "physiologic", unsaturated fatty acid (*6*). We are currently investigating the effect of unsaturation upon myocardial uptake by preparing 16-iodohexadecanoic acid for comparative studies.

In the near future we hope to further assess the potential of ^{123}I-labeled 16-iodo-9-hexadecenoic acid as a myocardial imaging agent by using larger doses (up to 10 mCi) in the dog, with and without experimentally induced infarction. If the results of these studies are favorable, clinical trials in man will begin.

CONCLUSION

The need for more sophisticated approaches to the design of radiopharmaceuticals continues to arise. In this sense, the application of gamma-emitting analog molecules to specific organ imaging and/or functional evaluation has much to offer (*12,13*). Labeled fatty acids continue to show promise as potential agents for imaging the myocardium. The use of fatty-acid analog molecules, radioiodinated in the ω-position, where the iodine atom substitutes (sterically)

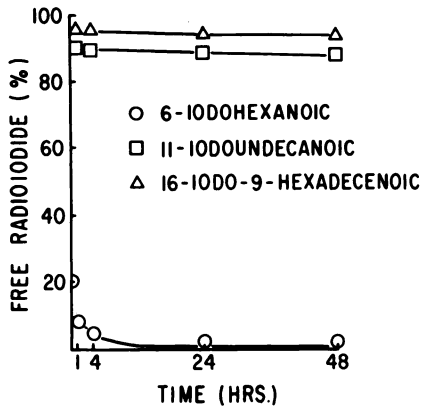


FIG. 5. Hydrolysis of radioiodinated "analog" fatty acids in 6% HSA solution.

for a terminal methyl group, may offer considerable improvement over unsaturated fatty acids labeled by the more traditional ICl addition for this purpose. The ease and rapidity of preparation of 16-iodo-9-hexadecenoic acid, coupled with its high myocardial extraction, implies that this agent or a similar analog molecule labeled with ^{123}I may prove to be a useful alternative to ^{43}K or ^{129}Cs for heart imaging.

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