RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Radioiodinated Branched-Chain Fatty Acids: Substrates for Beta Oxidation? Concise Communication

Charlotte A. Otto, Lawrence E. Brown, and Ann M. Scott

University of Michigan-Dearborn, Dearborn, Michigan, and University of Michigan Medical Center, Ann Arbor, Michigan

Branched-chain iodinated fatty acids have been proposed for use as myocardial imaging agents. Several ω-iodoalkyl and ω-iodoaryl β-methyl branched fatty acids have been synthesized and tested in rats. Myocardial activity levels at t = 5 min are affected by chain length for both alkyl and aryl acids, with chain lengths of 16 carbons possessing higher levels of activity than shorter lengths. Branching significantly lowers heart-to-blood ratios relative to straight-chain analogs. The degree of branching also affects radioactivity levels. Monoalkylation at the β carbon does not reduce the levels for ω-iodoalkyl fatty acids, but dialkylation reduces the levels significantly. Branching in the ω-iodoaryl series of fatty acids altered the time course of activity in the myocardium from a level of activity decreasing with time for the straight-chain acid to an essentially constant level of activity for the branched analogs.

J Nucl Med 25: 75–80, 1985

Since fatty acids constitute the major energy source of heart tissue through β oxidation catabolism, efforts have been made to radiolabel fatty acids to probe myocardial metabolism in normal and damaged tissue. Initial studies established the viability of terminally radioiodinated alkyl fatty acids as imaging agents (*1–3*). Recent work by Machulla (*4*) and Stöcklin (*5*) has shown that radiohalogenated phenyl fatty acids are also useful as myocardial probes. Both phenyl and alkyl fatty acids are expected to undergo β oxidation. Alkyl fatty acids should yield iodoacetyl SCoA or iodopropyl SCoA depending on chain length and absence of hydrolysis of iodine. Theoretically, halophenyl fatty acids should yield comparable halophenyl SCoA esters, but Coenen et al. (*5*) reported significant amounts of *p*-[^{75}Br]bromophenylpropionic acid in addition to *p*-[^{75}Br]bromobenzoic acid. The aromatic ring apparently inhibits complete β oxidation. It remains to be firmly established that loss of myocardial activity for either alkyl or phenyl halo fatty acids is a quantitatively related measure of β oxidation.

Manipulation of the normal, straight-chain fatty acid structure can give rise to a different class of fatty acids—those that inhibit and/or are not substrates for β oxidation. One such manipulation is the inclusion of tellurium at a central position within the chain. F. F. Knapp, Jr and colleagues are actively exploring myocardial imaging with this chain manipulation (*6–7*). A second manipulation is the alteration of the straight-chain structure by alkyl branching. Chemical modification of the fatty-acid molecular structure was originally suggested by Poe et al. (*8*) as a means of increasing myocardial retention of radioactivity in order to obtain better imaging.

An examination of the essential enzymatic steps of β oxidation (see Fig. 1) reveals several sites for interference in the process by mono- or dialkylation. Interference can occur in two ways: (a) the fatty acid is a potential substrate but acts as an inhibitor, and (b) the fatty acid is theoretically not a substrate, and acts as an anti-metabolite. The initial dehydrogenation to yield trans-enoyl SCoA, Step a, requires minimally a hydrogen on both α and β carbons, as shown in bold face in Fig. 1. Dial-

Received May 19, 1983; revision accepted Sept. 15, 1983.

For reprints contact: Charlotte A. Otto, PhD, Assistant Prof. of Chemistry, Dept. of Natural Sciences, University of Michigan-Dearborn, Dearborn, MI 48128.

kylation at either position would yield a fatty acid that would then be an anti-metabolite. Monoalkylation at either the α or β carbon would yield a fatty acid that may be an inhibitor. The second dehydrogenation reaction to yield β-keto acyl SCoA, Step c, requires the presence of at least one β hydrogen; thus monoalkylation at the β position would yield a fatty acid that is an anti-metabolite. Additionally, Steps b and d may be inhibited by fatty acids monoalkylated at the α carbon. Fatty acid activation to SCoA esters and fatty acid transport into the cell and into the mitochondria may also be inhibited by alkylation at the α and β positions.

A preliminary evaluation of branching at the β position was reported by this laboratory (*9*), using 13-[^{125}I]iodo-3-methyltridecanoic acid. The results were not encouraging, as myocardial uptake of radioactivity was low and residence time short. Blood and thyroid radioactivity levels were high, suggesting extensive deiodination. Recently Livni et al. (*10*) reported the synthesis and evaluation of a β-branched fatty acid, β-methyl-[1-^{11}C]heptadecanoic acid. Radioactivity levels in the myocardium were high and essentially constant over 1 hr. It was suggested that the branched fatty acid was trapped in the myocardium as a result of β oxidation. Since the use of a β-methyl C-11 branched fatty acid resulted in prolonged myocardial retention, the iodo β-methyl compound previously evaluated in our laboratory apparently suffered extensive deiodination due to chemical hydrolysis or enzymatic cleavage of the primary alkyl iodide bond.

Prior work (*11*) showed that myocardial radioactivity levels for straight-chain ω-iodo fatty acids at t = 5 min were dependent on chain length. Natural length and longer fatty acids showed higher activity levels and longer myocardial residence times. In the work reported here, our intent was to develop a radioiodinated branched-chain fatty acid that would possess high uptake and long retention in the myocardium. To achieve this goal, we evaluated several parameters: (a) chain length, (b) degree of alkylation at the β carbon, and (c) stabilization of the carbon-iodide bond by incorporation of carbon into an aromatic ring.

MATERIALS AND METHODS

Elemental analysis. Analysis for carbon, hydrogen and halogen was performed commercially.*

Spectroscopic analyses. Proton magnetic resonance (PMR) spectra and infrared (ir) spectra were obtained.

Chemicals. The following compounds or reagents were obtained from commercial sources: 15-phenylpentadecanoic acid, Na^{125}I, 3-methyl glutaric anhydride and 11-bromoundecene.

Chromatography. Thin-layer chromatography was done on cellulose† or silica gel† plates using the solvents indicated. Chromatograms of radioiodinated compounds were analyzed with a radiochromatogram scanner. High-pressure liquid chromatography was carried out using a reverse-phase column with tetrahydrofuran/acetonitrile/water (25/45/35) as solvent (flow rate: 2.0 ml/min).

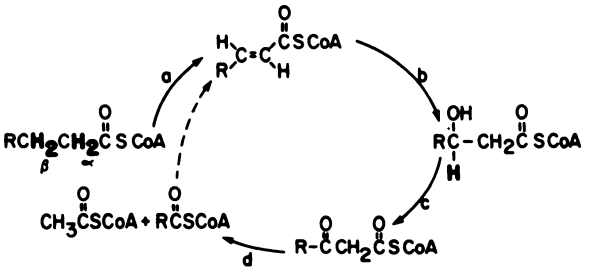

FIG. 1. Scheme for β oxidation of fatty acids: a = acyl-CoA dehydrogenase; b = enoyl-CoA hydrase; c = 3-L-hydroxyacyl-CoA dehydrogenase; d = thiolase.

Synthesis of unlabeled compounds. All new compounds synthesized were fully characterized by NMR, ir, and elemental analysis.

The 13-carbon β-methyl fatty acid was synthesized as previously described (*9*). The 16-carbon ω-bromoalkyl β-methyl fatty acid was synthesized by either a dialkenyl cadmium or a copper (I) iodide catalyzed alkenyl Grignard condensation with the acid chloride of methyl 3-methyl-glutarate. Reduction of the 5-keto-3-methyl alkenyl ester with hydrazine hydrate in the presence of base was followed by addition of hydrogen bromide catalyzed by benzoyl peroxide.

The ω-bromo-β,β-dimethyl fatty acid was synthesized via a condensation of an alkenyl Grignard with diethyl isopropylidene malonate, catalyzed by copper(I) iodide. After ester saponification and decarboxylation, hydrogen bromide was added in the presence of benzoyl peroxide to yield the ω-bromo-β,β-dimethyl fatty acid.

The syntheses of 15-(*p*-iodophenyl)pentadecanoic acid (IPPA) and 8-(*p*-iodophenyl)-3-methyloctanoic acid (8-IPβM) (unpublished data).

Synthesis of radiolabeled compounds. Radiosyntheses in this study were completed using Method A (below) for primary alkyl bromides and Method B (below) for aryl iodides except for 15-(*p*-[^{125}I]iodophenyl)-3-methylpentadecanoic acid (15-IPβM) which was synthesized as described elsewhere (*12*).

Radioiodide exchange. *Method A.* The brominated primary alkyl fatty acids were exchange-labeled as described previously. The residue was formulated by dissolution in absolute ethanol, then dilution with 5% human serum albumin (HSA) to give a final solution of 4% ethanol by volume. To remove free radioiodide, the formulation was passed through a glass column packed with about 1.0 g AG1-X8 anion-exchange resin (chloride form) and washed with formulation. Removal of free radioiodide was confirmed by repeat chromatography

TABLE 1. RADIOACTIVITY LEVELS IN HEART, BLOOD, AND THYROID FOR BRANCHED-CHAIN FATTY ACIDS*

Compound structure/number	Time (min)	Heart	Blood	Thyroid
$[^{125}I](CH_2)_{10}CH(CH_3)CH_2CO_2H$†	5	0.13	0.30	3.12
	10	0.09	0.21	4.46
1	20	0.07	0.18	11.26
$[^{125}I](CH_2)_{13}CH(CH_3)CH_2CO_2H$	5	0.34 ± 0.04	0.19 ± 0.01	1.22 ± 0.06
	10	0.22 ± 0.02	0.14 ± 0.01	2.68 ± 0.35
2	20	0.25 ± 0.04	0.14 ± 0.00	6.30 ± 0.42
$[^{125}I](CH_2)_{11}C(CH_3)_2CH_2CO_2H$	5	0.03 ± 0.00	0.07 ± 0.01	0.76 ± 0.21
	10	0.02 ± 0.00	0.05 ± 0.00	1.69 ± 0.15
3	20	0.02 ± 0.00	0.04 ± 0.01	2.21 ± 0.46

* % kg dose/g ± standard error of the mean by tissue; average of five rats.
† Average of three rats (minimum).

of an aliquot. If loss of radioactivity was >5%, the formulation was rechromatographed with AG1-X8.

Method B. The cold iodophenyl fatty acids were exchange-labeled with $^{125}I^-$ and purified (unpublished data).

Isolated yields ranged from approximately 60–80% for Method A and from 43–95% for Method B. Specific activities were about 1.0 mCi/mmol.

Purity determination. The radiochemical purity was determined by thin-layer radiochromatography using two chromatogram systems as reported (*11*). The iodoalkyl acids were >95% pure; the iodoaryl acids were >98% pure.

Tissue distribution studies. All radioiodinated fatty acids were evaluated in female Sprague-Dawley rats as described previously (*11*).

Analysis of the heart. Analysis was performed as before (*11*) at 5 min after injection for each of two fatty acids, IPPA, and 8-IPβM. Percentages of radioactivity in the pellet, aqueous, and organic fractions of heart homogenates were obtained.

RESULTS AND DISCUSSION

The effects of alkylation and chain length on myocardial activity levels at 5, 10, and 20 min are summarized in Table 1. At all time intervals the radioactivity levels are higher for the natural-length, 16-carbon β-methyl fatty acid, **2**. Activity levels are approximately three times that of the comparable 13-carbon β-methyl compound, **1**. Chain length affects myocardial activity values for branched as well as for straight-chain fatty acids (*11*). The value at 5 min for 16-[^{125}I]-3-methylhexadecanoic acid, 0.34 ± 0.04 % kg dose/g, is experimentally equivalent to 0.39 ± 0.04, the value for the straight-chain acid 16-[^{125}I] iodohexadecanoic acid (*10*). The experimental equivalence of activity levels suggests that the presence of the β-methyl substituent does not significantly alter myocardial concentration for alkyl fatty acids.

Note that the data used above for 16-[^{125}I] iodohexadecanoic acid, 16-IHDA, were obtained using ethanol-Tween 80 as a solubilizing agent (*11*), and that ethanol-HSA was used for **1–3**. A blank experiment was performed using 16-IHDA to determine whether the biodistribution was affected by the formulation change. At 5 min after injection, heart values were 0.39 ± 0.03 % kg dose/g for ethanol-Tween 80 and 0.42 ± 0.03 for ethanol-HSA; blood values were 0.08 ± 0.02 for Tween 80 and 0.09 ± 0.00 for HSA. The change in solubilizing agent apparently does not affect biodistribution, since the values are experimentally equivalent.

Dialkylation virtually eliminates myocardial extraction, since activity levels are reduced by a factor of ten in comparison with the 16-carbon, β-methyl fatty acid. Liver concentration for the dialkyl fatty acid is high compared with other fatty acids, both straight- and branched-chain. At t = 5 min, the value is 1.71 ± 0.39 % kg dose/g for the dimethyl acid **3**, 0.54 ± 0.02 for the

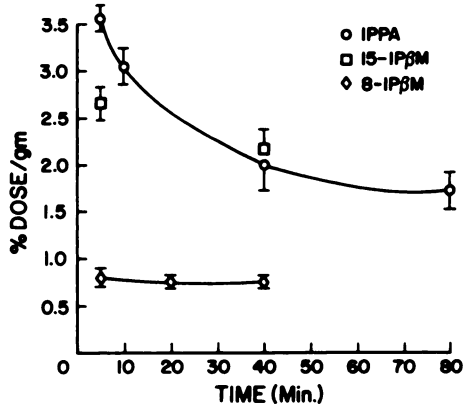

FIG. 2. Time course of radioactivity in myocardium of Sprague-Dawley rats after i.v. injection of 15-(*p*-iodophenyl)pentadecanoic acid and 8-(*p*-iodophenyl)-3-methyloctanoic acid.

monomethyl (16-carbon) acid **2**, and 0.85 ± 0.09 for the straight-chain acid (*11*).

Thyroid radioactivity values are a rough indication of deiodination. At all time intervals, the 13-carbon acid **1**, had higher thyroid activity levels than either **2** or **3**. Blood activity levels followed the same order, with the highest levels associated with **1** and the lowest with **3**. Tissue samples were also obtained from lung and muscle. Radioactivity values in both of these tissues were comparable with those published elsewhere (*11*) for straight-chain ω-iodoalkyl fatty acids. The data are available on request.

The decrease of myocardial radioactivity levels with time was not expected. If the β-methyl alkyl fatty acids were not substrates for the second dehydrogenation step of β oxidation, one would expect to observe a linear or slowly decreasing relationship between radioactivity levels and time. This type of relationship was observed by Livni et al. (*10*) for β-methyl-[^{11}C] heptadecanoic acid. The difference in behavior suggests chemical or enzymatic deiodination of the ω-iodo branched-chain alkyl fatty acids independent of β oxidation. Similar deiodination processes presumably can occur for the ω-iodo straight-chain fatty acids. In either case, the interpretation of myocardial imaging in terms of β oxidation metabolism is compromised.

A similar chain-length dependence for myocardial activity is found for the iodide-stabilized, ω-iodophenyl β-methyl fatty acids. Figure 2 illustrates the time course of activity for 15-(*p*-[^{125}I]iodophenyl)pentadecanoic acid (IPPA), and 8-(*p*-[^{125}I]iodophenyl)-3-methyloctanoic acid (8-IPβM). Data at 5 and 40 min from 15-IPβM are included for purposes of comparison. A time course for this acid in Fischer rats is published elsewhere (*12*). Myocardial activity is reduced by shortening the alkyl chain length. For 15-IPβM at 5 min, the activity level is 2.66 ± 0.18 % dose/g whereas the level for 8-IPβM is 0.80 ± 0.10. Branching also reduced myocardial activity levels, since myocardial activity for IPPA at 5 min was 3.56 ± 0.14 % dose/g whereas that of the branched analog was 2.66 ± 0.18. We did not expect that branching would reduce myocardial activity levels significantly for the iodoaryl and not for the iodoalkyl fatty acids. Possible differences in lipophilicity between aryl and alkyl fatty acids may account for these observations.

TABLE 2. CHEMICAL STRUCTURES, HEART VALUES AND HEART-TO-BLOOD RATIOS AT 5 min

Structure	Heart*	Heart/ Blood
$[^{125}I](CH_2)_{10}CH(CH_3)CH_2CO_2H$	0.13	0.4
$[^{125}I](CH_2)_{13}CH(CH_3)CH_2CO_2H$	0.34	1.8
$[^{125}I](CH_2)_{15}CO_2H$	0.39	5.6
$[^{125}I]C_6H_4(CH_2)_5CH(CH_3)CH_2CO_2H$	0.17	0.5
$[^{125}I]C_6H_4(CH_2)_{14}CO_2H$	0.69	4.6

* % kg dose/g by tissue; average of four rats (minimum).

The difference in the time course of activity for the branched-chain ω-iodophenyl fatty acids compared with the straight-chain ω-iodophenyl acids is clear. IPPA is expected to behave as 15-(*p*-[^{75}Br]bromophenyl)pentadecanoic acid (BPPA) (*5*), which has been shown to undergo β oxidation. The activity of IPPA in the heart decreases with time, as was observed for BPPA. Both aryl branched-chain fatty acids remain in the heart at experimentally constant levels from 5 to 40 min. F. F Knapp and co-workers report similar constant levels of radioactivity from 5 to 60 min for 15-IPβM in Fischer rats (*12*, M. M. Goodman, G. Hirsh, F. F. Knapp, unpublished data). These results parallel data using β-methyl-[^{11}C] heptadecanoic acid (*10*), discussed above.

The time-course data presented here for both aryl and alkyl β-methyl fatty acids, coupled with the β-methyl-[^{11}C] alkyl fatty acid data (*10*), are evidence for deiodination of the ω-iodoalkyl β-methyl fatty acids.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN HEART HOMOGENATES AT 5 min

Compound	% Pellet	% Aqueous	% Organic	% Recovered
$[^{125}I]C_6H_4(CH_2)_{14}CO_2H$*	12.4 ± 1.0	6.4 ± 1.6	58.1 ± 2.1	80.4 ± 2.8
$[^{75}Br]C_6H_4(CH_2)_{14}CO_2H$			57† 55‡	
$[^{125}I]C_6H_4(CH_2)_5CH(CH_3)CH_2CO_2H$*	9.2 ± 1.7	15.9 ± 3.1	45.9 ± 5.4	72.6 ± 7.5

* Data based on six hearts per compound.
† At 0.5 min (*5*).
‡ At 10 min (*5*).

Thus side-chain manipulation designed to change the metabolic fate of these acids had no significant effect on preventing deiodination. The similarity in time courses for the aryl β-methyl fatty acids and their alkyl C-11 counterparts (*10*) may reflect differences in lipophilicity, and thus potential differences in substrate availability rather than a similarity in metabolism. Chemical identification of metabolites and comparison of subcellular distribution data would be necessary to clarify this point. The differences in time-course data for the ω-iodoaryl straight-chain fatty acid and ω-iodoaryl branched fatty acids (see Fig. 2) cannot readily be attributed to lipophilic differences. The slower rate (or lack) of metabolism suggested by the data for aryl branched acids can be explained by assuming that these acids are not substrates for β oxidation. The data presented here, however, do not preclude other explanations.

Heart-to-blood ratios at 5 min are tabulated in Table 2. Radiolabeled fatty acids need to have high heart-to-blood ratios in order to obtain myocardial rather than blood-pool images. The ratios are highest for the straight-chain iodoalkyl and iodoaryl fatty acids and are significantly lower for branched-chain analogs. Note that the β-methyl substituted fatty acids are all racemic mixtures of R and S optical isomers. F. F. Knapp has suggested that the myocardium may extract only one of the optical isomers, which would explain the high blood activity levels (*12*). A definitive answer awaits synthesis and evaluation of optically pure R and/or S β-methyl fatty acids.

Homogenates of the heart were extracted with chloroform-methanol as discussed in Materials and Methods. The distribution of radioactivity for IPPA and 8-IPβM is summarized in Table 3. Similar distribution was observed for both compounds. The percent activity in the organic fraction for IPPA is experimentally equivalent to that reported by Coenen et al. for BPPA (*5*). The distribution between pellet, aqueous, and organic fractions is similar to that previously reported for 19-[^{125}I]iodononadecanoic acid (*11*).

In conclusion, the chain lengths of β-methyl branched-chain iodoalkyl and iodoaryl fatty acids have been shown to affect myocardial activity levels at 5 min after injection. Natural-length fatty acids of about 16 carbons have higher radioactivity levels than shorter-length fatty acids. Myocardial activity levels for straight-chain fatty acids are also affected by chain length (*11*). The degree of alkylation has also been shown to affect myocardial radioactivity levels. Monomethyl iodoalkyl fatty acids are similar to straight-chain acids, but dimethylation severely reduced myocardial activity values. That extensive deiodination of the β-methyl iodoalkyl fatty acids occurs is also supported by the data, which suggest that a similar deiodination process may occur for the straight-chain iodofatty acids. Finally, the data presented here suggest that the aryl β-methyl fatty acids do not appear to be substrates for β oxidation. These aryl branched fatty acids may be useful as myocardial imaging agents and possibly useful as probes of metabolic processes other than β oxidation.

ACKNOWLEDGMENTS

Research was supported by Grant No. HL25459-03 from the National Heart, Lung and Blood Institute, by D.O.E. contract #DE-ACO-2-76EV02031 and by a Student Research Fellowship (1981) for Ann M. Scott from the Michigan Heart Association.

The authors are particularly grateful to F. F. Knapp, Jr. and M. M. Goodman at Oak Ridge National Laboratory for the gift of 15-IPβM. The authors thank D. D. Marsh and K. Lapanowski-Netzel for technical assistance and L. Markham for help in preparing the manuscript.

FOOTNOTES

* Spang Microanalytical Laboratory, Eagle Harbor, Michigan.
† Whatman K2F cellulose plates and Whatman K6F silica gel plates.

REFERENCES

1. POE ND, ROBINSON GD JR, MACDONALD NS: Myocardial extraction of labeled long-chain fatty acid analogs.

Proc Soc Exp Biol Med 148:215-218, 1975

2. MACHULLA H-J, STÖCKLIN G, KUPFERNAGEL C, et al: Comparative evaluation of fatty acids labeled with C-11, Cl-34m, Br-77, and I-123 for metabolic studies of the myocardium: Concise communication. *J Nucl Med* 19:298-302, 1978
3. ROBINSON GD JR, LEE AW: Radioiodinated fatty acids for heart imaging: iodine monochloride addition compared with iodide replacement labeling. *J Nucl Med* 16:17-21, 1978
4. MACHULLA H-J, MARSMANN M, DUTSCHKA K: Biochemical concept and synthesis of a radioiodinated phenylfatty acid for in vivo metabolic studies of the myocardium. *Eur J Nucl Med* 5:171-173, 1980
5. COENEN HH, HARMAND M-F, KLOSTER G, et al: 15-(*p*-[^{75}Br]-bromophenyl)-pentadecanoic acid: Pharmacokinetics and potential as heart agent. *J Nucl Med* 22:891-896, 1981
6. GOODMAN MM, KNAPP FF JR: Synthesis of 15-(*p*-iodophenyl)-6-tellurapentadecanoic acid: A new myocardial imaging agent. *J Org Chem* 47:3004-3006, 1982
7. GOODMAN MM, KNAPP FF JR, CALLAHAN AP, et al: Synthesis and biological evaluation of 17-[^{131}I]-iodo-9-telluraheptadecanoic acid, a potential imaging agent. *J Med Chem* 25:613-618, 1982
8. POE ND, ROBINSON GD JR, GRAHAM LS, et al: Experimental basis for myocardial imaging with ^{123}I-labeled hexadecanoic acid. *J Nucl Med* 17:1077-1082, 1976
9. OTTO CA, BROWN LE, WIELAND DM, et al: Structure-distribution study of I-125-ω-iodofatty acids. *J Labelled Cpd Radiopharm* 18:43-44, 1981
10. LIVNI E, ELMALEH DR, LEVY S, et al: Beta-methyl [1-^{11}C] heptadecanoic acid: a new myocardial metabolic tracer for positron emission tomography. *J Nucl Med* 23:169-175, 1982
11. OTTO CA, BROWN LE, WIELAND DM, et al: Radioiodinated fatty acids for myocardial imaging: Effects of chain length. *J Nucl Med* 22:613-618, 1981
12. GOODMAN MM, KIRSCH G, KNAPP FF JR: Synthesis of radioiodinated ω-*p*-(iodophenyl)-substituted methyl branched long-chain fatty acids. *J Labelled Cpd Radiopharm* 19: 1316-1318, 1982