CONF-8510195--1

New Radioiodinated Methyl-Branched Fatty Acids for Cardiac Studies

CONF-8510195--1

DE86 002213

F. F. Knapp, Jr., K. R. Ambrose and M. M. Goodman

Nuclear Medicine Group
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831 U.S.A.

Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

For, "Proceedings," Symposium on Assessment of Myocardial Metabolism by Cardiac Imaging, Vienna, Austria, October 26, 1985.

ABSTRACT

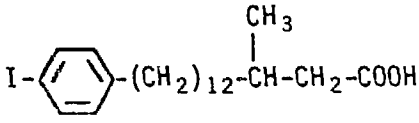
The effects of 3-methyl-substitution on the heart retention and metabolism of 3-R,S-methyl-(BMIPP) and 3,3-dimethyl-(DMIPP) analogues of 15-(p-iodophenyl)-pentadecanoic acid (IPP) have been studied in rats. Methyl-substitution considerably increased the myocardial half-time values in fasted rats: IPP, 5-10 min; BMIPP, 30-45 min; DMIPP, 6-7 h. Because of the observed differences in the relative myocardial uptake and retention of these agents, an evaluation of the subcellular distribution profiles and the distribution of radioactivity within various lipid pools extracted from cell components was performed. Studies with DMIPP in fasted rats have shown high levels of the free fatty acid and only slow conversion to triglycerides. These data are in contrast to the rapid clearance of the straight chain IPP analogue and rapid incorporation into triglycerides. These data suggest that the prolonged myocardial retention observed ith DMIPP in vivo may result from innibition of β-oxidation. Subcellular distribution studies have shown predominate association of DMIPP and BMIPP with the mitochondrial and microsomal fractions, while IPP was primarily found in the cytoplasm. Because of the unique "trapping" properties and the high heart:blood ratios, [^{123}I]DMIPP should be useful for evaluation of aberrations in regional myocardial uptake.

INTRODUCTION

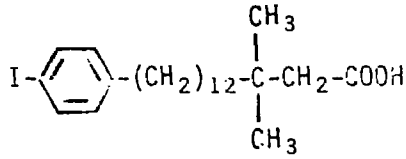
Structurally-modified fatty acids that show normal myocardial extraction but are not readily catabolized through the oxidative chain can be used to evaluate regional fatty acid uptake since they should not be catabolized and thus show prolonged myocardial retention. We have recently investigated the use of methyl-branching in the 3-position of the alkanoic acid chain of terminal iodophenyl fatty acids to inhibit β-oxidation and prolong myocardial retention.[1] The effect of methyl-branching at the 3-position on myocardial retention in rats was assessed by a comparison of the distribution of the methyl-branched agent with the myocardial uptake of the corresponding straight chain analogue, 15-(p-[^{125}I]iodophenyl)pentadecanoic acid (IPP). The increased myocardial uptake and retention of radioactivity following injection of 15-(p-[^{125}I]-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP) in comparison with [^{125}I] IPP suggested that methyl-branching at position-3 could be an effective means of inhibiting myocardial metabolism. The BMIPP agent shows longer myocardial retention in rats than the straight chain IPP analogue, but does not show irreversible retention. The results of initial clinical studies with [^{123}I] BMIPP are described in another section of these proceedings by Dudczak et al. We have now prepared and evaluated the 3,3-dimethyl-analogue (DMIPP) and found that this new agent exhibits negligible washout over 1-2 h after administration to rats. In this paper we describe the relative retention properties of the IPP analogues. In addition, the results of detailed comparative subcellular distribution and lipid analyses are described. The goal of these studies was to correlate in vivo retention with the molecular fate and metabolism of these agents.

METHODS

To gain insight into the metabolism of the 3-methyl-branched fatty acids, subcellular distribution studies and lipid analyses have now been performed using radioiodinated IPP, BMIPP, and DMIPP (Figure 1). Fischer rats were injected intravenously with either [^{125}I]IPP, [^{125}I]BMIPP or [^{125}I]DMIPP



15-(p-Iodophenyl)-3-R,S-Methylpentadecanoic Acid (BMIPP)



15-(p-Iodophenyl)-3,3-Dimethylpentadecanoic Acid (DMIPP)

Figure 1. Structures of 3-methyl-branched iodinated fatty acid analogues.

complexed with 6% bovine serum albumin. In some studies dual and triple label mixtures were also used (vide infra). For subcellular distribution studies, rat hearts were excised and homogenized and the crude pellet, mitochondrial, microsomal and cytoplasmic fractions obtained by differential centrifugation. Lipids were extracted from either whole heart homogenates or the isolated subcellular fractions, using chloroform:methanol (2:1) by the usual Folch procedure. The extracted lipids were analyzed by thin-layer chromatography using a petroleum ether-ethyl ether-acetic acid (80:20:1) solvent system; polar lipids (R_f = 0.00), diglycerides (R_f = 0.20), free fatty acids (R_f = 0.50), and triglycerides (R_f = 0.75).

RESULTS AND DISCUSSION

The distribution of radioactivity in the lipid pools from whole hearts of fasted rats up to 1 h after administration of the radioiodinated fatty acid was evaluated. Injection of the unbranched [^{125}I]IPP analogue results in initial high myocardial extraction followed by rapid washout (Figure 1a). Lipid analysis has demonstrated that the majority of the radioactivity is initially (1-3 min) present in the free fatty acid fraction. As expected, there is a rapid increase of radioactivity associated with the triglyceride fraction which is maximal at 10 min. Radioactivity in the diglyceride fraction is maximal at 5 min (20% total activity) and decreased slowly over the remainder of the assay period. Similar data for BMIPP and DMIPP are also presented in Figure 1. With the BMIPP monomethyl-branched analogue the majority of radioactivity is found at all assay times in the triglyceride fraction (Figure 1b). Radioactivity in the free fatty acid fraction is significant only at the earliest time periods. The

radioactivity in the diglyceride fraction is maximal at 3-5 min (14% extracted activity), but decreases to a level similar for the free fatty acid fraction. With the DMIPP dimethyl-branched analogue, the free fatty acid fraction initially contains the majority of radioactivity (Figure 1c). At later time periods, the majority of the radioactivity is found in triglyceride fraction. The rate of "conversion," however, is comparatively slower than with IPP. Similar studies with the three analogues were also conducted with unfasted rats demonstrating a faster incorporation into the triglyceride fraction with IPP and BMIPP and somewhat slower incorporation with DMIPP.

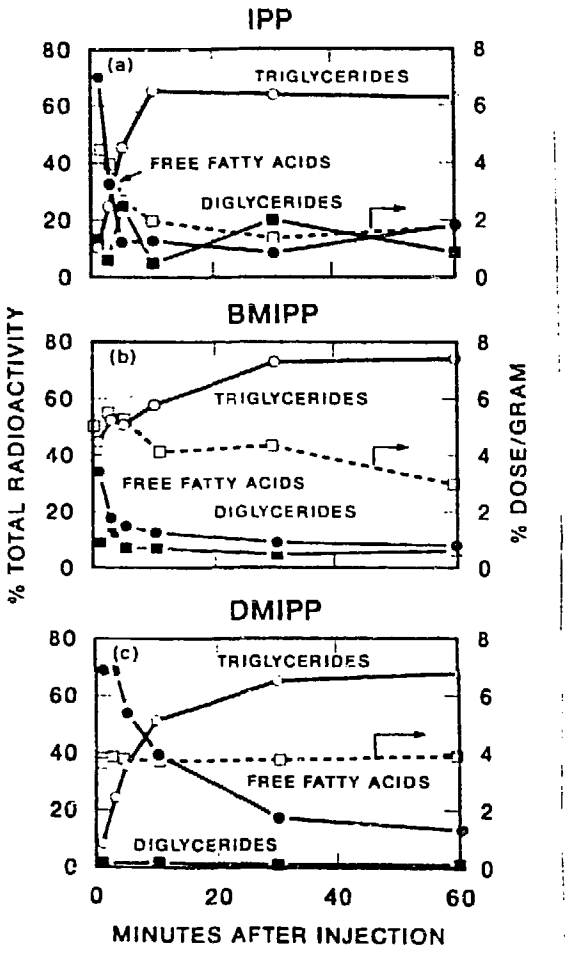


Figure 2. Heart uptake (---) and distribution of radioactivity within the lipid pools of rat hearts (——) following intravenous administration of (a) 15-(p-[^{125}I]iodophenyl)-pentadecanoic acid (IPP), (b) 15-(p-[^{125}I]iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP), or (c) 15-(p-[^{125}I]iodophenyl)-3,3-dimethylpentadecanic acid (DMIPP).

From these preliminary tissue distribution studies, the 5 and 30 min time periods were chosen for examination of the lipid pools in the hearts of rats injected with the [^{125}I]IPP/[^{131}I]BMIPP/[^{123}I]DMIPP triple-labeled mixture. This study was designed to eliminate the possible errors inherent in a comparison of the differences that may be detected in subcellular distribution and lipid pools between different groups of rats. In this manner, each measurement has essentially an internal control and differences observed with the three analogues can be directly compared. The mass of each fatty acid injected was very similar to eliminate any effects of possible mass differences on myocardial uptake. The details of this experiment are given in Table 1. For the triple-labeling experiments, the I-123 (159 keV) and I-131 (262 keV) photopeaks were counted simultaneously in two windows and the samples then stored in the cold until the I-123 contribution to the I-125 x-ray photopeak region was less than 4-5%. The samples were then counted again to determine the distribution of I-125.

Table 1. Summary of experimental details for the evaluation of [^{125}I]IPP/[^{131}I]BMIPP/[^{123}I]DMIPP triple-labeled fatty acid mixture administered to female Fischer rats.

	Radioiodinated fatty acid		
	[^{125}I]IPP	[^{131}I]BMIPP	[^{123}I]DMIPP
Specific activity (μCi/nmole)	2.74	0.47	1.07
μCi/rat	89	15	53
nmole/rat	32.5	32	49.5
μgm/rat	16	16	24

The major differences in lipid pools of the three analogues are seen at 5 min post-injection (Figure 3). At this time the relative proportion of radioactivity in the diglyceride fraction ranks in this order: IPP>BMIPP>DMIPP, whereas the relative distribution of radioactivity in the free fatty acid pool is reversed: DMIPP>BMIPP>IPP. At 30 min the majority of radioactivity (64-82%) chromatographs with the triglyceride standard for all three analogues.

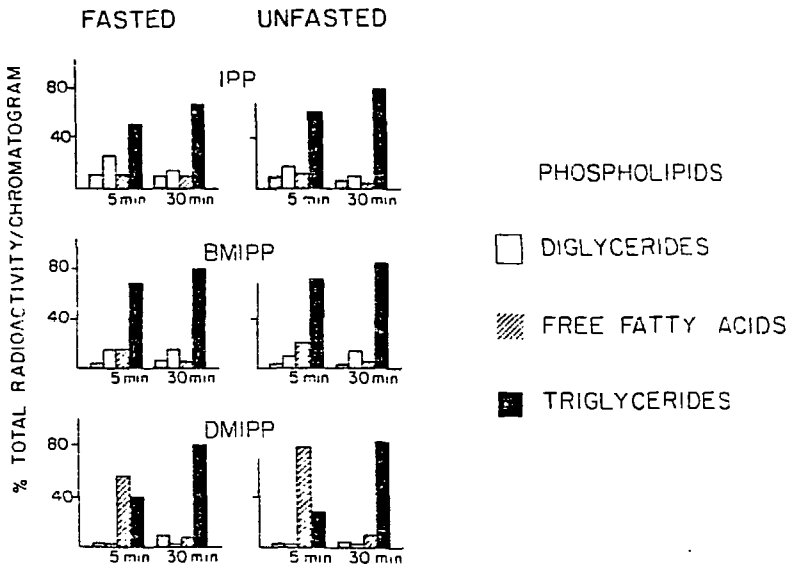


Figure 3. Comparison of the distribution of ^{125}I, ^{131}I and ^{123}I in lipid pools of heart extracts after administration of the [^{125}I]IPP/[^{131}I]BMIPP/[^{123}I]DMIPP mixture to fasted and unfasted female Fischer rats.

Other investigators have evaluated the myocardial lipid distribution of various radiolabeled fatty acids, including the straight chain IPP agent. In one study, 75-80% of the extracted activity from the hearts of unfasted rats injected with IPP was found in the triglyceride fraction.[3] In later reports,[4] between 45-57% of the activity was in the triglyceride fraction with approximately 10% in each of the diglyceride and free fatty acid fractions in fasted rats. These data are similar to the results reported here although specific assay times cannot be compared. In a study by Otto et al[5] terminally iodinated long chain fatty acids [I-$(CH_2)_n$COOH, where n = 18 or 21] appeared to be fated primarily for triglyceride storage, whereas shorter chain fatty acids ($n \leq 15$) were subject to β-oxidation. The fatty acids in this study did not contain the terminal phenyl moiety and were not branched. It is thus difficult to draw a comparison, although it appears that the phenylpentadecanoic analogues have a chain length that would favor triglyceride storage.

To further investigate the metabolism of the 3-methyl-branched and straight chain analogues, subcellular distribution experiments were performed in independent studies with extracts of hearts excised from fasted and nonfasted rats 30 min after injection of the [^{125}I]labeled analogues. Comparison of the results obtained in nonfasted rats (Figure 4) shows little difference in the relative subcellular distribution of each compound at 30 min. In rats fasted

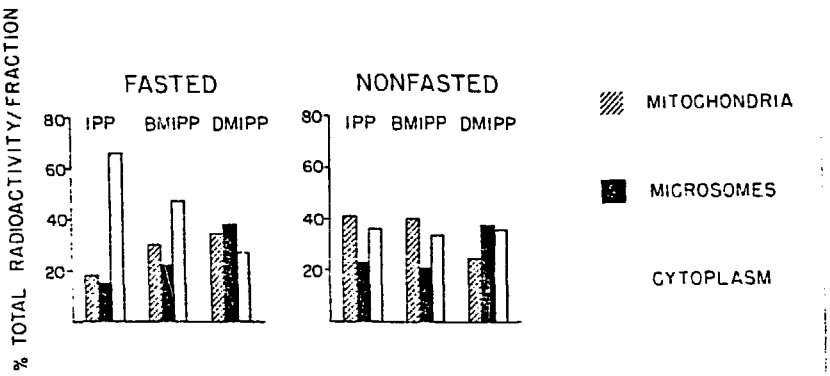


Figure 4. Distribution of radioactivity in subcellular fractions from heart homogenates 30 min after intravenous administration of either [^{125}I]IPP, [^{125}I]BMIPP or [^{125}I]DMIPP to separate groups of fasted and nonfasted female Fischer rats.

for 24 h, however, there are major differences in the subcellular distribution profiles of the branched and unbranched analogues in the subcellular fractions isolated 30 min after injection. The IPP straight chain analogue, which shows rapid washout in the hearts of fasted rats, resulted in a proportionally greater amount of radioactivity in the cytoplasmic fraction with the corresponding proportional lower activity in the mitochondrial and microsomal fractions.

Although this subcellular distribution profile of IPP obtained in fasted rats was significantly different from that observed with unfasted rats, comparison of the % dose/fraction values demonstrated similar levels of IPP in the cytoplasm. Thus, the apparent increase of radioactivity in the cytoplasm of the hearts of fasted rats injected with IPP is actually a proportional increase due to the loss of activity from the mitochondrial and microsomal fractions. It should be noted that relative retention of all three analogues is prolonged in unfasted rats and the subcellular distribution profiles are also similar. The monomethyl-branched BMIPP analogue, which shows longer myocardial retention in vivo than IPP, showed comparatively higher percentages of radioactivity in the mitochondrial and microsomal fractions. The DMIPP dimethyl-branched analogue, which shows the longest in vivo myocardial retention of the three analogues, also has the highest proportion of radioactivity associated with the microsomal and mitochondrial fractions. From these results and from previous studies in which the subcellular distribution profiles of a number of fatty acids were compared, it appears that the length of retention in the hearts of fasted rats correlates with the relative proportion of radioactivity found in the mitochondrial and microsomal fractions 30 minutes after injection.

These subcellular studies were expanded to include both the 5 and 30 min assay periods and also lipid analysis of the subcellular fractions. Because the 3-4 day period required to complete these analyses made it impractical to use ^{123}I-labeled fatty acids, ^{131}I/^{125}I-labeled mixtures of two analogues [^{131}I]IPP/[^{125}I]DMIPP and [^{131}I]BMIPP/[^{125}I]DMIPP) were evaluated in fasted rats in two separate experiments. The comparative differences in the 30 min subcellular profiles observed in the earlier experiments were reproduced in these dual label experiments, and the subcellular distribution patterns at 5 min resembled those found at 30 min. It thus appears that shifts in the relative proportion of radioactivity within the subcellular fractions do not occur between 5 and 30 min (Figure 5). As would be expected from previously described lipid analysis of "whole hearts" (Figure 3), however, there were differences observed in the lipid pools within these cellular fractions at the different time intervals. Lipid analysis of the pellet of the centrifugation of the crude homogenate (see Figures 6 and 7) was performed and may approximate the lipid profile of unfractionated heart tissue. With all cell fractions for the three analogues, there was a shift to a predominance of radioactivity in the triglyceride fraction 30 min after injection (Figures 6 and 7). The comparison of radioactive lipid pools between the different cell fractions of hearts of rats injected with the same compound or the comparison of the same subcellular component of hearts with different compounds injected, however, showed a number of observable differences.

With the straight-chain IPP analogue (Figure 6), the radioactive lipid profiles of the microsomal and crude pellet fractions are quite similar, whereas the mitochondrial fraction shows a greater proportion of free fatty acid. In the cytoplasmic fraction 5 min after injection, the majority of the extractable

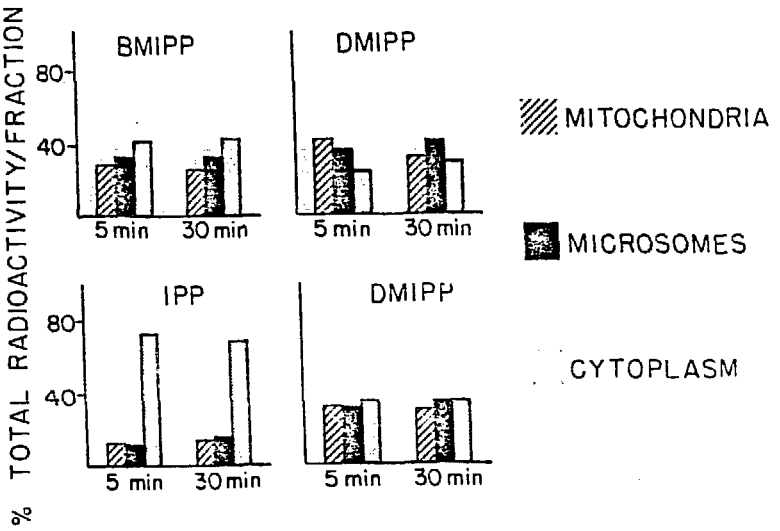


Figure 5. Comparison of the subcellular distribution of ^{125}I and ^{131}I from heart homogenates at 5 min and 30 min following the intravenous administration of either [^{131}I]IPP/[^{125}I]DMIPP or [^{131}I]BMIPP/[^{125}I]DMIPP mixtures to fasted female Fischer rats.

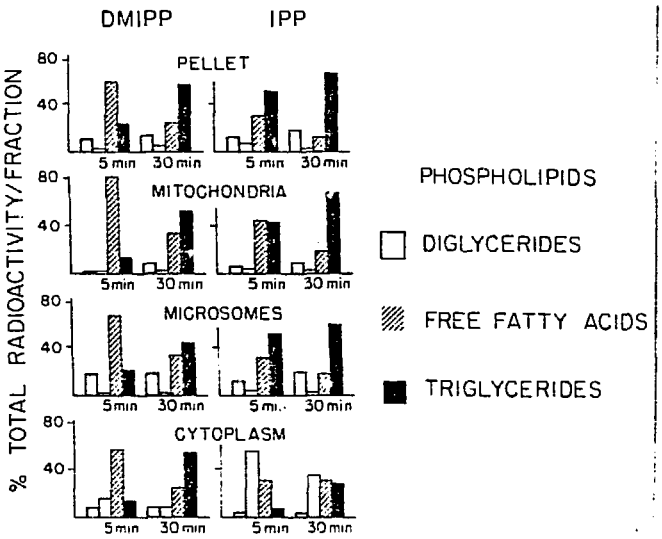


Figure 6. Distribution of radioactive lipids from the subcellular fractions of the [^{131}I]IPP/[^{125}I]DMIPP study described in Figure 5.

radioactivity chromatographs with the diglyceride standard. With the monomethyl-branched BMIPP analogue (Figure 7), the distribution of radioactive lipids in the microsomal fraction again resembles the crude pellet. For both the cytoplasmic and mitochondrial fractions, however, radioactivity in the free fatty acid component becomes a more predominant feature of the lipid profile particularly 5 min after injection. With DMIPP (Figures 6 and 7) both the microsomal and cytoplasmic fractions of rat hearts injected with this dimethyl-branched fatty acid show lipid profiles similar to the crude pellet. In the mitochondrial fractions from these hearts, however, almost 80% of the extractable activity is in the form of free fatty acid.

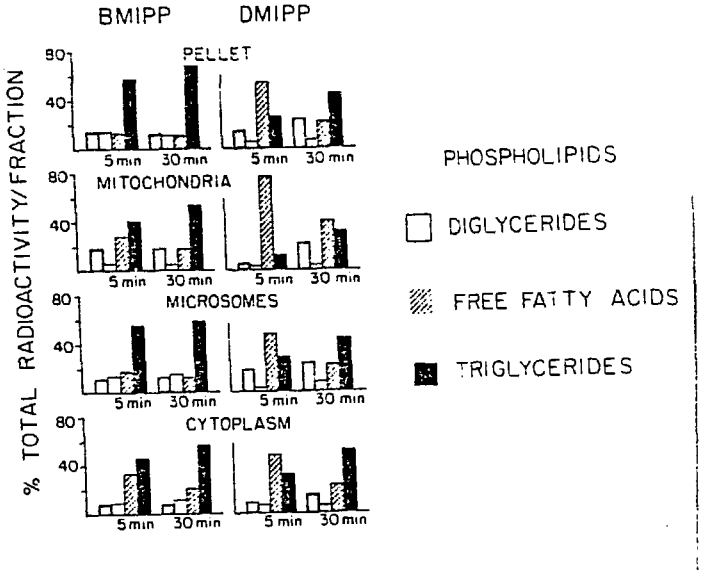


Figure 7. Distribution of radioactive lipids from the subcellular fractions of the [^{131}I]BMIPP/[^{125}I]DMIPP study described in Figure 5.

The progressively longer myocardial retention observed with increasing methyl-substitution on the β-carbon suggests some interference with the catabolism of these modified fatty acids. As shown in Figure 8, monomethyl-substitution at the β-carbon would be expected to interfere with β-oxidation at the stage of formation of the β-keto acid intermediate (4). However, after initial α-hydroxylation (1-5), subsequent β-oxidation would be possible following the loss of propionic acid (7-11). Thus, the moderate loss of BMIPP could be explained by the "modified" catabolism of this agent. In contrast, DMIPP should not be catabolized by the usual α- or β-oxidative routes (Figure 9), and should demonstrate unique myocardial retention. There thus appears to be a relation beween myocardial retention and inhibition of oxidative catabolism. Despite this analysis, a component behaving chromatographically like hippuric acid has been observed in the urine of rats after administration

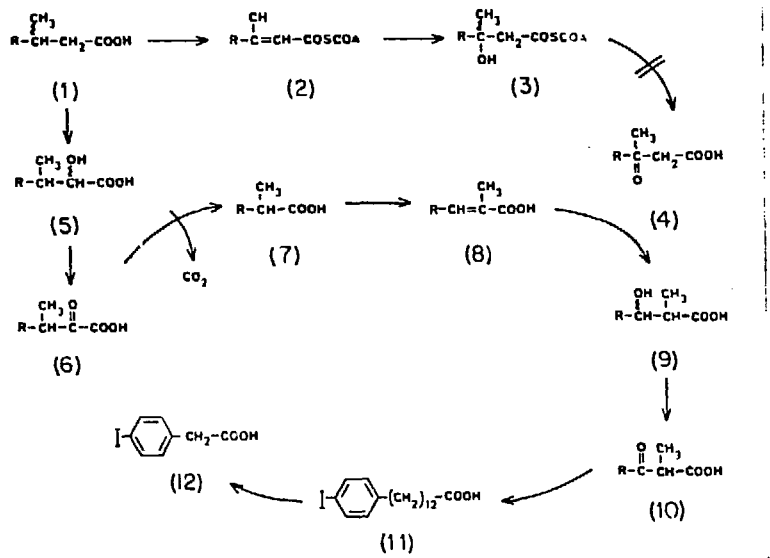


Figure 8. Possible catabolism of 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP) by either α- or β-oxidation; R = the remainder of the (p-iodophenyl)alkyl chain.

of BMIPP and DMIPP (F. F. Knapp et al, unpublished data). Such a component has also been detected in the urine from patients after injection of [^{123}I] BMIPP[6] and these data are discussed in another section of these proceedings by Dudczak et al. Such catabolic products probably result from a different metabolic pathway in the liver involving perhaps an initial biotin-catalyzed methyl carboxylation.

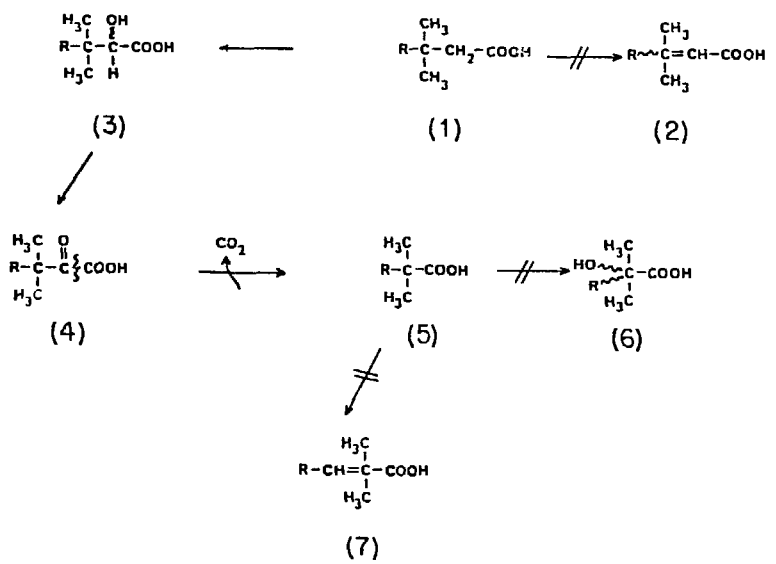


Figure 9. Potential catabolism of 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPP) by α- or β-oxidation. R = remainder of the (p-iodophenyl)alkyl chain.

An evaluation of the subcellular distribution profiles and the distribution of radioactive lipids extracted from the cell components shows distinct differences for the three analogues. Both the relative association within the mitochondrial versus the cytoplasmic fractions and the lipid profiles of these two cell components appear to reflect major differences that can be correlated between the structures and the observed differences in the in vivo behavior of these analogues. The IPP straight chain analogue shows poor myocardial in vivo retention and its metabolic products seem to be primarily associated with the cytoplasm, initially found predominantly in the diglyceride pool. In contrast, DMIPP shows the best myocardial retention of the three analogues and shows an early association with the mitochondria where the extractable radioactivity is almost exclusively in the free fatty acid fraction. Finally, BMIPP shows primarily equal distribution in the mitochondrial, microsomal and cytoplasmic fractions and seems to demonstrate the most rapid incorporation into triglycerides. Further analyses and identification of metabolites is being pursued. The present studies demonstrate clear differences in the metabolic basis for myocardial retention of these methyl-branched fatty acids.

SUMMARY

Our comparison of the relative myocardial retention properties of the unmethylated and methylated analogues led to two major questions: how are these differences reflected at the molecular level, and how may the new [^{123}I] DMIPP agent be useful for the evaluation of regional myocardial fatty acid uptake. The present studies of the effects of methyl-substitution on the myocardial retention, subcellular distribution and lipid pool profiles of the radioiodinated modified-fatty acids have shown major differences in these properties which can be directly correlated with the relative degree of myocardial retention. Major differences include the "rate" of incorporation into triglycerides (IPP>BMIPP>DMIPP) and the apparent incorporation into diglycerides (IPP>BMIPP>DMIPP). In addition, large differences in the levels of radioactivity in the free fatty acid pool (DMIPP>BMIPP>IPP) have been observed which can be directly compared with the degree of retention observed in vivo. Also, the differences observed in the subcellular distribution profiles appear to mirror the relative retention characteristics. While IPP shows rapid myocardial wash-out in vivo, this agent appears to be primarily associated with the cytoplasmic fraction in fasted rats. In contrast, DMIPP exhibits prolonged retention and is principally associated with the mitochondrial and microsomal particulate fractions. The prolonged retention of DMIPP suggests that the [^{123}I]-labeled agent may be useful as both a research and clinical tool for the evaluation of aberrations of regional fatty acid uptake. The prolonged retention and high heart:blood levels are desirable for single-photon-emission-computerized tomographic studies (SPECT). In addition to the potential use for the study of various cardiomyopathies, the recent demonstration of the apparent uncoupling of regional perfusion with fatty acid uptake in autoradiographic studies with salt sensitive Dahl rats suggests that this agent may be useful in studying chronic hypertension.[7] In these cases it may be possible to study the properties of fatty acid metabolism in the chronic hypertensive state which are necessary to preserve ventricular function.

ACKNOWLEDGEMENTS

Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc. The authors thank A. P. Callahan, E. B. Cunningham and B. A. Owen for technical assistance, and L. S. Ailey for typing the manuscript.

REFERENCES

1. Goodman MM, Kirsch G, Knapp FF Jr, "Synthesis and evaluation of radioiodonated terminal p-iodophenyl-substituted α- and β-methyl-branched fatty acids," J Med Chem, 25, 390-397, 1984.

2. For a description of the synthesis, radioiodination and tissue distribution properties of the new DMIPP agent in laboratory animals see: Knapp FF Jr, Goodman MM, Kirsch G, Callahan AP, "Radioiodinated 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid (DMIPP): a new agent to evaluate regional myocardial fatty acid uptake," J Nucl Med, 26, P 123, 1985a; Knapp FF Jr, Goodman MM, Callahan AP, Kirsch B, "Radioiodinated 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid: a useful new agent to evaluate myocardial fatty acid uptake," J Nucl Med, 1985b (submitted); Knapp FF Jr, Goodman MM, Ambrose KR, Proceedings, European Nuclear Medicine Congress, London, Sept. 2-6, 1985 (Nucl Med Comm, 6, 551, 1985).

3. Reske SN, Machulla H-J, Winkler C, J Nucl Med, 23, P 10, 1982.

4. Reske SN, Saver W, Machulla H-J, Winkler C, J Nucl Med, 25, 1335-1342, 1984.

5. Otto CA, Brown LE, Wieland DM, Beierwaltes WH, J Nucl Med, 22, 613-618, 1981.

6. Knapp FF Jr, Goodman MM, Ambrose KR, Som P, Brill AB, Yamamoto K, Kubota K, Yonekura Y, Dudczak R, Angelberger P, Schmoliner R, "The development of radioiodinated 3-methyl-branched fatty acids for evaluation of myocardial disease by single photon techniques. In: van der Wall EE (ed) Noninvasive imaging of cardiac metabolism. Martinus Nijhoff Medical Publishers, Amsterdam (in press).

7. Yonekura Y, Brill AB, Som P, Yamamoto K, Srivastava Suresh C, Iwai J, Elmaleh DR, Livni E, Strauss HW, Goodman MM, and Knapp FF, Jr, "Quantitative autoradiographic measurement of regional myocardial substrate utilization in hypertensive rats," Science, 227, 1494-1496, 1985; Yonekura Y, Tamaki N, Torizuka K, Brill AB, Som P, Yamamoto K, Srivastava S, Iwai J, Elmaleh DR, Livni E, Strauss HW, Goodman MM and Knapp FF, Jr, "Quantitative autoradiographic measurement of regional myocardial substrate uptake in hypertensive rats," 31st Annual Meeting, Society of Nuclear Medicine, Los Angeles, California, June 5-8, 1984, J Nucl Med, 27, P 24, 1984; Yonekura Y, Som P, Srivastava SC, Yamamoto K, Meinken GE, Brill AB, Elmaleh DR, Strauss HW, Goodman MM, and Knapp FF, Jr, "Autoradiographic studies of labeled fatty acids analogs as potential myocardial imaging agents," J Nucl Med, 24, P 125, 1983.