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RADIOIODINATED METHYL-BRANCHED FATTY ACIDS - EVALUATION OF CATABOLITES FORMED IN VIVO

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SUMMARY

Radioiodinated terminal iodophenyl-substituted long-chain fatty acids containing either racemic mono-methyl or geminal dimethyl-branching in the alkyl chain have been shown to exhibit delayed myocardial clearance properties which make these agents useful for the SPECT evaluation of myocardial fatty acid uptake patterns. Although the myocardial clearance rate of 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP) is considerably delayed, in comparison with the IPPA straight-chain analogue, analysis of the radioiodinated lipids present in the outflow tract of isolated rat hearts administered BMIPP have clearly demonstrated the presence of a polar metabolite. The synthesis of β-hydroxy fatty acids has been developed to allow investigation of the possible formation of β-hydroxy catabolites in vivo. The preparation of β-hydroxy BMIPP and β-hydroxy IPPA are described, and the possible significance of their formation in vivo discussed.

INTRODUCTION

The use of iodine-123-labeled fatty acid analogues for the clinical evaluation of fatty acid uptake and/or clearance patterns has stimulated much interest. To obtain maximal information of regional distribution with minimal interference from radioactivity in adjacent regions of the myocardium or surrounding tissues, single photon computerized tomographic imaging (SPECT) must be used. The 10-20 minute rotation periods required for SPECT evaluation of regional radiotracer distribution necessitates minimal redistribution or loss from the target tissue during the imaging period. For SPECT evaluation of regional myocardial fatty acid uptake patterns, the two approaches which have been pursued to inhibit rapid radiotracer loss involve either inhibition of oxidative turnover of aliphatic fatty acids by increase in arterial lactate levels (1-2), or possible inhibition of β-oxidation (Figure 1) by introduction of methyl-substitution at strategic locations in the fatty acid chain (3-8).

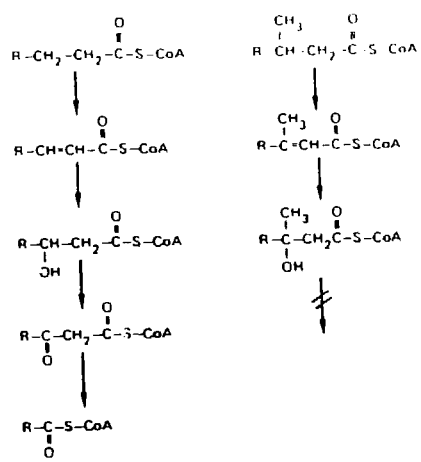

Figure 1. Possible inhibiton of fatty acid β-oxidation by methyl-substitution in the β-position.

The latter approach has required the development of synthetic strategies for preparation of new methyl-branched fatty acids (3, 6-9). Structures of representative radioiodinated 3-monomethyl-branched fatty acids which we have developed and evaluated are shown in Figure 2.

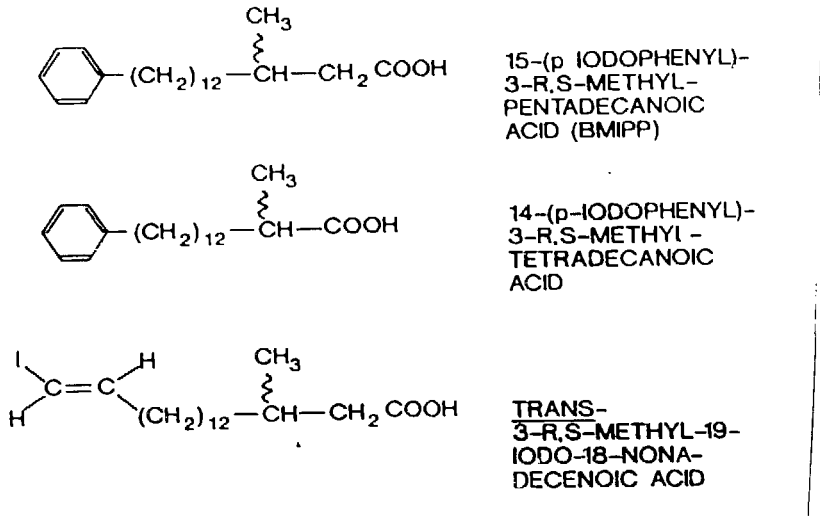

Figure 2. Structures of iodophenyl- and iodovinyl-substituted 3-R,S-methyl-branched fatty acids.

As a result of the ease of radioiodination and the high myocardial extraction and good retention, I-123 BMIPP has been chosen as a model agent by several groups and has been evaluated in a variety of animal models (9-10). More importantly, studies in humans have clearly demonstrated good myocardial uptake and significantly increased retention of BMIPP in comparison with the 15-(p-iodophenyl)pentadecanoic acid (IPPA) analogue under resting conditions (11-12), thus permitting SPECT evaluation of the regional pattern of fatty acid uptake (13-14). Because the behavior of BMIPP in man is very similar to that observed in rat (9), hamster (10) and canine (11) models, the behavior of this agent in humans under a variety of physiological conditions can be accurately predicted from in vivo animal studies. In our view, this is an

important property of BMIPP if such agents are to be further developed for human use.

Although BMIPP shows good retention in both humans and animals, the slow wash-out of radioactivity from the myocardium has suggested that this agent is lost from the myocytes by either back diffusion or as a result of an unexpected, and possibly unknown, metabolic process. The latter possibility was further suggested by the chromatographic identification of unknown polar components in the plasma and urine of patients following administration of I-123 BMIPP (11,12). Experimental evidence in support of the latter possibility has been recently obtained by our studies with Langendorff perfused rat hearts which have clearly shown the presence of a metabolite in the outflow tract following administration of radioiodinated BMIPP (15,16). We have suggested the possible formation of a β-hydroxy catabolite which cannot be metabolized further since such an intermediate may be expected to accumulate because of the impediment of the 3-methyl substituent (1). Formation of this intermediate would preclude oxidation to the obligatory 3-keto product prior to carbon-carbon bond cleavage (Figure 1).

In this paper we discuss the possible identity of this material and our work directed at the development of new synthetic techniques for the preparation of β-hydroxy fatty acids. The availability of the authentic β-hydroxy analogues of aliphatic fatty acids will also allow an evaluation of the potential significance of their expected accumulation in ischemic heart tissue.

3-Hydroxy-3-Methyl Fatty Acids

The radioiodinated component observed in the outflow tract of Langendorff-perfused rat hearts administered radioiodinated BMIPP under

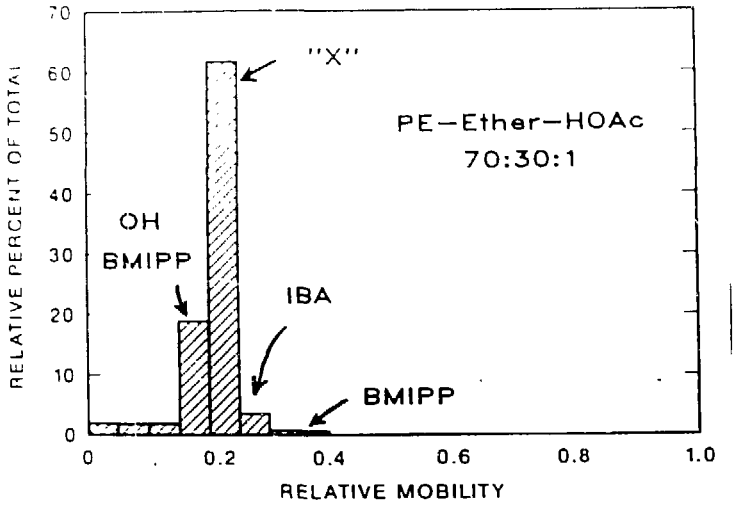

Figure 3. A major polar product is present in the outflow tract from Langendorff perfused hearts administered radioiodinated BMIPP.

normoxic conditions (Figure 3) appears to represent a single component ("X") which is considerably more polar than BMIPP (15,16). As discussed earlier, a prime candidate for this component could be a racemic 3-hydroxy catabolite (Figure 1). Since authentic standards are not available, a synthetic strategy for the preparation of such compounds has been developed as summarized in Figure 4. Briefly, this approach is based upon introduction of the racemic methyl group via a protected malonic acid ("Meldrum" acid) (17). Acylation with 13-phenyltridecanoyl chloride followed by acid treatment readily gives the methyl ketone. Reformatsky treatment and hydrolysis then provides the 15-phenyl-3-(R,S)-hydroxy-3-methylpentadecanoic acid (HBMIPP) which can then

be iodinated in the usual manner. The HBMIPP is more polar by chromatographic comparison than the material obtained from isolated rat

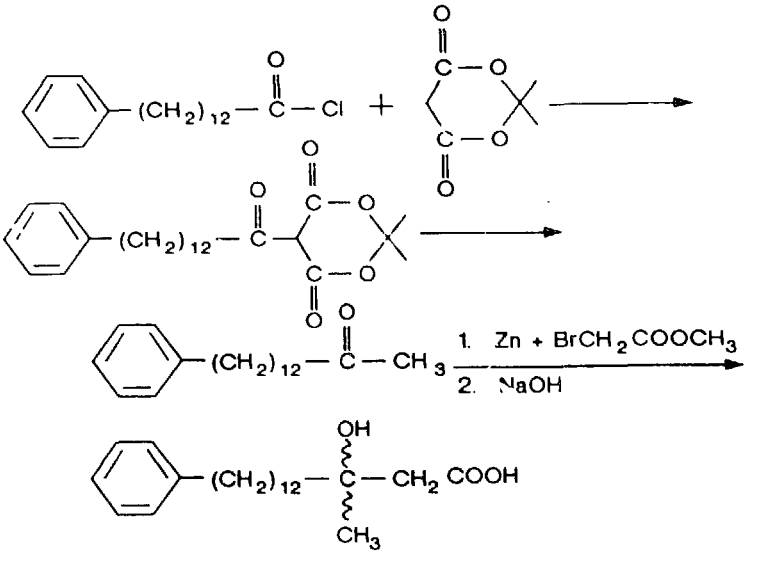

Figure 4. Synthesis of β-hydroxy BMPPA via acylation of Meldrum's acid.

hearts. Thus, studies are now directed at isolating sufficient quantities of the unknown catabolite from isolated hearts for mass spectral analysis.

3-Hydroxy Fatty Acids

As a result of oxygen deprivation during ischemia, leading to deficiency of oxidized pyridine nucleotides, oxidation of the obligatory 3-hydroxy intermediates to the 3-keto products is impaired (Figure 1). Under such ischemic conditions, the 3-hydroxy catabolites would be expected to accumulate. This has clearly been demonstrated to occur in both isolated rat heart (18) and rabbit heart studies (19). The 3-hydroxy intermediates of palmitic, myristic and stearic acids have been isolated and identified by gas-liquid chromatography-mass spectrometry as the major products. Although these products are only minor components of the out-flow tract, they are major

components of the lipids extracted from the homogenized myocardial tissue. The term "leaky hose pipe" has been used to describe the accumulation of these products. The evaluation of the delayed regional clearance curves of IPPA and PA in ischemia has not addressed the possible presence of these expected catabolites. The formation of these metabolites, however, and their influence on an interpretation of these data are important. A major factor which has delayed such an analysis has been the unavailability of the necessary chromatographic standards. In addition, iodobenzoic acid is the usual catabolite of IPPA under normoxic conditions, and we expect it has mobility similar to β-hydroxy-IPPA and diglycerides using the usual thin-layer chromatographic systems.

Using an adaption of the approach with "Meldrum's Acid" described earlier, we have also now synthesized the racemic 3-R,S-hydroxy form of IPPA (Figure 5). The approach involves acylation following ring opening in methanol to provide the methyl ester of the 3-ketone. This product is then reduced with borohydride and hydrolyzed with base to provide 15-phenyl-3-R,S-hydroxypentadecanoic acid which can be iodinated in the usual manner to give 3-hydroxy-IPPA. Studies are now in progress to prepare 3-hydroxy forms of other fatty acids of current interest and to evaluate the expected formation of 3-OH-IPPA in ischemic myocardium.

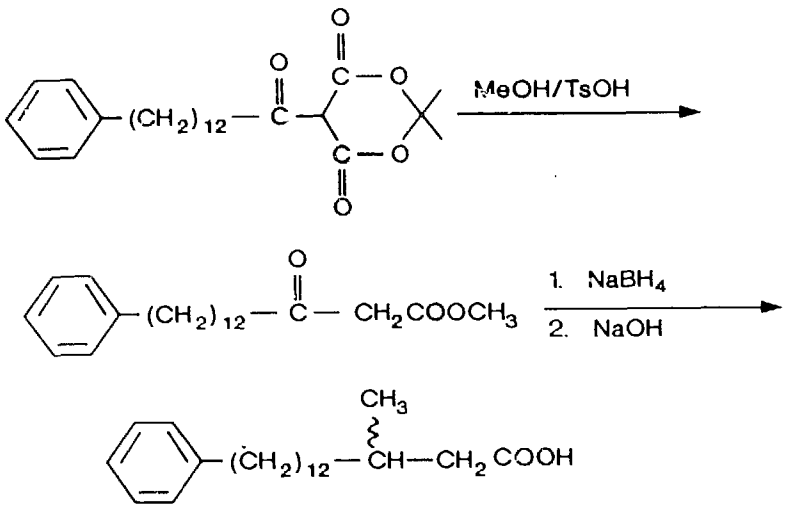

Figure 5. Synthesis of β-hydroxy PPA.

DISCUSSION

The large number of radioiodinated fatty acids currently available suggests that the synthetic problems associated with making these interesting agents available have been solved. It is important to now evaluate in some detail the physiological behavior and metabolism of the best candidates to better understand their behavior in vivo. In addition to an evaluation of the metabolism of agents such as BMIPP, we feel it is now crucial to evaluate other important factors affecting fatty acid uptake and metabolism on the molecular level, which up to now have not been evaluated in detail. These factors include albumin binding properties and the significance and potential importance of membrane bound and intracellular fatty acid binding proteins on the uptake and fate of these agents. In addition to the practical significance of understanding how these factors fit into the total picture of the use of radioiodinated fatty acids for imaging the myocardium, it could be possible that a simple screening test could be developed to screen new fatty

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