Effects of Configuration on the Myocardial Uptake of Radioiodinated 3(R)-BMIPP and 3(S)-BMIPP in Rats

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Radioiodinated 3(R)-(+)- and 3(S)-(−)-15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP) were prepared and evaluated in rats to investigate the effects of absolute configuration of the 3(β)-methyl group on myocardial uptake and release kinetics. **Methods:** The 3(R)-(+)-BMIPP analog was synthesized by initial acylation of a thiophene template with the acid chloride of ethyl 3(R)-methylglutarate. 3(S)-(−)-BMIPP was obtained by separation from the mixture of diastereomeric amides prepared from reaction of the acid chloride of racemic BMIPP with the S-(−)-α-methylbenzylamine. The amide of synthetic 3(R)-BMIPP prepared from S-(−)-α-methylbenzylamine was identical to the chromatographically more polar isomer. Free acids were obtained by acid hydrolysis of the amides, fully characterized and then converted to the radioiodinated BMIPP isomers. **Results:** Biodistribution studies in rats with the dual-labeled [^{131}I]−3(S)-BMIPP/[^{125}I]-3(R)-BMIPP mixture demonstrated greater myocardial uptake of 3(R)-BMIPP compared with the 3(S)-BMIPP isomer [60 min: 3(R)-BMIPP = 4.37 %ID/g; 3(S)-BMIPP = 3.44; $p < 0.05$; 180 min, 2.31 and 1.78 %ID/G, respectively, $p < 0.01$], although both isomers had similar myocardial washout curves (5–180 min). Percent ID/g values for other tissues which were examined (blood, lungs, thyroid) were similar. **Conclusion:** Higher myocardial uptake of the 3(R)-BMIPP isomer observed in these animal studies may suggest differences in carrier-mediated myocyte uptake of the two isomers. These studies suggest that [^{123}I]−3(R)-BMIPP is a candidate for clinical evaluation and may show greater myocardial uptake than the 3(S)-BMIPP isomer and may thus require reduced injected dose.

Key Words: iodine-123-BMIPP; 3(R)- and 3(S)-BMIPP; fatty acid imaging

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Clinical studies with ^{123}I-labeled fatty acids are currently primarily focused on myocardial imaging with 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP; Fig. 1) (*1–5*). The protocols that are currently most widely used involve comparison of the regional uptake of [^{123}I]-BMIPP with the distribution of flow tracers such as [^{99m}Tc]-sestamibi (*6–9*) or ^{201}Tl (*10–12*). The combined results from these studies conducted in patients at several clinical centers have clearly demonstrated that important information on myocardial viability can be determined by evaluation of the "mismatch" ratio between BMIPP and flow tracer distribution (i.e., BMIPP < flow = viable but threatened myocardium).

The preparation of [^{123}I]-BMIPP for current clinical studies involves radioiodination of the racemic BMIPP mixture, which is based on the original synthesis of BMIPP reported by Knapp and colleagues in 1984 (*13*), and involved the preparation of the racemic mixture of the 3(R)-BMIPP and 3(S)-BMIPP using a thiophene template approach. Although the potential importance of the effects of the absolute configuration of the 3-methyl group on myocardial uptake and release kinetics had been recognized (*2,14,15*), the requisite optically active methyl-substituted substrates required for preparation of the 3(R)-BMIPP and 3(S)-BMIPP enantiomers were not readily available at that time. Through 1996, however, over 100,000 clinical studies have been completed (information provided by Nihon Medi-Physics, Ltd.) with [^{123}I]-BMIPP in Japan alone (for examples, see refs. *16–21*). Applications include SPECT imaging in cases of myocardial infarction (*16*), ischemic heart disease (*17,18*), coronary vasospasm (*19*) and cardiomyopathy (*20,21*). In addition to extensive clinical use in Japan, [^{123}I]-BMIPP is also currently used for clinical evaluation of myocardial viability in Belgium (*6–9*) and Germany (*10–12*). The widespread clinical use of [^{123}I]-BMIPP has prompted us to explore the effects of configuration of the C-3-methyl group on the biological properties of radioiodinated BMIPP. In this paper we report the synthesis of 3(R)-BMIPP, which was used to aid in resolution of the 3(R)- and 3(S)-isomers from racemic BMIPP. Radioiodinated 3(R)- and 3(S)-BMIPP have been prepared and evaluated in fasted female Fischer rats.

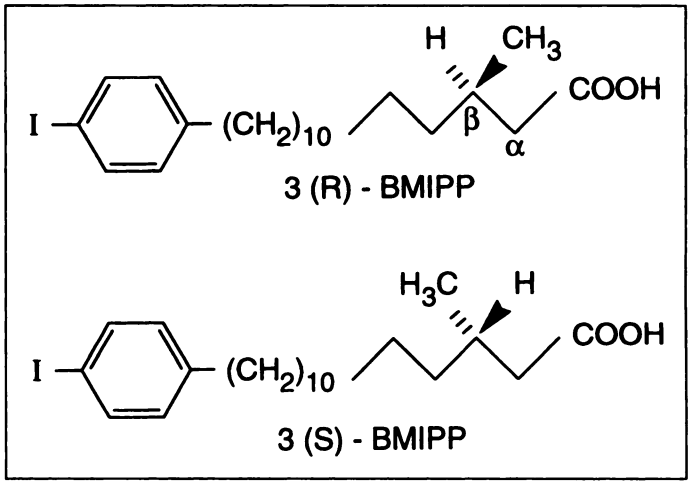

FIGURE 1. Fatty acid structures. The angular 3-methyl group results in prolonged myocardial retention of radioiodinated BMIPP. Racemic BMIPP is commercially available as Cardiodine^{R} as a clinical agent in Japan and consists of a racemic mixture of the 3(R)- and 3(S)-BMIPP isomers.

MATERIALS AND METHODS

General

The S-(−)-α-methylbenzylamine was 99% enantiomeric excess determined by gas liquid chromatography; $[\alpha]_D = -39°$ (manufacturer), −39° (observed). The (R)-1-ethyl hydrogen 3-methylglutarate [designated as ethyl 3(R)-methyl glutarate] was >99% pure by gas liquid chromatography with an R:S ratio of >99:1

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determined by high performance liquid chromatography (HPLC); $[\alpha]_D = -1.5°$ (manufacturer), $-1.5°$ (observed). The petroleum ether had a boiling range of 30–60°C. Anhydrous toluene was purchased from Aldrich Chemical Co. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide before use. All other chemicals and solvents were analytical grade and used without further purification. Melting points (mp) were determined in capillary tubes using a Thomas Hoover Capillary Melting Point apparatus and are uncorrected.

The ^{1}H-NMR and ^{13}C-NMR were obtained using a Varian Gemini 200 instrument with $CDCl_3$ as the solvent. The proton spectra are reported downfield from the tetramethylsilane (TMS) internal standard (0 ppm). The carbon spectra were measured using chloroform as the internal standard [77.0 parts per million (ppm)] and were consistent with the proposed structures. All mass spectra were obtained with an Extrel-FTMS (Madison, WI) 2000 Fourier transform mass spectrometer (3 Telsa magnet) equipped with a Spectra-Physics DCR-11 Nd:YAG laser as previously described (*22*). The $(M+K)^+$ molecular ions were primarily detected, but in some cases $(M+Na)^+$ and $(M+H)^+$ ions were also observed. Accurate mass measurements for molecular ions were determined by using internal calibrant ions such as K^+ and C_{60}^+ to calibrate the mass spectrometer. Optical rotations were recorded using a Model MDL SR6 half-circle polarimeter from Polysciences (Niles, IL) and a Perkin–Elmer Model 243 automatic polarimeter. Concentrations of the specific rotation values are reported as milligrams per milliliter. The elemental analyses were determined by Galbraith Laboratories (Knoxville, TN). Sodium [^{131}I]- and [^{125}I]-iodide were purchased from DuPont/New England Nuclear.

Chromatography

Thin-layer chromatographic (TLC) analyses were performed using silica gel GF-254 coated on glass plates, using 50% ether–hexane for amides and 6% methanol–chloroform for evaluation of free acids or other solvents where indicated. Purification by absorption column chromatography was performed either on silica gel 200–400 mesh or silicic acid 60–200 mesh with the solvents indicated. Analytical HPLC analyses were performed using Whatman Partisil 5 (3.9 mm × 30 cm) or Whatman Partisil 10 ODS 3 columns with monitoring at 254 nm with an online variable wavelength detector or an absorbance detector (*4*). Purification and analysis of radioiodinated compounds was performed using an on-line Beckman Model 170 radioisotope detector and UV detection at 254 nm. For preparative purification of compounds, semi-preparative HPLC columns were used with the same solvent systems indicated above with UV monitoring at 254 nm. The solvents and conditions for the determination of radiochemical purity are summarized in the legends for Figure 4.

Radioactivity Measurements

Tissue samples were counted in a Packard Minaxi 5000 sodium iodide gamma counter. An aliquot (2%–5%) of the injected dose was counted for calculation of the percent injected dose per gram of tissue values. For dual-label studies, two 20% windows were used for detection of the 35 keV (^{125}I) and 365 keV (^{131}I) photo peaks. Corrections were made for the 7% spillover of ^{131}I in the 35 keV window. For determination of the injected values, a calibrated HpGe solid state detector (EG&G ORTEC, Oak Ridge, TN) coupled to a PC-based multichannel analyzer system (Nuclear Data/Canberra, Inc.) was used. Data were analyzed using Nuclear Data Accu-Spec Spectroscopy software.

Synthesis of 3(R)-BMPPA (8A) and 3(R)-BMIPP (9A)

The 3(R)-BMPPA (15-phenyl-3-methylpentadecanoic acid) isomer was synthesized (Fig. 2) by a route similar to that described earlier for synthesis of racemic 3-(R,S)-BMPPA (*13*), based on the initial acylation of a thiophene template with 6-phenylhexanoic acid (**1**). Chromatography of the crude product on silica gel by elution with 5% ethyl acetate-hexane gave 3.94 g (74% yield) of 2-(6-phenyl-1-oxohexyl)thiophene (**3**) as a brown-colored oil; TLC (10% ethyl acetate-hexane) R_f 0.37; ^{1}H-NMR 1.2–1.9 (m, 6H, $-CH_2-$), 2.5 (t, 2H, CH_2Ph), 2.87 (t, 2H, $-CH_2CO$), 7.2 (m, 6H, Ar), 7.57 (d, 1H, Ar), 7.67 (d, 1H, Ar). Hydrazine reductive desulfurization of the 2-(6-phenyl-1-oxohexyl)thiophene (**3**) gave after column chromatography of the crude product on silica gel by elution with 5% ethyl acetate-hexane, 2.61 g (72.5% yield) of 2-(6-phenyl-1-hexyl)thiophene (**4**) as a brown-colored oil; TLC (10% ethyl acetate-hexane) R_f 0.68; ^{1}H-NMR 1.1–1.7 (m, 6H, $-CH_2-$), 2.52 (t, 2H, CH_2Ph), 2.72 (t, 2H, thienyl CH_2), 6.67 (d, 1H, Ar), 6.82 (t, 1H, Ar), 7.11 (m, 6H, Ar). Subsequent acylation of (**4**) with the acid chloride of (R)-1-ethyl hydrogen-3-methyl glutarate (**5**) provided crude (**6**). After work-up and chromatography on silica gel by elution with 5% ethyl acetate-hexane, 3.54 g (86.4% yield) of 2-[3(R)-methyl-1-oxo-4-carboethoxybutyl]-5-(6-phenylhexyl)thiophene (**6**) was obtained as a brown-colored oil; TLC (20% ethyl acetate-hexane) R_f 0.53; ^{1}H-NMR 1.03 (d, 3H, $CHCH_3$), 1.22 (t, 3H, OCH_2CH_3), 1.3–3.05 (m, 17H), 4.1 (q, 2H, OCH_2CH_3), 6.78 (d, 1H, Ar), 7.20 (m, 5H, Ar), 7.55 (d, 1H, Ar). Hydrazine reduction of the keto ester (**6**) gave 1.49 g (83% yield) of 2-[3(R)-methyl-4-carboxylbutyl]-5-(6-phenylhexyl)-thiophene (**7**) obtained as a brown-colored oil; TLC, single spot R_f 0.26 (CH_2Cl_2-1% CH_3COOH) and R_f 0.16 [petroleum ether (PE)-5% Et_2O-1% CH_3COOH]; ^{1}H-NMR 1.0 (d, 3H, $HCCH_3$), 1.1–2.8 (m, 1 9H), 6.55 (m, 2H, Ar), 7.2 (m, 5H, Ar), 10.5 (br, 1H, COOH). Finally, W-2 Raney Ni catalyst (10 g, Grade W-2; freshly prepared from Ni-Al alloy) reductive desulfurization of the acid (**7**) (1.29 g, 3.6 mmol) provided crude 15-phenyl-3(R)-methylpentadecanoic acid [**8A**; 3(R)-BMPPA]. Because the ^{1}H-NMR showed the presence of olefinic hydrogens (~10% of mixture), the product was

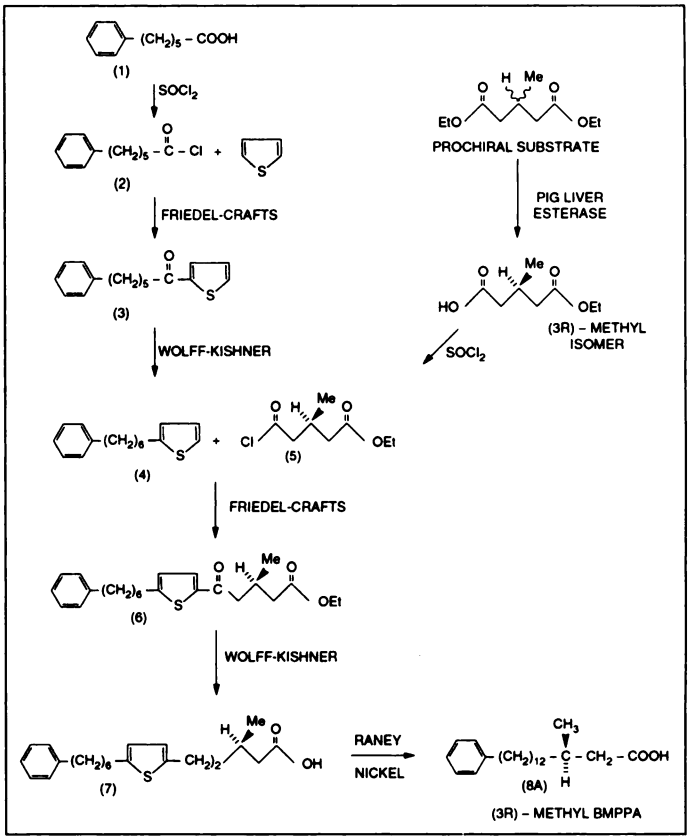

FIGURE 2. Synthesis of 3(R)-BMIPP using commercially available ethyl-3(R)-methylglutarate.

TABLE 1
Summary of High Pressure Liquid Chromatographic Retention Times of BMIPP- and BMPPA-Free Acids and Amides Prepared from (S)-(−)-α-Methylbenzylamine

Compound	Free acid* retention time, min (C18 column)	Amide† retention time, min (silica gel column)
3-(R,S)-BMPPA	2.86	1.59 and 1.49
3(R)-BMPPA		
Synthetic	2.86	1.59
From amide mixture	2.85	1.59 ± 0.02
3(S)-BMPPA	2.86	1.49 ± 0.014
3-(R,S)-BMIPP	3.69	1.57 and 1.47
3(R)-BMIPP		
Synthetic	3.69	...
From amide mixture	3.69	1.57 ± 0.006
3(S)-BMIPP	3.69	1.47 ± 0.007

Whatman Partisil 10 ODS column; solvent = ethanol-isoproponal-water-acetic acid, 80:5:15:1.
*Relative to the acetone solvent peak.
†Relative to the chloroform solvent peak.

therefore catalytically reduced with 10% Pd/C and hydrogenated at 40 pounds per square inch for 18 hr (Parr apparatus) to provide the crude product (**8A**), which was chromatographed on silicic acid eluted with petroleum ether–6% ethyl ether (750 ml) to give 0.65 g of white crystals (54.6% yield); mp 42–42.5°C; $[\alpha_D]$ + 1.47°; analysis calculated for $C_{22}H_{36}0_2$: C, 79.46; H, 10.91; found: C, 79.60; H, 10.96; TLC, single spot, R_f 0.14 (PE-10% Et_2O-1% CH_3COOH); HPLC (Table 1); high resolution mass spectral analysis of $(M+K)^+$: calculated for $C_{22}H_{36}O_2K$: 497.131; found: 497.132; ^{1}H-NMR 0.97 (d, 3H, $HCCH_3$), 1.27 (s, 22H, $-CH_2-$), 2.17 (d, 2H, $HCCH_2COOH$), 2.60 (t, 2H, CH_2Ph), 7.22 (m, 5H, Ar). Para-iodination of the acid (**8**) was then accomplished with thallium(III)trifluoroacetate in trifluoroacetic acid to provide 15-(p-iodophenyl-3(R)-methylpentadecanoic acid [**9A**; 3(R)-BMIPP]. Recrystallization from MeOH gave 0.277 g (60.4% yield) of white crystals, mp 52–53°C; $[\alpha_D]$ + 0.74°; analytical calculations for $C_{22}H_{35}0_2I$: C, 57.64; H, 7.70; found: C, 57.56; H, 8.00; TLC, R_f 0.19 (PE-10% Et_2O-1% CH_3COOH); HPLC, see Table 1; high resolution mass spectral analysis of $(M+K)^+$: calculations for $C_{22}H_{35}O_2IK$: 371.235; found: 371.257; ^{1}H-NMR 1.02 (d, 3H, $HCCH_3$), 1.1–2.5 (m, 25H), 2.60 (t, 2H, CH_2Ph), 6.98 (d, 2H, Ar), 7.63 (d, 2H, Ar).

Resolution of 3R- and 3S-BMPPA and 3R- and 3S-BMIPP from Racemic BMPPA and BMIPP

The racemic BMPPA and BMIPP mixtures were converted to the corresponding amide mixtures by reaction with (S)-(−)-α-methylbenzylamine and the diastereomeric amides and then separated by chromatography (Fig. 3). Abbreviations for the diastereomeric amides designate the fatty acid asymmetric center first and the amine asymmetric center second.

Preparation of the Amide Mixture of Racemic BMPPA with (S)-(−)-α-Methylbenzylamine and Separation of the (R,S)- and (S,S)-Diastereomers (**10**). Racemic BMPPA (99.6 mg, 0.3 mmol) and thionyl chloride (106.3 mg, 0.87 mmol) were added to anhydrous chloroform (3 ml) and the mixture stirred at 55°C for 2 hr under an Argon atmosphere. Excess thionyl chloride was removed in vacuo and the crude acid chloride dissolved with (S)-(−)-α-methylbenzylamine (43.2 mg, 0.36 mmol) and triethylamine (0.165 g, 1.63 mmol) in chloroform (6 ml). The mixture was stirred at room temperature for 0.5 hr. The mixture was washed with 1 N HCl (20 ml), saturated $NaHCO_3$ (30 ml), saturated NaCl (30 ml) and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo to give 128 mg of white solid. For TLC purification, the mixture of BMPPA (R,S) amides (100 mg) was dissolved in acetone (2 ml) and spotted on six preparative TLC plates (1000 μ). The plates were developed using 20% ethyl acetate

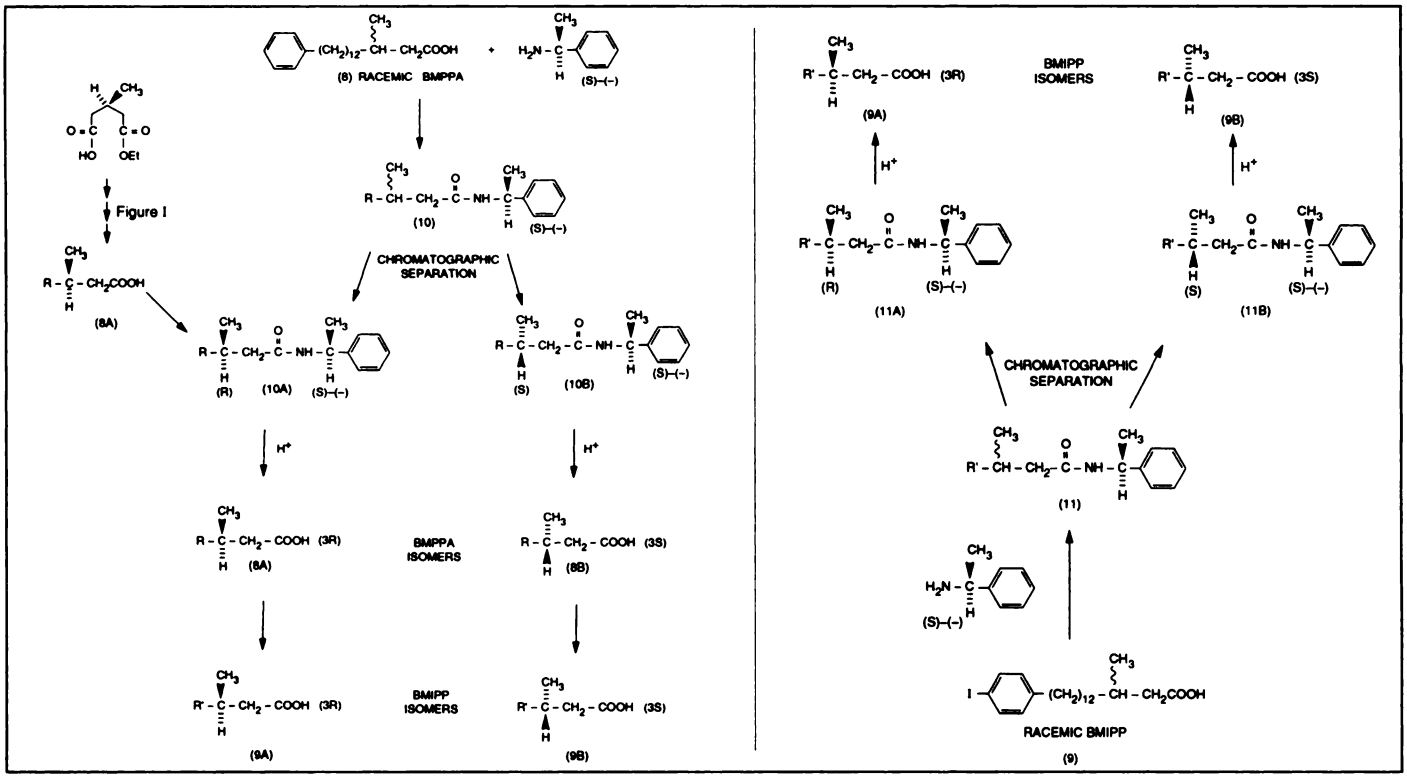

FIGURE 3. Resolution of the amides of 3(R)- and 3(S)-BMPPA and BMIPP by reaction of the acyl chlorides of the racemic BMPPA and racemic BMIPP mixtures with (S)-(−)-α-methylbenzylamine.

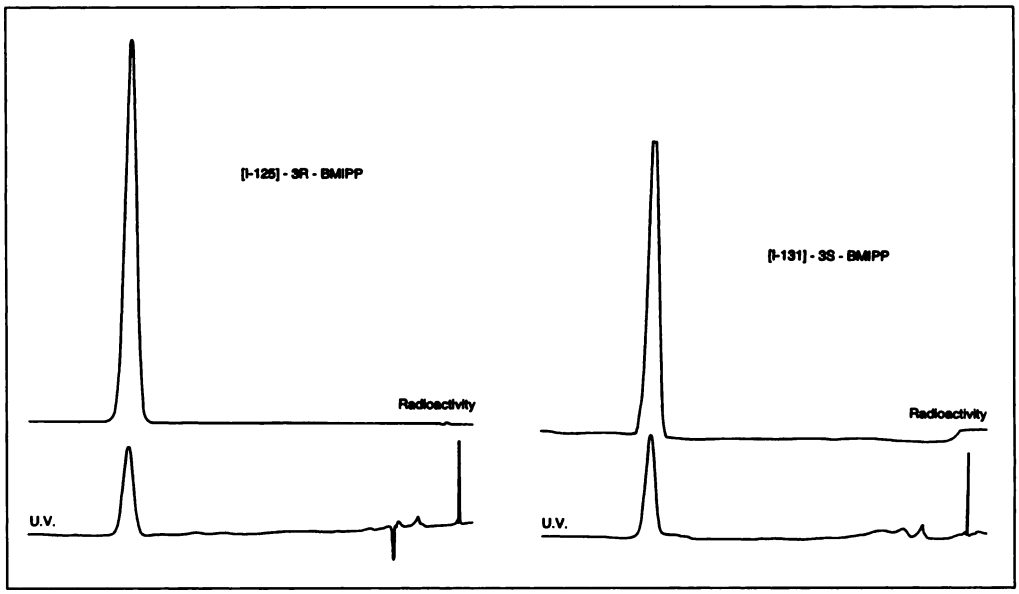

FIGURE 4. HPLC analysis of HPLC-purified [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP, which were used in the dual-label experiment to obtain the tissue distribution data summarized in Table 2. Whatman Partisil 10 ODS column; solvent = ethanol-isopropanol-water-acetic acid-water, 80:5:15:1.

in hexane. The bands at R_f 0.24 and 0.26 were scraped off and eluted with diethyl ether (3 × 30 ml), respectively. The solvent was evaporated in vacuo to give the **(R,S) amide (10A)** as a white solid (39.2 mg), mp 74–74.5°C. In another study, the amides were separated by column chromatography on a column (2 × 81 cm) loaded with 200–400 mesh silicic acid by elution with 30% ether in hexane. TLC, R_f 0.24; HPLC, see Table 1; high resolution mass spectral analysis of $(M+K)^+$: calculated for $C_{30}H_{45}NOK$: 474.313; found: 474.328; ^{1}H-NMR 0.93 (d, 3H, $HCCH_3$), 1.24–1.48 (b, 22H), 1.53 (d, 3H, $NHCHCH_3$), 1.60–2.19 (m, 4H), 2.60 (t, 2H, CH_2Ph), 5.16 (q, 1H, CH_3CHNH), 5.62 (d, 1H, NH), 7.13–7.36 (m, 10H, Ar). The **(S,S) amide (10B)**: (23.9 mg); TLC R_f 0.26; HPLC, see Table 1; mp 76–77°C; MS, same as above for (R,S) isomer; ^{1}H-NMR in ppm: 0.895 (d, 3H, $HCCH_3$), 1.11–1.48 (b, 22H), 1.53 (d, 3H, $NHCHCH_3$), 1.60–2.19 (m, 4H), 2.60 (t, 2H, CH_2Ph), 5.16 (q, 1H, CH_3CHNH), 5.62 (d, 1H, NH), 7.13–7.36 (m, 10H, Ar).

*Preparation of the (S)-(−)-α-Methylbenzylamine Amide of 3(R)-BMPPA (***10A***).* The synthetic 3R-BMPPA isomer (**8A**) was converted to the corresponding amide by reaction of the acid chloride with (S)-(−)-α-methylbenzylamine as described above. The product, mp 70–73°C, exhibited HPLC and other physical data, which were identical to that of (**10A**) isolated from 3-(R,S)-BMPPA amide mixture as described above.

*Preparation of the (S)-(−)-α-Methylbenzylamine Amide of Racemic BMIPP (***11A** and **11B***).* Preparation and separation of the amide was similar to that described above for preparation of the 3-(R,S)-BMPPA amide mixture. For TLC purification, the amide was dissolved in chloroform and spotted on preparative TLC plates and eluted with 25% EtOAc-hexane.

The (R,S)-amide (11A), mp 87–89°C; TLC, R_f 0.34; HPLC, see Table 1; high resolution mass spectral analysis of $(M+K)^+$. Calculated for $C_{30}H_{44}NOIK$: 600.210; found: 600.239; ^{1}H-NMR 0.92 (d, 3H, $HCCH_3$), 1.10–1.47 (b, 22H), 1.48 (d, 3H, $NHCHCH_3$), 1.53–2.11 (m, 4H), 2.53 (t, 2H, CH_2Ph), 5.15 (q, 1H, CH_3CHNH), 5.62 (d, 1H, NH), 6.92 (d, 2H, Ar), 7.25–7.34 (m, 5H, Ar), 7.57 (d, 2H, Ar).

The (S,S)-amide, mp 108–110°C; TLC, R_f 0.39; HPLC, see Table 1; high resolution mass spectral analysis of $(M+K)^+$. Calculated for $C_{30}H_{44}NOIK$: 600.210; found: 600.198; ^{1}H-NMR 0.89 (d, 3H, $HCCH_3$), 1.10–1.47 (b, 22H), 1.48 (d, 3H, $NHCHCH_3$), 1.53 (m, 4H), 2.53 (t, 2H, CH_2Ph), 5.15 (q, 1H, CH_3CHNH), 5.62 (d, 1H, NH), 6.92 (d, 2H, Ar), 7.25–7.34 (m, 5H, Ar), 7.57 (d, 2H, Ar).

Acid Hydrolysis of Purified Diastereomers Prepared from (S)-(−)-α-Methylbenzylamine (General Procedure). To determine the optimal conditions for acid hydrolysis of the amides, 5 mg aliquots of the amide of 3(R)-BMPPA were heated with 1–2 ml of concentrated HCl and heated in a Teflon-lined bomb in an oven for 1 hr at temperatures beginning at 100°C increasing in 25° increments to 200°C. The products were analyzed by TLC and proton NMR. Since the doublet for the β-methyl group of the free acid resonated downfield from the doublets for either amide, integration of this region provided an estimate of the degree of hydrolysis. Based on these studies, a hydrolysis temperature of 175°C for 2 hr was chosen for preparative-scale hydrolysis of the S-amide, the 3(S)-BMPPA. The amide (10–70 mg) was combined with concentrated HCl (1.5 ml) and the mixture heated at 175°C for 2 hr. The hydrolyses were performed in a Teflon insert housed in a stainless steel "bomb" placed in an oven. The mixture was cooled to room temperature and extracted with diethyl ether (3 × 5 ml). The ether extracts were washed thoroughly with H_2O (3 times) and dried over anhydrous $MgSO_4$. The solvent was evaporated to provide the crude product that was chromatographed by TLC on silicic acid eluted with $CHCl_3$ or purified by column chromatography. The chromatographic properties of the 3(R)-BMPPA and 3(R)-BMIPP isomers prepared by hydrolysis of the more polar amides in each mixture were identical to the synthetic 3R-BMPPA and 3R-BMIPP (vide ante).

15-Phenyl-3(S)-Methylpentadecanoic Acid [8B; 3(S)-BMPPA]

After acid hydrolysis of the (S,S)-amide, column purification gave 18 mg of white crystals (69% yield); mp 40–41°C; $[\alpha_D]$ −3.90°; analysis calculated for $C_{22}H_{36}O_2$: C, 79.46; H, 10.91; found: C, 79.95; H, 10.99; TLC, R_f 0.14 (PE-10% Et_2O-1% CH_3COOH); HPLC, see Table 1; high resolution mass spectral analysis of $(M+K)^+$. Calculated for $C_{22}H_{36}O_2K$: 497.131; found: 497.132; ^{1}H-NMR 0.97 (d, 3H, $HCCH_3$), 1.27 (s, 22H, $-CH_2-$), 2.17 (d, 2H, $HCCH_2COOH$), 2.60 (t, 2H, CH_2Ph), 7.22 (m, 5H, Ar).

15-(p-Iodophenyl-3(S)-Methylpentadecanoic Acid [9B; 3(S)-BMIPP]

After acid hydrolysis and column chromatography, 13.5 mg of (**9B**) were obtained (77% yield). Crystallization from MeOH gave white crystals, mp 45–46°C; $[\alpha_D]$ −2.50°; analysis calculated for $C_{22}H_{35}O_2I$: C, 57.64; H, 7.70; found: C, 58.40; H, 8.03; TLC, R_f 0.19 (PE-10% Et_2O-1% CH_3COOH); HPLC, see Table 1; high

resolution mass spectral analysis of $(M+K)^+$. Calculated for $C_{22}H_{35}O_2IK$: 371.235; found: 371.257; ^{1}H-NMR 1.02 (d, 3H, $HCCH_3$), 1.1–2.5 (m, 25H), 2.60 (t, 2H, CH_2Ph), 6.98 (d, 2H, Ar), 7.63 (d, 2H, Ar).

Preparation of Radioiodinated BMIPP Isomers

The radioiodinations were performed as described earlier using the thallation-iodide displacement method (*13,24*). The phenyl-substituted fatty acid substrate [i.e., 15-phenyl-3(R)-, 3(S)- or 3-(R,S)-methylpentadecanoic acid, BMPPA; 1.5 mg, 0.005 mmol] was reacted with 5.4 mg (0.01 mmol) of thallium trifluoroacetate in 2 ml of trifluoroacetic acid. After 3–18 hr at room temperature in the dark, the solution was then heated with ^{131}I or ^{125}I solution in dilute base, was combined with 0.5 ml of a standard aqueous KI solution (0.01 mmol, 1.6 mg/ml), added to the substrate solution that was heated at 95–100°C for 15 min in a heating block and worked-up in the usual manner (*24*). The radioiodinated BMIPP products were purified by HPLC on a semipreparative C18 column using an ethanol-isopropanol-water-acetic acid (80:5:15:0.1) solvent system. After purification, radiochemical purity was assessed with the same system. Examples are shown in Figure 4. The radioiodinated products were then stored as dry solids in the freezer until use.

Biodistribution Studies

The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and were reviewed and approved by the Oak Ridge National Laboratory Animal Care and Use Committee. The radioiodinated BMIPP isomers were dissolved in approximately 100 μl of warm ethanol and the solution then added slowly with stirring to a 6% BSA in normal saline solution at 40–45°C. After filtration through a 0.22 μ Millipore filter, the solutions were administered by intravenous injection (0.5 ml) into a lateral tail vein of groups (n = 5 per group) of metophane-anesthetized rats (1–2 μCi). The rats were fasted 24 hr before injection but were allowed food and water ad libitum during the course of the experiment. At various time points the animals were euthanized by cervical fracture after Metofane anesthesia. The various organs were removed, rinsed with saline, blotted dry and weighed in tared vials. A section of the trachea containing the thyroid gland was removed and the weight calculated as 0.7% of the total animal weight. Blood samples were obtained from the thoracic cavity after removal of the heart. For the dual-label studies, the radioiodinated fatty acids were individually formulated as described above and filtered through a 0.22 μ Millipore filter. Aliquots were then counted in the MCA and the approximate volumes determined for combination. After combining appropriate aliquots from each fatty acid solution, the combined 6% BSA solutions were then combined, diluted to the appropriate volume for injection of 0.5 ml per animal and filtered again through a 0.22 μm Millipore filter. Aliquots of this injection solution were then taken for the counting standards. Data are presented as the mean ± s.d. values. Statistical evaluation was performed using the Student's t-test for small n values with significance level of p = 0.05.

RESULTS

Synthesis of 3(R)-BMIPP and Resolution of Racemic BMPPA and BMIPP

The 3(R)-BMPPA was synthesized as shown in Figure 1 using a thiophene template for carbon–carbon bond formation as described earlier (*13,24*). The commercial availability of enantiomerically pure ethyl-3(R)-methylglutarate substrate provided an optically active substrate for the synthesis of 3(R)-BMPPA. The ^{1}H and ^{13}C NMR and MS data were consistent with the proposed structures of all compounds. Availability of authentic (**8A**) and (**9A**) allowed the identification and assignment of configuration of the 3(R)-isomers obtained by chromatographic separation of the amide mixtures prepared by reaction of racemic BMPPA (**8**) and BMIPP (**9**) with S-(−)-α-methylbenzylamine (Fig. 3). For this (R,S) and (S,S) notation for the diastereomeric amides, the fatty acid asymmetric center is designated first and the α-methylbenzylamine asymmetric center second.

The methyl-substituted substrate required for preparation of the 3(S)-methyl isomer of BMIPP [3(S)-BMPPA; **8B**] was not available and attempts to fractionally crystallize the salts prepared by combining the racemic BMPPA or BMIPP with S-(−)- or R-(+)-α-methylbenzylamine were unsuccessful. For these reasons, a different route was pursued that involved chromatographic separation of the diastereomeric mixture of amides prepared from racemic BMPPA by reaction with the optically pure (S)-(−)-α-methylbenzylamine, as shown in Figure 3. For this approach, racemic BMPPA (**8**) was converted to the acid chloride, which was then reacted with optically pure (S)-(−)-α-methylbenzylamine to provide the mixture of amides (**10A** and **10B**). The (R,S)- (**10A**) and (S,S)- (**10B**) diastereomers formed from racemic BMPPA readily separated and could be purified by preparative TLC, HPLC or column chromatography. In the same manner, the diastereomeric amides (**11A** and **11B**) formed from reaction of the acyl chloride of racemic BMIPP (**9**) with (S)-(−)-α-methylbenzylamine were readily separated. The diastereomeric amides formed from reaction of (S)-(−)-α-methylbenzylamine with authentic 3(R)-BMPPA or 3(R)-BMIPP isomers synthesized as shown in Figure 1 had identical spectral and chromatographic properties as the more polar amides formed from the racemic BMPPA and BMIPP mixtures, respectively. From a chromatographic comparison it was thus deduced that the less polar amides from the racemic mixtures must therefore represent the 3(S)-BMPPA (**10B**) and 3(S)-BMIPP (**11B**) isomers.

Because the upfield methyl doublet represents the (S,S)-diastereomer of each amide mixture and the downfield doublet represents the (R,S)-diastereomer, the ^{1}H-NMR spectra of the amides provided an unanticipated and important bonus, which aided in the assignment of the R configuration to the chromatographically more polar component in the BMPPA and BMIPP amide mixtures. The chemical shift values for the methyl doublets representing the secondary methyl group at the C-3 chiral center of the fatty acid moieties of the amides were considerably further downfield in the ^{1}H-NMR spectra of the (R)-isomers [**10A**, 3(R)-BMPPA = 0.932 and 0.901 ppm; **11A**, 3(R)-BMIPP = 0.934 and 0.903 ppm] compared with the S-methyl amides [**10B**, 3(S)-BMPPA = 0.906 and 0.874 ppm; **11B**, 3(S)-BMIPP = 0.901 and 0.870 ppm]. The downfield doublets in the multiplet patterns observed in the ^{1}H NMR spectra for the secondary methyl groups in the (**10**) and (**11**) amide mixtures can thus be attributed to the amides of the 3(R)-BMPPA (**10A**) and 3(R)-BMIPP (**11A**) isomers.

The 3(S)-BMPPA (**8A**) was then formed by acid hydrolysis of the (**10A**) amide, which had been purified by column chromatography from the racemic mixture. Subsequent thallation iodination then provided the authentic 3(S)-BMIPP isomer (**9A**). The amides (**10A** and **11A**) formed from reaction of (S)-(−)-α-methylbenzylamine with either 3(R)-BMPPA or 3(R)-BMIPP synthesized as shown in Figure 2, had identical spectral and chromatographic properties as the more polar amides of BMPPA (**10A**) and BMIPP (**11A**) formed from the racemic BMPPA mixtures, respectively. In this manner, the more polar diastereomer was assigned the 3(R)-configuration since it was identical to the amide formed from the authentic

TABLE 2
Comparison of the Distribution of Radioactivity in Tissues of Groups of Fasted Female Fisher Rats (n = 5 per Group) after Intravenous Administration of a Dual-Labeled Mixture of [^{125}I]-3(R)-BMIPP and [^{131}I]-3(S)-BMIPP

		Mean % injected dose per gram of tissue values ± s.d.					
Minutes after injection	BMIPP isomer	Blood	Heart	Heart p value	Liver	Lungs	Thyroid
5	[I-125]-3(R)-	2.27 ± 0.13	5.25 ± 0.77	$p > 0.05$	6.41 ± 0.81	2.06 ± 0.15	18.79 ± 9.3
	[I-131]-3(S)-	2.16 ± 0.20	4.51 ± 0.50		7.18 ± 0.92	1.98 ± 0.10	17.42 ± 8.7
30	[I-125]-3(R)-	2.09 ± 0.15	4.37 ± 1.22	$p > 0.05$	2.70 ± 0.28	1.51 ± 0.15	21.12 ± 8.9
	[I-131]-3(S)-	2.07 ± 0.19	3.44 ± 0.79		2.76 ± 0.29	1.53 ± 0.12	18.73 ± 8.2
60	[I-125]-3(R)-	2.12 ± 0.14	3.36 ± 0.61	$p < 0.05$	2.02 ± 0.21	1.33 ± 0.14	20.60 ± 8.0
	[I-131]-3(S)-	2.09 ± 0.13	2.51 ± 0.29		2.03 ± 0.19	1.36 ± 0.13	19.41 ± 8.9
180	[I-125]-3(R)-	1.70 ± 0.10	2.31 ± 0.51	$p < 0.01$	1.03 ± 0.06	1.23 ± 0.09	16.85 ± 10.4
	[I-131]-3(S)-	1.69 ± 0.09	1.78 ± 0.26		1.05 ± 0.05	1.22 ± 0.09	15.45 ± 9.2

Five fasted rats were studied for each group. Each rat was injected through a lateral tail vein with a mixture of 3.95 μCi [^{125}I]-3(R)-BMIPP and 1.07 μCi [^{131}I]-3(S)-BMIPP complexed to a 6% bovine serum albumin solution.

3(R)-BMPPA isomer (**10A**) synthesized from ethyl-3(R)-methylglutarate. From a chromatographic comparison, the less polar amides from the (**10**) and (**11**) racemic mixture thus represent the amides of 3(S)-BMPPA (**10B**) and 3(S)-BMIPP (**11B**). Table 1 shows the clear separation of the diastereomeric amides by HPLC. In contrast, the free acids could not be separated using the normal and reverse-phase systems which were evaluated.

While the R- and S-amides of BMPPA and BMIPP could be separated by HPLC, the 3(R)-BMPPA and 3(S)-BMPPA free acid pair and 3(R)-BMIPP and 3(S)-BMIPP could not be separated. As well, ^{1}H NMR analyses of the pure enantiomers demonstrated that the doublets for the secondary methyl substituents have essentially the same chemical shift values for both the 3(R)- and 3(S)-isomers of BMPPA and BMIPP. Other than the differences observed in the chemical shift values for the secondary methyl group of the amides, other regions of the proton NMR spectra were essentially identical.

The availability of 3(R)-BMIPP and 3(S)-BMIPP has now permitted an evaluation of the expected important effects of the stereochemistry of the C-3 asymmetric center on the heart uptake of the radioiodinated 3(R)- and 3(S)-BMIPP isomers. The 3(S)-methyl and 3(R)-methyl isomers were freed from the diastereomeric amides by acid treatment. The products were >95% pure by TLC and HPLC, and the ^{1}H and ^{13}C NMR spectra and MS data were consistent with the proposed structures. The iodinated BMIPP isomers were prepared from the purified 3(R)- and 3(S)-methyl isomers and exhibited spectral properties that were consistent with the proposed structures.

Animal Tissue Distribution Studies with Radioiodinated 3(R)-BMIPP and 3(S)-BMIPP

Radioiodinated 3(R)-BMIPP and 3(S)-BMIPP were prepared and evaluated in groups (n = 5 per group) of fasted female Fisher rats, and these data were initially compared with the tissue distribution data obtained with racemic [^{125}I]−3-(R,S)-BMIPP. An initial experiment involved evaluation of ^{125}I-labeled 3(S)-BMIPP, 3(R)-BMIPP and racemic 3-(R,S)-BMIPP in separate groups of fasted female Fisher rats. The results from this initial study suggested that the 3(R)-BMIPP isomer had considerably greater myocardial uptake than the 3(S)-isomer, although levels of radioactivity in other tissues evaluated appeared similar.

To further evaluate the relative tissue uptake values at various time intervals to eliminate inherent differences in comparing data from different groups of animals, [^{131}I]-3(S)-BMIPP and [^{125}I]-3(R)-BMIPP were synthesized and purified by preparative HPLC for use in the dual-label experiment. The purified samples (Fig. 4) were formulated together in 6% BSA solution and the dual-labeled mixture then administered to groups of fasted rats (n = 5). In this manner, each rat served as a control to eliminate differences between groups of rats. The results (Table 2) confirmed the earlier study and clearly demonstrated higher myocardial uptake of the 3(R)-BMIPP isomer compared to 3(S)-BMIPP ($p = 0.05$). The percent dose per gram values were similar for the other tissues evaluated, although the early time points suggested higher liver uptake of the 3(S)-BMIPP isomer. One group of rats was also individually housed in metabolism cages and the urine and feces collected daily. Radioactivity from both isomers was principally excreted through the urinary bladder, and >95% of the excreted radioactivity was found in the urine. About 65% of the injected dose was excreted in the initial 24 hr period after administration.

DISCUSSION

The importance of molecular asymmetry on biological behavior is well established for drugs, enzyme substrates, receptor ligands and many other biologically important molecules. Because racemic 3-(R,S)-BMIPP is a mixture of the 3(R)- and 3(S)-BMIPP isomers (*2,14,15*), the isomers may show significantly different myocardial extraction and/or pharmacokinetic properties. Binding to plasma proteins for transport in the blood, movement through the interstitial space and transfer through the myocyte membrane are events that do not represent solely a simple passive diffusion process. Involvement of fatty acid binding proteins or carrier proteins (*25,26*) for transport through the myocyte plasma membrane may have specific stereochemical requirements. In addition, activation to the requisite fatty acyl intermediates important for incorporation of intracellular fatty acids into glyceride storage products, carnitine derivative for mitochondrial transport and incorporation into complex lipids may be expected to require certain stereochemical features. Furthermore, disturbances in diffusion and carrier-mediated processes of fatty acid uptake may be asymmetrically altered in different myocardial diseases. For these

reasons, this study was focused on the synthesis of the radioiodinated 3(R)-BMIPP and 3(S)-BMIPP isomers and initial evaluation in rats. Since initial attempts to separate the diastereomeric salts formed by combination of the 3-(R,S)-BMIPP racemic mixture with an optically active amine were unsuccessful, an approach involving chromatographic separation of the diastereomeric amides was pursued (*28,29*). We found that the diastereomeric amides prepared by reaction of the acyl chlorides of racemic 3(R,S)-BMPPA and 3(R,S)-BMIPP with the optically active (S)-(−)-α-methylbenzylamine could be chromatographically separated into the (R,S) and (S,S) amides. The more polar diastereomer was thus assigned the (R) configuration, since it was identical to the amide formed from 3(R)-BMPPA synthesized from ethyl-3(R)-methylglutarate (**10A**).

The results of these studies have demonstrated for the first time that the 3(R)-BMIPP isomer has greater myocardial uptake in rats compared with the 3(S)-BMIPP isomer. Although it may have been expected that only one BMIPP isomer would have significant myocardial extraction and/or that the myocardial release kinetics for the isomers would be drastically different, the results suggest that the observed difference is reflected in the myocardial extraction of the two radioiodinated BMIPP isomers, since the rates of myocardial release of radioactivity are essentially the same for both isomers. The percent injected dose per gram values for the other tissue examined were similar for the two isomers (Table 2), which provide further evidence for the differences in absolute myocardial extraction for 3(R)-BMIPP and 3(S)-BMIPP. Future studies will be required to determine the relative incorporation of the 3(R)-BMIPP and 3(S)-BMIPP isomers into intracellular lipids (*30,31*) and to evaluate the relative formation of the p-iodophenylacetic acid metabolite and other expected oxidative products, which have been identified as metabolites from racemic 3-(R,S)-BMIPP (*34,35*).

CONCLUSIONS

Although the relative myocardial uptake and pharmacokinetic behavior of these isomers in humans cannot be predicted, significantly increased myocardial uptake and/or different pharmacokinetic properties of one isomer may have certain benefits. If the relative uptake and properties of the 3(R)-BMIPP and 3(S)-BMIPP isomers observed in animal studies are reproduced in humans, then [^{123}I]-3(R)-BMIPP may represent the preferred isomer for expanded clinical studies focused on the use of ^{123}I-BMIPP in comparison with flow tracers for the evaluation of myocardial viability. Of course, differences in relative myocardial extraction and pharmacokinetics of 3(R)-BMIPP and 3(S)-BMIPP may be more, or less, pronounced in humans than observed in these initial animal studies. The advantages of increased myocardial specificity of one BMIPP isomer are important because the costs associated with ^{123}I could be reduced. Increased retention could permit better statistics for serial SPECT at later time periods. In addition, the radiation exposure would be reduced, and lower relative nontarget tissue uptake in humans would improve visualization of the myocardium, for example, if the relative myocardial/hepatic ratio were increased, visualization of the inferior myocardial wall may be improved. The potential relevance of these results to clinical studies must await the evaluation of the ^{123}I-labeled 3(S)-BMIPP and 3(R)-BMIPP isomers in humans.

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REFERENCES

1. Knapp FF Jr, Kropp J. Iodine-123-labeled fatty acids for myocardial single-photon emission tomography: current status and future perspectives. *Eur J Nucl Med* 1995;22:361–368.
2. Knapp FF Jr. Myocardial metabolism of radioiodinated BMIPP ("cardiodine"). *J Nucl Med* 1995;36:1051–1054.
3. Knapp FF Jr, Franken P, Kropp J. Cardiac SPECT with iodine-123-labeled fatty acids: evaluation of myocardial viability with BMIPP. *J Nucl Med* 1995;36:1022–1030.
4. Knapp FF Jr, Kropp J, Goodman MM, et al. The development of iodine-123-methyl-branched fatty acids and their applications in nuclear cardiology. *Ann Nucl Med* 1993;7:1–14.
5. Tamaki N, Kawamoto M, Yonekura Y, et al. Assessment of fatty acid metabolism using iodine-123-branched fatty acid: comparison with positron emission tomography. *Ann Nucl Med* 1993;7:41–48.
6. De Geeter F, Franken PR, Knapp FF Jr, Bossuyt A. Relationship between blood flow and fatty acid metabolism in subacute myocardial infarction: a study by means to technetium-99m MIBI and iodine-123 beta-methyl iodophenyl pentadecanoic acid. *Eur J Nucl Med* 1994;21:283–291.
7. Franken PR, De Geeter F, Dendale P, Demoor D, Block P, Bossuyt A. Abnormal free fatty acid uptake in subacute myocardial infarction after coronary thrombolysis: correlation with wall motion and inotropic reserve. *J Nucl Med* 1994;35:1758–1765.
8. Franken PR, Demoor D, De Sadeleer C, Block P, Bossuyt A. Free fatty acid uptake in myocardium with postischemic dysfunction: comparison with dobutamine echocardiography to predict long-term functional recovery [Abstract]. *J Nucl Med* 1994;35:50P.
9. Franken PR, Dendale P, Flamen P, Bossuyt A, Knapp FF Jr. Incremental value of combined BMIPP and sestamibi SPECT to predict functional outcome after acute myocardial infraction. In: *Proceedings, Nuclear Medicine Today 1996—Role in Clinical Decision Making*. Cessena, Italy, in press.
10. Kropp J, Juergans M, Glaenzer K, Luederitz B, Biersack H-J, Knapp FF Jr. Evaluation of ischemia and myocardial viability in patients with coronary artery disease (CAD) with iodine-123-labeled 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP). *Ann Nucl Med* 1993;7:93–100.
11. Kropp J, Koehler H, Knapp FF Jr, Biersack H-J. 15-(p-[I-123]iodophenyl)-3-R,S-methylpentadecanoic acid to evaluate ischemia in patients with coronary artery disease [Abstract]. *Eur J Nucl Med* 1991;18:650.
12. Kropp J, Jorgens M, Glanzer K, Knapp FF Jr, Biersack H-J. Evaluation of ischemia and myocardial viability in patients with CAD with the fatty acid 15-(p-[I-123]-iodophenyl-3-R,S-methylpentadecanoic acid. *The First International Congress of Nuclear Cardiology*. Cannes, France: 1993.
13. Goodman MM, Kirsch G, Knapp FF Jr. Synthesis and evaluation of radioiodinated terminal p-iodophenyl-substituted α- and β-methyl-branched fatty acids. *J Med Chem* 1984;27:390–397.
14. Knapp FF Jr, Goodman MM, Ambrose KR, et al. 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*. Boston: Martinus Nijhoff Publishers; 1987:159–201.
15. Antar MA. Radiolabeled fatty acids for myocardial studies. In: Spencer RP, ed. *New Procedures for Nuclear Medicine*. Boca Raton, FL: CRC Press; 1989:95–126.
16. Tamaki N, Tadamura E, Kudoh T, et al. Prognostic value of iodine-123-labeled BMIPP fatty acid analog imaging in patients with myocardial infarction. *Eur J Nucl Med* 1995;23:272–279.
17. Taki J, Nakajima K, Matsunari I, Bunko H, Taka S, Tonami N, Hisada K. Impairment of regional fatty acid uptake in relation to wall motion and thallium-201 uptake in ischemic but viable myocardium: assessment with iodine-123-labeled beta-methyl-branched fatty acid. *Eur J Nucl Med* 1995;22:1385–1392.
18. Tamaki N, Tadamura E, Kawamoto M, et al. Decreased uptake of iodinated branched fatty acid analog indicates metabolic alterations in ischemic myocardium. *J Nucl Med* 1995;36:1974–1980.
19. Kodama K, Igase M, Kazatani Y, Matsuzaki K, Murakami E, Kokubu T. Detection of perioperative coronary vasospasm on iodine-123-MIBG, thallium-201 and iodine-123-BMIPP myocardial SPECT images. *J Nucl Med* 1995;36:2050–2052.
20. Nishimura T, Uehara T, Shimonagata T, Nagata S, Haze K. Clinical experience of

iodine-123-BMIPP myocardial imaging for myocardial infarction and hypertrophic cardiomyopathy. *Ann Nucl Med* 1993;7:35–40.
21. Kurata C, Tawahara K, Okayama K, Wakabayashi Y, Kobayashi A, Yamazaki N, Kaneko M. Myocardial imaging with radioiodinated beta-methyl-branched fatty acid in cardiomyopathy. *Ann Nucl Med* 1993;7:27–34.
22. Morita K, Yanagimoto S, Otsuka N, et al. Iodine-123-BMIPP scintigraphy in seven cases with cardiomyopathy. *Ann Nucl Med* 1993;7:101–107.
23. Buchanan MV, Hettich RL. Fourier transform mass spectrometry of high-mass biomolecules. *Anal Chem* 1993;65:245A–259A.
24. Knapp FF Jr, Goodman MM, Callahan AP, Kirsch G. Radioiodinated 15-(p-iodophenyl)-3,3-dimethylpentadecanoic acid: a useful new agent to evaluate myocardial fatty acid uptake. *J Nucl Med* 1986;27:521–531.
25. Stremmel W. Fatty acid uptake by isolated rat heart myocytes represents a carrier-mediated transport process. *J Clin Invest* 1988;81:844–852.
26. Glatz JFC, van der Vusse GJ, Reneman RS. Protective role of fatty acid-binding protein in ischemic and reperfused heart. *Circ Res* 1991;68:1490–1491.
27. Lam LKP, Hui RAHF, Jones JB. Enzymes in organic synthesis: stereoselective pig liver esterase catalyzed hydrolyses of 3-substituted glutarate diesters; optimization of enantiomeric excess through a reaction conditions control. *J Org Chem* 1986;51:2047–2050.
28. Konoike T, Araki Y. Practical synthesis of chiral synthons for the preparation of HMG-CoA reductase inhibitors. *J Org Chem* 1994;59:7849–7854.
29. Kiratake J, Inagaki M, Yamamoto Y, Oda J. Enaniotropic-group differentiation: catalytic asymmetric ring-opening of prochiral cyclic acid anhydrides with methanol, using chinchona alkaloids. *J Chem Soc Perkin Trans* 1987:1053–1058.
30. Ambrose KR, Owen BA, Goodman MM, Knapp FF Jr. Evaluation of the metabolism in rat hearts of two new radioiodinated 3-methyl-branched fatty acid myocardial imaging agents. *Eur J Nucl Med* 1987;2:486–491.
31. Ambrose KR, Owen BA, Callahan AP, Goodman MM, Knapp FF Jr. Effects of fasting on the myocardial subcellular distribution and lipid distribution of terminal p-iodophenyl-substituted fatty acids in rats. *Nucl Med Biol* 1988;15:695–700.
32. Knapp FF Jr, Goodman MM, Reske SN, et al. Radioiodinated methyl-branched fatty acids: evaluation of catabolites formed in vivo. *NucCompact/Eur Am Commun Nucl Med* 1990;21:229–231.
33. Kropp J, Ambrose KR, Knapp FF Jr, Nissen HP, Biersack H-J. Incorporation of IPPA and BMIPP fatty acid analogs into complex lipids from isolated rat hearts. *Nucl Med Biol* 1992;19:283–288.
34. Morishita S, Kusuoka H, Yamamichi Y, Suzuki N, Kurami M, Nishimura T. Kinetics of radioiodinated species in subcellular fractions from rat hearts following administration of iodine-123-labeled 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (^{123}I-BMIPP). *Eur J Nucl Med* 1996;23:383–389.
35. Yamamichi Y, Kusuoka H, Morishita K, et al. Metabolism of iodine-123-labeled 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP) in perfused rat hearts. *J Nucl Med* 1995;36:1043–1050.

Clinical Validation of Automatic Quantitative Defect Size in Rest Technetium-99m-Sestamibi Myocardial Perfusion SPECT

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We examined the relationships of automatic quantitative perfusion defect size and defect severity to rest left ventricular ejection fraction and semiquantitative visual sestamibi defect size in rest ^{99m}Tc-sestamibi SPECT in 40 consecutive patients with a history of myocardial infarction more than 30 days prior to testing. The purpose of this investigation was to validate the use of automatic quantitative rest sestamibi SPECT as a clinical measure of assessing relative infarction size. **Methods:** All patients received 20–30 mCi of ^{99m}Tc-sestamibi followed by SPECT imaging. Quantitative defect analysis used previously developed resting normal limits and an automatic version of a commercially available quantitative program (CEqual). Semiquantitative visual defect interpretation used a 20 segment/scan and five-point scoring analysis. First-pass (FP) radionuclide ventriculography (RVG) and gated sestamibi perfusion SPECT were each performed in 31 patients. **Results:** LVEF assessed by FP RVG was 37% ± 15% (range 14%–62%) and 37% ± 16% (range 12%–63%) by gated perfusion SPECT with high linear correlation ($r = 0.96$, $n = 22$) between the two methods. Myocardial perfusion defect size was 24% ± 15% of LV (range 0%–50%) and defect severity was 1103 ± 864 (range 0 to 2825) by automatic quantitative rest sestamibi. Perfusion defect size and defect severity both had close correlations with LVEF by FP RVG ($r = -0.78$, $r = -0.86$) and by gated perfusion SPECT ($r = -0.75$, $r = -0.79$). High linear correlations were observed between quantitative defect size and summed visual score of segments with score ≥2 ($r = 0.82$) and the number of visually abnormal segments ($r = 0.77$), as well as between defect severity and visual summed rest score ($r = 0.86$) and the number of visually abnormal segments ($r = 0.76$). **Conclusion:** Quantitation of rest sestamibi SPECT defect extent and severity using automatic CEqual correlates well with rest LVEF and with semiquantitative expert visual analysis. Results of this study define a strong relationship between measurements of ^{99m}Tc-sestamibi perfusion defect as measured by an automatic software program and global left ventricular function. The automatic quantitative program appears to be a useful measure of assessing infarct size in patients with remote myocardial infarction.

Key Words. technetium-99m-sestamibi; myocardial perfusion; myocardial infarction; left ventricular function; SPECT

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The extent of left ventricular (LV) perfusion defect on rest ^{99m}Tc-sestamibi SPECT has been shown to reflect the size of myocardial infarction (*1–3*). The size of myocardial infarction has been shown to be a strong predictor of long-term survival (*4*). Therefore, a readily available, accurate method for the noninvasive measurement of infarct size could provide important prognostic information. In clinical practice, assessment of perfusion defect size is most widely performed with subjective visual analysis (*5*). We have previously developed normal limits and criteria of abnormality for quantitative analysis of rest sestamibi perfusion SPECT, resulting in an automatic quantitative approach which could provide an objective reproducible way to assess perfusion defect size. Since left ventricular ejection fraction (LVEF) has been shown to be inversely proportional to infarction size (*6,7*), we sought to validate the automatic quantitative defect size in rest sestamibi myocardial perfusion SPECT as a clinical measure of assessing infarct size

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