Effect of Monounsaturation of a Branched Fatty Acid on Organ Selectivity

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1-[^{11}C]-3-R,S-methylheptadecanoic acid (BMHA) is a branched chain fatty acid analog that is transported into the myocardium. Due to incomplete metabolism, however, radiolabeled products are trapped within myocytes. Recently, we demonstrated that this compound is an excellent tracer to monitor fatty acid metabolism. **Method:** To evaluate the effect of mono-unsaturation on myocardial substrate utilization, we prepared 1-[^{11}C]-3-R,S-methyl-*trans*—heptadec-7-enoic acid (t-7-BMHA) and measured its biodistribution in rats. In addition, preliminary PET studies were performed on dogs. **Results:** Biodistribution studies demonstrated that myocardial-to-lung and myocardial-to-blood ratios for t-7-BMHA are higher than those for BMHA. Fifteen minutes after injection, heart-to-lung ratios were 5.23 compared to 2.92 and heart-to-liver ratios were 3.07 compared to 1.41 for t-7-BMHA and BMHA. By 30 min postinjection heart-to-lung ratios were 7.03 compared to 5.88 and heart-to-liver ratios were 4.43 compared to 1.09. The heart-to-blood ratio of t-7-BMHA was greater than 11:1. PET imaging with 1-[^{11}C]-t-7-BMHA demonstrated high myocardial extraction, prolonged retention of radioactivity and excellent image quality. Accumulation of radioactivity in the myocardium reached a plateau within 10 min postinjection, with heart-to-blood ratios exceeding 20:1 and heart-to-lung ratios exceeding 10:1. Blood clearance of radioactivity was biphasic with half-times of 1.46 and 14.7 min, respectively. **Conclusion:** These data suggest that introduction of a trans-double bond in BMHA improves myocardial selectivity and results in a potentially superior imaging agent.

Key Words: carbon-11-BMHA; positron emission tomography; fatty acid metabolism

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The primary substrates for myocardial energy metabolism are long-chain fatty acids. Radiolabeled fatty acids (e.g., [^{11}C] palmitate) have been widely investigated as probes for measuring regional myocardial oxidative metabolism (*1–11*). Unfortunately, the complexity of the myocardial kinetics of radiolabeled straight-chain fatty acids has significantly complicated the clinical application of these reagents. A major problem with the use of straight-chain fatty acids to measure myocardial metabolism is the complexity of the washout kinetics of these tracers. In healthy subjects, washout kinetics of straight-chain fatty acids are affected by physical and even emotional stress (*12*). In ischemic or infarcted myocardium, these effects are even more profound. Overall, the results of most studies with these radiopharmaceuticals have been more confusing than encouraging. The accumulation and clearance of ^{11}C-palmitate, as described by the late phase of myocardial radioactivity during ischemia, does not accurately reflect substrate oxidation since nearly 50% of the radioactivity corresponds to unmetabolized substrate (*12–14*).

The clinical application of ^{123}I-ω-labeled straight chain fatty acids for measuring myocardial metabolism has several shortcomings:

1. Quantitative analysis requires correction of the images for free ^{123}I (*15*).
2. Rapid deterioration of image quality due to fast myocardial clearance limits the imaging time to the first 30 min after injection (*16*).
3. Inferiority to ^{201}Tl for detecting myocardial ischemia (*17–20*).

These observations demonstrate the need for better tracers of myocardial metabolism. In this regard, beta-methylated fatty acids such as 3-R,S-methylheptadecanoic acid (BMHA) (Fig. 1), have been particularly promising. BMHA has been shown to have biological properties that are similar to naturally occurring straight-chain fatty acids (*21*). This substrate undergoes several cycles of beta oxidation and is eventually trapped in the cytosolic and mitochondrial compartments as an ^{11}C-labeled beta-ketoacyl-SCoA intermediate (*22*), similar to the intracellular "trapping" of phosphorylated deoxyglucose (*23*). Based on these considerations, [^{123}I] (p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPPA) was synthesized (*24*). This radiopharmaceutical has excellent in vivo properties (*24–26*) and was recently introduced into clinical practice as a SPECT tracer of myocardial metabolism (*27,28*). By performing dual-photon imaging after coinjection of [^{123}I]BMIPPA and ^{201}Tl, myocardial metabolism and perfusion can be evaluated simultaneously.

Recently, it was reported that there is a significant cor-

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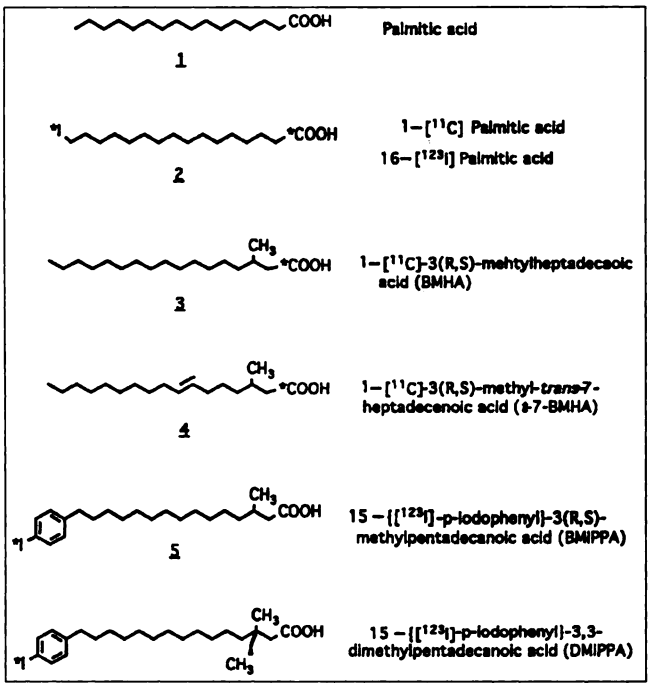

FIGURE 1. Structure and radiolabeling positions of straight- and branched-chain fatty acid analogs.

relation between risk of coronary artery disease and ingestion of trans fatty acids (*29*). Since these compounds are normally present at very low concentrations, it is possible that radiolabeled branched trans fatty acid may be useful probes for studying cardiac metabolism in health and disease. In this study, we report the synthesis and preliminary biological evaluation of 1-[^{11}C]-3-R,S-methyl-*trans*-heptadec-7-enoic acid (*t*-7-BMHA). Like BMHA, this branched chain unsaturated fatty acid analog is also trapped intracellularly as a product of beta oxidation.

MATERIALS AND METHODS

All chemical reagents were of analytical grade and used without further purification. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Proton magnetic resonance spectra (^{1}H-NMR) were recorded with a Varian T-60 NMR spectrophotometer using TMS as an internal standard. Thin-layer chromatography was performed on DC-Alufolien Kiesel gel 60 F (E. Merck) using hexane:diethyl ether:acetic acid, 70:30:1 (v/v/v) as the mobile phase. Lipids were visualized by spraying the plates with 5% $CuSO_4$ in water solution containing 15% concentrated phosphoric acid followed by drying and heating to (200°C) for 10 min.

Chemistry

Dodec-2-enyl bromide (2). Trans-dodec-2-ene-1-ol (15.46 g, 0.08 mole) and triphenylphosphine (43.8 g, 0.17 mole) were dissolved in 640 ml benzene and carbon tetrabromide (55.2 g, 0.17 mmole) in 120 ml benzene was added with stirring. After heating the mixture at reflux for 90 min, TLC indicated that all of the starting material was consumed. The reaction mixture was cooled to room temperature, filtered and the filter cake was washed with 300 ml petroleum ether. The petroleum ether wash was combined with the filtrate and evaporated to dryness. Petroleum ether (300 ml) was added to the residue and the mixture was refluxed for one hour. The mixture was then cooled to room temperature and filtered. The filtrate was evaporated to an oil which was distilled under vacuum. The fraction boiling at 73–80°C/0.2 mmHg was collected; 14.5 g (70% yield).

Tetradec-4-enoic Acid (3). Sodium metal (1.27 g, 0.05 mole) was dissolved in 50 ml absolute ethyl alcohol. Diethyl malonate (8.08 g, 0.05 mole) was added slowly with stirring, followed by a solution of dodec-2-enyl bromide (12.10 g, 0.05 mole) in 17 ml absolute ethyl alcohol and the reaction mixture was refluxed for 2 hr. No starting material was detected by TLC. Two-thirds of the ethyl alcohol was evaporated, 100 ml water and 200 ml petroleum ether were added and the aqueous layer was extracted with 200 ml petroleum ether. The combined petroleum ether extract was washed with 50 ml water, dried over sodium sulfate and filtered. The filtrate was evaporated to dryness to yield an oil. The diethyl ester (R_f = 0.75) was hydrolyzed without purification. A solution of potassium hydroxide (27.44 g, 0.49 mole) in 55 ml water was added to the residue and the mixture was refluxed for 3 hr with stirring. The mixture was then cooled in an ice bath, acidified to pH 1 with concentrated hydrochloric acid and extracted with petroleum ether (2 × 1000 ml). The petroleum ether extract was washed with brine (100 ml), dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The yield of 1-carboxyl-tetradec-4-enoic acid (a white solid) was 8.82 g (67%), melting point of 140–142°C.

Tetradec-4-enol (4). Tetradec-4-enoic Acid (3, 3.47 g, 0.02 mole) in 20 ml ether was added slowly to an ice cold suspension of lithium aluminum hydride (2.1 g, 0.06 mole) in 100 ml ether. The mixture was refluxed for 3 hr and cooled with an ice-bath. Water (5 ml) was added and the mixture was extracted with ethyl ether (2 × 50 ml). The ether extract was washed with water (2 × 25 ml), dried over sodium sulfate, filtered and evaporated to dryness to yield 3.21 g of crude tetradec-4-enol. The product was partitioned between dichloromethane (50 ml)/water (50 ml), and the dichloromethane layer was dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to dryness to yield 3.12 g of pure tetradec-4-enol (96%). TLC showed one spot, R_f = 0.25.

Tetradec-4-enyl Bromide (5). Carbon tetrabromide (14.6 g, 0.04 mole) in 30 ml benzene was added to a solution of compound 4 (4.65 g, 0.022 mole) and triphenyl phosphine (11.54 g, 0.044 mole) in 140 ml benzene (slowly with stirring) and the mixture was heated at reflux 90 min. TLC indicated that all of the starting material was consumed. The mixture was cooled to room temperature, filtered and washed with petroleum ether (3 × 100 ml). The filtrate was evaporated to dryness, and petroleum ether (75 ml) was added to the residue. The mixture was refluxed with stirring for 1 hr, cooled to room temperature, filtered, evaporated to dryness and distilled under reduced pressure to yield an oil, 5.48 g (91%).

2-Methylhexadec-6-Enoic Acid (6). Sodium metal (0.46 g, 0.02 mole) was dissolved in 21.6 ml absolute ethanol. Methyl malonate (3.54 g, 0.02 mole) followed by compound 5 (5.4 g, 0.02 mole) in 8.2 ml absolute ethanol were added and the mixture was refluxed for 2 hr. Subsequent procedures were the same as those described for the synthesis of compound 3. Compound 6 (5.26 g) was isolated (64% yield).

2-Methyl-trans-6-Hexadecen-1-ol (7). Lithium aluminum hydride (1.25 g) was suspended in 50 ml anhydrous ethyl ether and a solution of 6 (2.12 g, 7.9 mole) in 125 ml anhydrous ethyl ether was added drop-wise. The reaction mixture was refluxed for 3.5 hr and then cooled in an ice bath. Subsequent procedures were the same

as those described for the synthesis of 4. For compound 7, 1.51 g were obtained (76% yield). The overall yield of 7 from 1 was 14%.

2-Methyl-trans-hexadecenyl Bromide (8). This compound was prepared by an adaptation of the method of Hooz (*30*). A solution of carbon tetrabromide (2.0 g, 11.8 mmole) and triphenylphosphine (3.10 g, 11.8 mmole) in 10 ml benzene was added with stirring to a solution of compound 7 (1.51 g, 5.9 mmole) in 50 ml benzene and the mixture was heated at reflux for 90 min, cooled, filtered and washed extensively with petroleum ether. The filtrate, including washes, was concentrated in vacuo and the residue was diluted with petroleum ether. The mixture was heated for 1 hr, filtered and the filtrate was concentrated in vacuo. The residual liquid was distilled to yield 1.4 g (75%) of compound 8 (bp: 115°C/0.15 mmHg). Anal. calc. for $C_{17}H_{33}Br$ (317.34): C, 64.34; H; 10.48; Br; 25.18, found: C, 64.10; H, 10.34, Br, 25.02.

2-Methylheptadec-trans-7-enyl Cyanide (9). A mixture of 8 (1.48 g, 4.67 mmole) and NaCN (366 mg, 7.47 mmole) in 15 ml DMSO was stirred at 70–75°C for 4 hr and then poured into water. The aqueous solution was extracted with ether (150 ml × 3) and the extracts were combined, dried over Na_2SO_4 and concentrated to a waxy solid which was chromatographed on silica gel (9% HOAc/Et_20). The acid was obtained as a white solid, mp 58°C–59°C. Yield: 250 mg (19%).

3-Metylheptadec-trans-7-enoic Acid (10). 2-Methylheptadec-7-enyl cyanide (160 mg, 0.61 mmole), 1 ml 6-N NaOH and 1 ml DMSO were stirred in a sealed tube under Argon. The reaction mixture was heated in an oil-bath at 220°C for 4 hr, cooled to room temperature, acidified and extracted with ether. The ether extract was washed with brine, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. ^1H NMR ($CDCl_3$) 1.22 (d,3H), 0.68–2.5 (m,29H), 5,4 (m,2H) 11.1 (S,1H). MS, m/e 282 (m^+; 8%); 265 (m^+-H_2O; 23%), 222 (m^+-60; 100%). Anal. calcd. for $C_{18}H_{34}O_2$ (286.45); C, 76.54; H, 12.13; found: C, 76.16; H, 11.83. The configuration of 10 is trans because the synthesis started with the trans bromoderivative and none of the subsequent reactions alter stereochemistry.

1-[^{11}C]3-Methylheptadec-trans-7-enoic Acid (11). A mixture of 8 (200 mg, 0.73 mmol) and magnesium metal (16.9 mg) in 5 ml ethyl ether (freshly distilled from CaH_2) was heated at reflux under argon for 90 min and then transferred to a radiolabeling reaction funnel which had been flushed with helium. Carbon-11 labeling of the final product was achieved using $^{11}CO_2$, as previously reported (*31,32*). Typically, the specific activity of the final product was between 100 and 200 mCi/mmole as estimated from the specific activity of $^{11}CO_2$. The decay-corrected radiochemical yield was 30%–40% and the radiochemical purity of the final product exceeded 98% by TLC.

Tissue Distribution Studies

CD Fisher rats (175–225 g) were anesthetized with ether and 80–100 μCi radiolabeled compound 11 were injected through the femoral vein. The rats were killed by ether asphyxiation at 5, 15 and 30 min postinjection. Samples of the blood, heart, lung and liver were collected and radioactivity was measured with a well-type automatic gamma counter. All results were expressed as the percentage injected dose per gram (mean ± s.e.m., six animals per group).

PET

Images were acquired with a PC-384 PET camera (Scanditronix AB, Uppsala, Sweden). This instrument is well-described in the literature (*33*). The primary imaging are an in-plane resolution of 7.0 mm FWHM, axial resolution of 12 mm, 5 contiguous slices of 14 mm separation and a sensitivity of 22,000 cps/μCi.

Two mongrel dogs (~20 kg) were fasted overnight, anesthetized with sodium pentobarbital (2.9 mg/kg) and placed supine on a plexiglas imaging cradle with the heart oriented so that the central imaging plane transversed the mid left ventricle perpendicular to its long axis. A transmission scan for attenuation correction was recorded using a cylindrical annulus containing a solution of $^{68}GaCl_3$. Compound 11 (4.69 mCi) was injected through a femoral vein and serial PET images were recorded every 20 sec for 9 min and then every minute for a total imaging time of 48 min. Serial 1.0-ml blood samples were drawn from a contralateral femoral vein catheter (every minute for the first 10 min and at 5-min intervals thereafter) and whole blood radioactivity was measured with a well-type automatic gamma counter.

All images were reconstructed using a conventional filtered back projection algorithm to an in-plane resolution of 7 mm FWHM. The projection data were corrected for nonuniformity of detector response, deadtime, random coincidences and scattered radiation. Irregular regions of interest (ROIs) were drawn over the left ventricular lateral wall, septum and left ventricular cavity. Circular ROIs were placed over the lungs and dorsal paravertebral muscles.

The PET camera was cross-calibrated with a well scintillation counter by comparing the PET camera response from a uniform distribution of an ^{18}F solution in a 20-cm cylindrical phantom with the response of the well counter to an aliquot of the same solution.

Statistical Analysis

The unpaired Student t-test was used to compare tissue concentrations of *t*-7-BMHA with previously reported concentrations of BMHA. Probability values of less than 0.05 were considered to be statistically significant. The blood clearance data were fit to a bi-exponential function by nonlinear least squares.

RESULTS

Radiochemical Synthesis

Figure 2 summarizes the synthesis of the title compound. Synthesis of the bromide derivatives was performed by modification of the method of Hooz, which was used to prepare alkyl chlorides (*30*). Briefly, *trans*-2-dodecen-1-ol 1 was brominated with triphenyl phosphine and carbon tetrabromide in benzene to give the corresponding bromide 2 in 75% yield. Compound 2 was alkylated with diethylmalonate followed by saponification and decarboxylation to produce *trans*-4-tetradecenoic acid 3 in 45% yield. Lithium aluminum hydride reduction of 3 gave the primary alcohol 4 which was converted to the corresponding bromide 5, as described above.

Alkylation of 5 with diethylmethylmalonate and subsequent saponification and decarboxylation gave 2-methyl-*trans*-6-hexadecenoic acid 6. Reduction of 6 with lithium aluminum hydride gave the primary alcohol 7, which underwent bromination in the prescribed fashion to give the corresponding primary bromide 8. Compound 8 was converted to the corresponding nitrile which was hydrolyzed to yield 3-methyl-trans-7-heptadecenoic acid 9. Carboxylation of the Grignard reagent of 8 with $^{11}CO_2$ yielded radiochemically pure (>98%) compound 11, with a specific activity of 100–200 mCi/mmol. Structural identification of the title

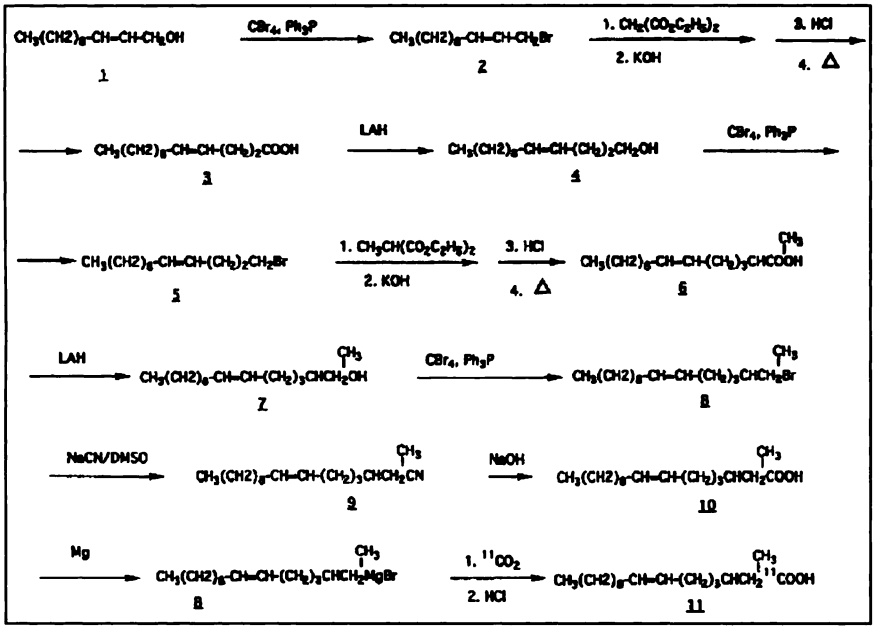

FIGURE 2. Synthetic scheme for the preparation of *t*-7-BMHA.

compound was confirmed by mass spectral analysis of the unlabeled compound. Elemental analyses of all intermediates were within 0.4% of the theoretical values.

Biodistribution in Rats

Table 1 summarizes the tissue biodistribution of BMHA and *t*-7-BMHA in rats. The initial myocardial extraction of both substances was similar. At 30 min postinjection, myocardial concentration of BMHA was 2.96 ± 0.7 compared with 2.04 ± 0.62 for *t*-7-BMHA. In the lung and liver, concentrations of *t*-7-BMHA were lower than BMHA. Figures 3 and 4 illustrate the heart-to-tissue ratios for BMHA and *t*-7-BMHA. At all three times, significantly higher heart-to-liver ratios were observed with *t*-7-BMHA ($p < 0.05$) and higher heart-to-lung ratios were measured. The greatest difference was observed at 5 min postinjection.

PET

Figure 4 shows serial 1-min images of [^{11}C]-*t*-7-BMHA accumulation in canine myocardium at one ventricular level and Figure 5 illustrates a 3-min image acquired between 8 and 11 min postinjection at five ventricular levels. Figure 6 shows the time dependence of [^{11}C]-*t*-7-BMHA concentration in the blood pool, left ventricular lateral wall, septum, lung and back muscle; the time dependence of the heart-to-lung radioactivity ratio is also illustrated. These data indicate that [^{11}C]-*t*-7-BMHA clears from the blood rapidly and enters the myocardium to provide excellent quality images. Canine myocardial *t*-7-BMHA radioactivity plateaued within 10 min postinjection and remained at this level for at least 48 min. The value of the heart-to-lung ratio at plateau exceeded 10:1 and the heart-to blood ratio was greater than 20:1 (data not shown). Radioactivity in the relaxed paravertebral muscles also showed a plateau pattern: muscle-to-blood ratio ~2:1. Figure 7 illustrates that the blood clearance of ^{11}C-*t*-7-BMHA radioactivity was well described by a biexponential function (half-times = 1.46 and 14.7 min). Similar results were obtained in the other dog.

DISCUSSION

The exact mechanisms of cellular uptake and intracellular transport of different fatty analogs acids remain unclear, but several processes appear to affect extraction, retention

TABLE 1
Biodistribution of BMHA and *t*-7-BMHA in Rats* (mean ± s.e.m., n = 6)

	[1-^{11}C]BMHA†			[1-^{11}C] *t*-7-BMHA		
	5 min	15 min	30 min	5 min	15 min	30 min
Blood	0.41 ± 0.06	0.19 ± 0.02	0.29 ± 0.05	0.26 ± 0.05	0.18 ± 0.06	0.16 ± 0.01
Heart	2.32 ± 0.32	2.16 ± 0.68	2.94 ± 0.7	2.47 ± 0.49	2.09 ± 0.58	2.04 ± 0.62
Lung	1.43 ± 0.10	0.74 ± 0.08	0.50 ± 0.12	0.43 ± 0.13	0.40 ± 0.11	0.29 ± 0.04
Liver	1.64 ± 0.23	1.53 ± 0.29	1.62 ± 0.21	0.82 ± 0.24	0.68 ± 0.09	0.47 ± 0.04

*Expressed as percent injected dose per gram.
†From reference 31.

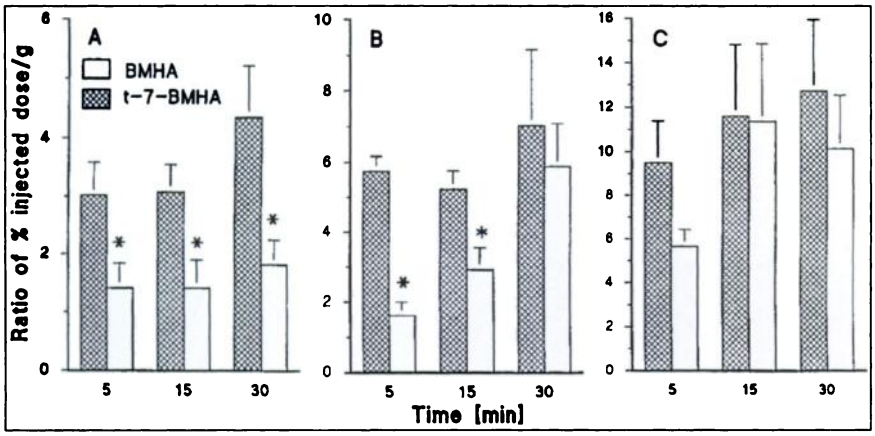

FIGURE 3. Heart-to-blood (A), heart-to-lung (B) and heart-to-liver (C) ratios for *t*-7-BMHA (cross-hatched bars) and BMHA- (open bars) at 5, 15 and 30 min postinjection. Each value is the mean ± s.e.m. for six rats. *$p < 0.05$.

and metabolism of fatty acids and their analogs. For a given metabolic state, factors such as lipophilicity, chain length, affinity for carriers and key enzymes (i.e., acylCoA synthase) determine rate of uptake and metabolism. Earlier observations have demonstrated that the chain length of straight-chain fatty acids is an important determinant of myocardial extraction (*34,35*). In addition, mono-unsaturation significantly increases fatty acid extraction by the myocardium; the extraction of 16-iodo-9-hexadecenoic acid is twice that of the saturated analog (*36,37*). In the course of evaluating the effect of beta-dimethylation on myocardial extraction, we verified that chain length is an important factor in extraction. For example, myocardial extraction of 3-R,S-methylheptadecanoic acid is higher than that of heptadecanoic (HDA) but myocardial extraction of the beta-gem-dimethylated analog (DMHA) is lower (*38*). Furthermore, when straight-chain fatty acids were compared with beta-mono or beta-dimethylated analogs, a steady increase in myocardial extraction was observed for ^{18}C or longer fatty acids and ^{15}C fatty acids containing a vinyl group (*38,39*).

At the level of cellular extraction, fatty acid binding proteins (FABPs) may play a major role. FABPs are low molecular weight cytosolic proteins (14–15 kD) that are present in endothelium, intestinal epithelial cells, muscle and other tissue. These proteins serve as a transport system for medium- and long-chain fatty acids. In endothelial cells, FABPs are responsible for the transport of fatty acids from the vascular bed to various tissues where they are further transported into cytosolic compartments by cell-specific FABPs. Distinct differences in the structures and immunogenicity of FABPs isolated from the heart and liver of different species, including humans, have been reported (*40,41*). Both proteins bind free fatty acids; 2 moles of oleic or palmitic acid/mole of protein (*42*). The findings of recent studies indicate that the capacity to oxidize fatty acids is correlated with cellular concentration of FABPs, particularly in heart and skeletal muscle (*43,44*). Although little is known about the differential affinity of these molecules for branched-chain or unsaturated fatty acids, the differences between heart and liver FABPs may explain the differences

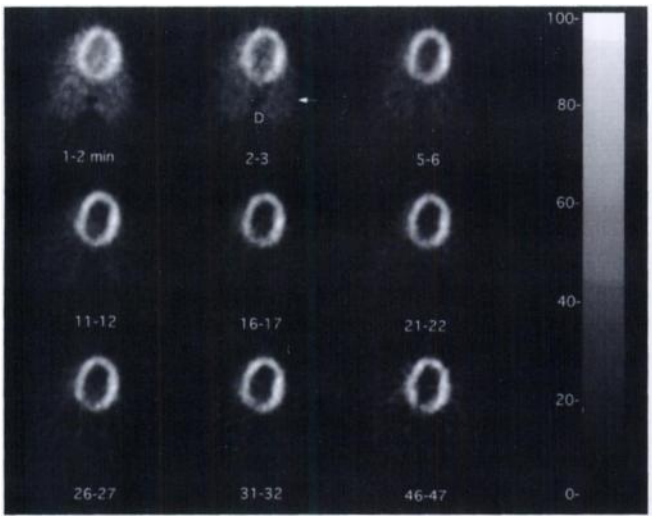

FIGURE 4. Sequential 1-min images of dog heart and thoracic structures acquired after intravenous injection of 4.6 mCi t-7-BMHA. The first two images show blood-pool activity in the lungs and left ventricular cavity which clears after 10–15 min. V = ventral; D = dorsal.

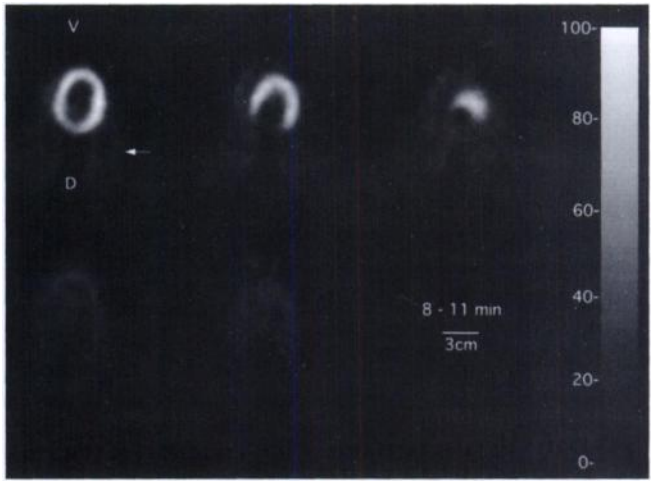

FIGURE 5. Dog hearts imaged by PET at five levels acquired for 3 min starting 8 min after intravenous injection of 4.6 mCi t-7-BMHA. The arrow indicates pulmonary activity. V = ventral; D = dorsal.

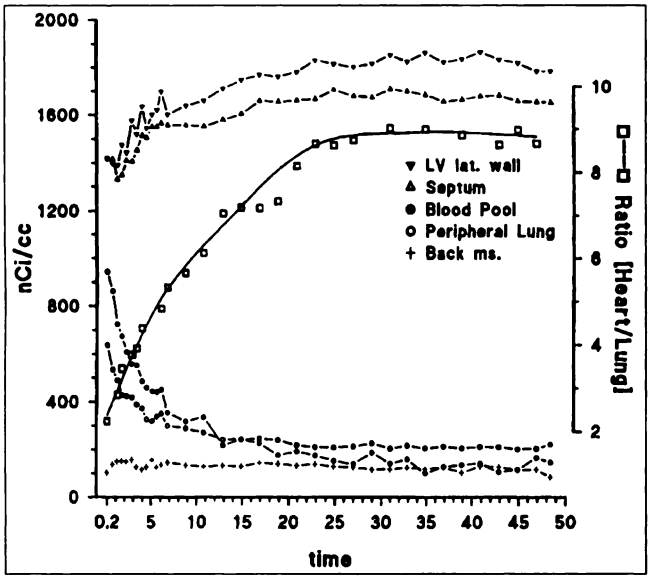

FIGURE 6. Time-activity curves (nCi/cc) for canine myocardium (triangles), blood (closed circles), lung (open circles) and paravertebral muscles (plus sign) dervived from the PET data. Open squares represent the heart-to-lung ratio which plateaued after 15–20 min.

between heart-to-liver ratios observed for saturated compared to unsaturated BMHA in this study.

CONCLUSION

The results of this study establish that the introduction of a trans double bond at the 7 position of BMHA results in a radiopharmaceutical with improved heart-to-lung and heart-to-liver ratios in normal rats and dogs. The utility of *t*-7-BMHA and other unsaturated analogs to evaluate myocardial disease remains to be tested.

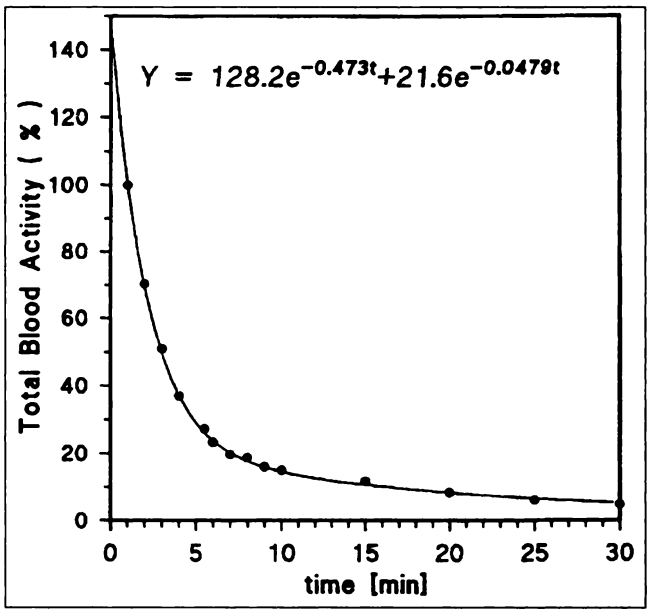

FIGURE 7. Clearance of *t*-7-BMHA from the circulation. The data are normalized to the measurement at 1 min postinjection. The solid curve represents a fit of the data to a biexponentail function (half-times = 1.4 and 14.6 min, respectively).

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