Technetium-99m-Labeled Long Chain Fatty Acid Analogues Metabolized by β-Oxidation in the Heart

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The development of ^{99m}Tc-labeled fatty acid analogues metabolized by β-oxidation in the myocardium constitutes an unsolved challenge. On the basis of our recent findings that [^{188}Re]tricarbonyl(cyclopentadienylcarbonate)rhenium ([^{188}Re]CpTR-COOH) was recognized as an aromatic compound and was metabolized as such in the body, [^{99m}Tc]cyclopentadienyltricarbonyltechnetium ([^{99m}Tc]CpTT) was conjugated at the ω-position of pentadecanoic acid to prepare [^{99m}Tc]CpTT-PA. When injected into rats, [^{99m}Tc]CpTT-PA exhibited the maximum myocardial accumulation and heart-to-blood ratio of 3.85 %ID/g at 1 min and 4.60 at 10 min postinjection, respectively. The metabolic study using isolated Langendorff perfused rat hearts demonstrated that approximately 67% of perfused [^{99m}Tc]CpTT-PA was incorporated and [^{99m}Tc]-CpTT-propionic acid, the metabolite after six cycles of β-oxidation of [^{99m}Tc]CpTT-PA, was detected as the major radiometabolite in the perfusate and myocardium. These findings indicate that [^{99m}Tc]CpTT-PA was recognized, transported, and metabolized as a long chain fatty acid analogue for energy production in the myocardium.

Introduction

Long chain fatty acids constitute a major source of energy in normal myocardium.[1,2] Since regional alternations in the myocardial fatty acid metabolism usually occur in ischemic heart disease and cardiomyophathies, radiolabeled long chain fatty acid analogues play an important role in the diagnosis of heart disease[3] and have been proven useful in the differential diagnosis of unstable angina or severe heart ischemia.[4] Carbon-11 (^{11}C) labeled palmitic acid, iodine-123 (^{123}I) labeled 15-(*p*-[^{123}I]-iodophenyl)pentadecanoic acid ([^{123}I]IPPA,[a] Figure 1A), and 15-(*p*-[^{123}I]iodophenyl)-3-(*R*,*S*)-methylpentadecanoic acid ([^{123}I]-BMIPP) are the representative fatty acid analogues used in clinical studies.[5,6] However, an on-site cyclotron is required to produce ^{11}C-labeled palmitic acid, and radioiodinated compounds must be obtained from radiopharmaceutical companies. Since heart disease generally requires an urgent examination, it would be useful if an on-site radiopharmaceutical could be used for clinical diagnosis. Thus, efforts have been made to develop ^{99m}Tc-labeled fatty acid analogues.[7−14] However, these ^{99m}Tc-labeled fatty acid analogues suffered from poor myocardial uptake. In addition, it remains uncertain whether they were metabolized by β-oxidation in the heart.

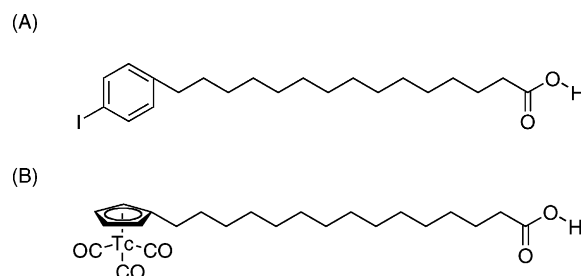

Figure 1. Chemical strucures of IPPA (A) and CpTT-PA (B).

We have previously observed that ^{99m}Tc-labeled *N*-[[[(2-mercaptoethyl)amino]carbonyl]methyl]-*N*-(2-mercaptoethyl)-6-aminohexanoic acid ([^{99m}Tc]MAMA-HA), a medium chain fatty acid analogue, was metabolized by β-oxidation in the liver[15] and that [^{99m}Tc]MAMA-conjugated hexadecanoic acid (HDA), a long chain fatty acid analogue of [^{99m}Tc]MAMA-HA, showed heart-to-blood ratios of 3.6 at 2 min postinjection.[16] The metabolic study also indicated that [^{99m}Tc]MAMA-HDA was metabolized by β-oxidation in the body. These findings suggest that an incorporation of an appropriate ^{99m}Tc chelate to a long chain fatty acid would provide ^{99m}Tc-labeled fatty acid analogues recognized and metabolized by the heart.

We have also observed that [^{188}Re]tricarbonyl(cyclopentadienylcarbonate)rhenium ([^{188}Re]CpTR-COOH) was recognized as an aromatic compound and was metabolized as such in the body.[17] The organometallic rhenium compounds have chemical properties similar to technetium counterparts.[18−20] These results suggest that cyclopentadienyltricarbonyltechnetium (CpTT) may constitute an appropriate molecule to prepare a ^{99m}Tc-labeled long chain fatty acid that reflects fatty acid metabolism in the heart.

In this study, [^{99m}Tc]CpTT was introduced at the ω-position of a pentadecanoic acid to prepare [^{99m}Tc]CpTT-PA (Figure 1B). The biodistribution of radioactivity after injection of [^{99m}Tc]-CpTT-PA was compared with [^{125}I]IPPA in rats. The myocar-

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[a] Abbreviations: CpTR-COOH, tricarbonyl(cyclopentadienylcarbonate)-rhenium; CpTT, cyclopentadienyltricarbonyltechnetium; IPPA, 15-(*p*-iodophenyl)pentadecanoic acid; BMIPP, 15-(*p*-iodophenyl)-3-(*R*,*S*)-methylpentadecanoic acid; CpTT-PA, tricarbonyl(15-cyclopentadienyl pentadecanoic acid)technetium; CpTR-PA, tricarbonyl(15-cyclopentadienyl pentadecanoic acid)rhenium; CpTT-propionic acid, tricarbonyl(3-cyclopentadienyl propionic acid)technetium; CpTR-propionic acid, tricarbonyl(3-cyclopentadienyl propionic acid)rhenium; MAMA-HA, *N*-[[[(2-mercaptoethyl)amino]carbonyl]methyl]-*N*-(2-mercaptoethyl)-6-aminohexanoic acid; MAMA-HDA, *N*-[[[(2-mercaptoethyl)amino]carbonyl]methyl]-*N*-(2-mercaptoethyl)-conjugated hexadecanoic acid.

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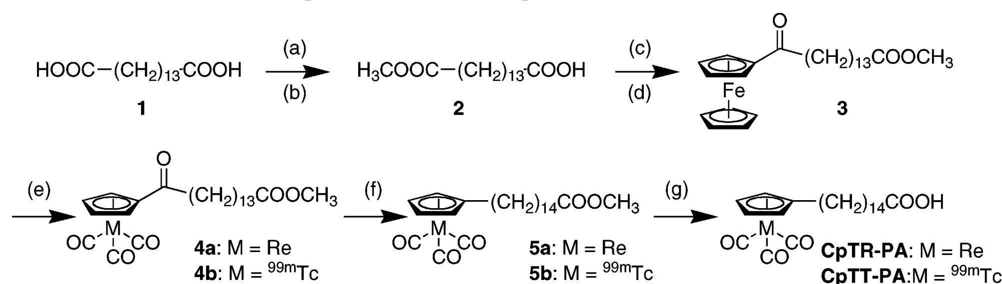
Scheme 1. Synthetic Procedure for [$^{185/187}$Re]CpTR-PA and [^{99m}Tc]CpTT-PA[a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH; (b) $Ba(OH)_2$; (c) $SOCl_2$; (d) $AlCl_3$, ferrocene; (e) $CrCl_3$, $Cr(CO)_6$, $^{99m}TcO_4^-$ or $^{185/187}ReO_4^-$; (f) $TiCl_4$, Et_3SiH; (g) 2 N NaOH.

Table 1. RP-HPLC Retention Times of [^{99m}Tc]CpTT-PA, [$^{185/187}$Re]CpTR-PA, and Their Synthetic Precursors

compd	retention time (min)
4a	14
4b	15.5
5a	23
5b	24
[$^{185/187}$Re]CpTR-PA	15.5
[^{99m}Tc]CpTT-PA	17.5

dium metabolism of [^{99m}Tc]CpTT-PA was also compared with [^{125}I[IPPA using the Langendorff rat heart model. The molecular design of [^{99m}Tc]CpTT-PA for measuring fatty acid metabolism in the heart was estimated.

Results

Synthesis of [$^{185/187}$Re]CpTR-PA, [^{99m}Tc]CpTT-PA, and Their Derivatives. Both nonradioactive [$^{185/187}$Re]CpTR-PA and [^{99m}Tc]CpTT-PA were synthesized under similar procedures as outlined in Scheme 1. Compound **3** was prepared by acylation of ferrocene with acid chloride of compound **2** in the presence of $AlCl_3$.[21] The double ligand transfer reaction of the ferrocene precursor, compound **3**, with nonradioactive $^{185/187}ReO_4^-$ or $^{99m}TcO_4^-$ produced compound **4a** or **4b**.[20] The carbonyl group in compounds **4a** and **4b** was then reduced according to the procedure of Bhattacharyya[22] using titanium(IV) chloride and triethylsilane. Afterward, the methyl ester of compounds **5a** and **5b** was saponified to produce [$^{185/187}$Re]CpTR-PA and [^{99m}Tc]-CpTT-PA. After purification by RP-HPLC, [^{99m}Tc]CpTT-PA was obtained with a radiochemical yield and purity of 10.1% (not decay corrected) and over 93%. The comparative RP-HPLC retention times of CpTM (M = ^{99m}Tc or $^{185/187}$Re) derivatives are summarized in Table 1.

Nonradioactive tricarbonyl(3-cyclopentadienyl propionic acid)-rhenium ([$^{185/187}$Re]CpTR-propionic acid) and its ^{99m}Tc counterpart ([^{99m}Tc]CpTT-propionic acid) were synthesized according to the procedure as described above. [$^{185/187}$Re]CpTR-propionic acid showed a retention time of 5.0 min on RP-HPLC (system 2), while [^{99m}Tc]CpTT-propionic acid had a retention time of 5.5 min under similar conditions.

Biodistribution Study. The biodistribution of radioactivity after simultaneous injection of [^{99m}Tc]CpTT-PA and [^{125}I]IPPA to rats is summarized in Table 2. [^{99m}Tc]CpTT-PA showed the maximum myocardial accumulation of 3.85 %ID/g at 1 min postinjection, followed by a gradual washout from the heart. [^{125}I]IPPA registered the time-course of radioactivity in the heart similar to [^{99m}Tc]CpTT-PA except that [^{125}I]IPPA reached the maximum radioactivity level of 7.59 %ID/g at 1 min post-injection. [^{99m}Tc]CpTT-PA showed a slow elimination rate of radioactivity from the blood at earlier postinjection time, whereas a gradual increase in radioactivity in the blood was observed with [^{125}I]IPPA from 2 to 30 min postinjection. As a result, while [^{125}I]IPPA exhibited the highest heart-to-blood ratio of the radioactivity of 12.46 at 2 min postinjection, [^{99m}Tc]-CpTT-PA reached the highest ratio of 4.60 at 10 min postinjection. In contrast, the radioactivity levels in the liver remained unchanged after injection of [^{125}I]IPPA, whereas [^{99m}Tc]CpTT-PA showed a gradual increase with times. The radioactivity levels in the stomach were low for the two radiolabeled fatty acid analogues.

Metabolic Analysis. Metabolite analysis was carried out in an isolated perfused rat heart model. After completion of the perfusion, the rat heart contained 34.5 $\pm$ 3.83% of perfused radioactivity for [^{99m}Tc]CpTT-PA and 90.5 $\pm$ 4.59% for [^{125}I]-IPPA. More than 93% of ^{99m}Tc and ^{125}I radioactivity in the heart homogenates was extracted into the organic phase. Figure 2 depicts TLC radiochromatograms of the rat heart extract. The highest radioactivity counts were detected in fractions of higher R_f values than intact [^{99m}Tc]CpTT-PA or [^{125}I]IPPA (55% for [^{99m}Tc]CpTT-PA and 76% for [^{125}I]IPPA). The minor components were observed as [^{99m}Tc]CpTT-PA (15%) and more hydrophilic compounds (30%) for [^{99m}Tc]CpTT-PA, [^{125}I]IPPA (14%) and more hydrophilic compounds (10%) for [^{125}I]IPPA.

After hydrolysis of the rat heart extract, more than 92% of ^{99m}Tc and ^{125}I radioactivity was recovered in the organic phase. Figure 3a shows RP-HPLC radiochromatograms of the hydrolyzed rat heart extract of ^{99m}Tc radioactivity trace (left) and ^{125}I radioactivity trace (right). Besides [^{99m}Tc]CpTT-PA, multiple radioactivity peaks were observed at retention times shorter than [^{99m}Tc]CpTT-PA. A polar component (5.5 min) showed a retention time similar to that of [^{99m}Tc]CpTT-propionic acid, as determined by co-chromatography. Similarly, the ^{125}I radioactivity trace of the hydrolyzed heart extract exhibited some radioactivity peaks with the major radioactivity peak being observed at a retention time of 26 min, similar to that of 13-(*p*-iodophenyl)tridecanoic acid, as determined by co-chromatography.

The rat heart perfusate was extracted by organic solvents with more than 92% efficiency for both ^{99m}Tc and ^{125}I radioactivity. Figure 3b shows the radiochromatograms of the perfusate. [^{99m}Tc]CpTT-PA accounted for approximately 50% of the radioactivity in the perfusate. Besides [^{99m}Tc]CpTT-PA, the ^{99m}Tc radioactivity trace showed two major radioactivity peaks at retention times of 2.5 min and 5.5 min. The radioactive peak at a retention time of 5.5 min was coeluted with [^{99m}Tc]CpTT-propionic acid. The ^{125}I radioactivity trace showed [^{125}I]IPPA along with a radioactivity peak at a retention time of 5 min, which was coeluted with *p*-iodobenzoic acid. Approximately 40% of the radioactivity in the perfusate was detected at [^{125}I]-IPPA fraction.

Table 2. Biodistribtuion of Radioactivity in Rats after Co-Injection of [^{99m}Tc]CpTT-PA and [^{125}I]IPPA[a]

	time after injection				
	1 min	2 min	5 min	10 min	30 min
			[^{99m}Tc]CpTT-PA		
blood	4.59 (0.20)	2.70 (0.15)	0.93 (0.12)	0.41 (0.04)	0.38 (0.10)
heart	3.85 (0.58)	3.64 (0.48)	2.71 (0.44)	1.87 (0.14)	1.27 (0.28)
liver	3.04 (0.30)	5.01 (0.33)	6.44 (0.30)	7.56 (0.43)	7.86 (1.84)
kidney	1.07 (0.11)	1.15 (0.11)	1.30 (0.20)	1.76 (0.23)	1.79 (0.40)
stomach[b]	0.34 (0.04)	0.43 (0.02)	0.42 (0.05)	0.40 (0.05)	0.39 (0.10)
heart/blood	0.84 (0.15)	1.35 (0.15)	2.93 (0.36)	4.60 (0.46)	3.44 (0.55)
			[^{125}I]IPPA[c]		
blood	1.28 (0.10)*	0.57 (0.09)*	0.78 (0.15)*	0.78 (0.18)*	0.92 (0.13)*
heart	7.59 (1.00)*	6.90 (1.02)*	5.67 (1.03)*	5.22 (0.57)*	4.19 (1.66)*
liver	2.60 (0.38)	3.56 (0.30)*	3.56 (0.38)*	3.71 (0.39)*	2.65 (0.61)*
kidney	1.45 (0.08)	1.55 (0.12)	1.41 (0.29)	1.30 (0.12)	1.16 (0.26)
stomach[b]	0.64 (0.11)	0.64 (0.05)	0.52 (0.05)	0.51 (0.05)	0.48 (0.14)
heart/blood	5.98 (1.12)*	12.46 (3.29)*	7.59 (2.33)*	7.11 (2.57)*	4.52 (1.88)

[a] Tissue radioactivity is expressed as % ID/g for each group ($n = 5$); results are expressed as mean (SD). [b] Expressed as %ID. [c] Significances determined by unpaired student's t-test; (*) $p < 0.05$ compared to [^{99m}Tc]CpTT-PA.

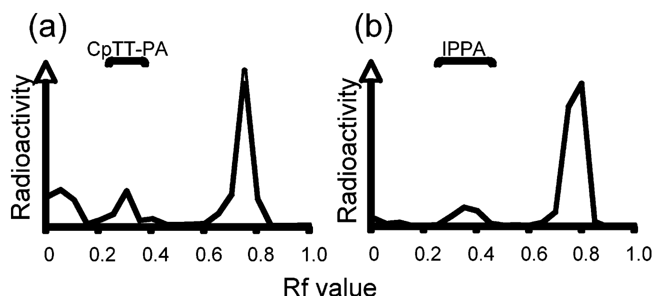


Figure 2. TLC radioactivity profiles of rat heart extracts after 2 h perfusion of [^{99m}Tc]CpTT-PA (a) and [^{125}I]IPPA (b). Under these conditions, [^{99m}Tc]CpTT-PA and [^{125}I]IPPA had R_f values of 0.30 and 0.35, respectively.

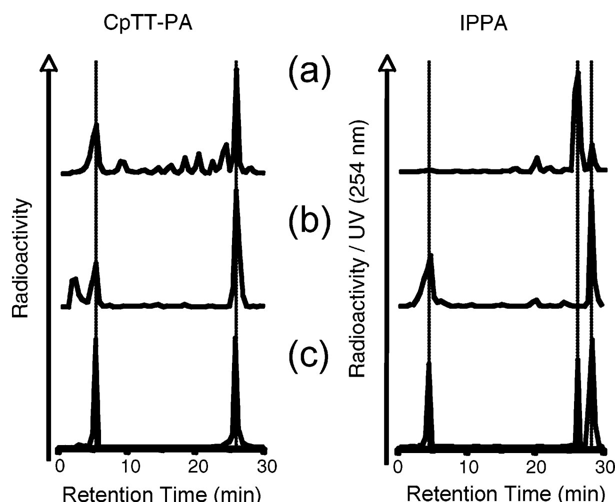


Figure 3. RP-HPLC radioactivity profiles of hydrolyzed rat heart lipids (a) and rat heart perfusate (b) after 2 h perfusion of [^{99m}Tc]CpTT-PA and [^{125}I]IPPA. RP-HPLC profiles of authentic samples are also shown in (c). Under these conditions, [^{99m}Tc]CpTT-PA, [^{99m}Tc]CpTT-propionic acid, [^{125}I]IPPA, 13-(p-[^{125}I]iodophenyl)tridecanoic acid, and p-[^{125}I]-iodobenzoic acid had retention times of 26, 5.5, 28.5, 26.5, and 5 min, respectively.

Discussion

[^{99m}Tc]CpTT-PA and its precursors were synthesized according to the procedures similar to those of nonradioactive rhenium compounds, as outlined in Scheme 1, and were characterized by RP-HPLC using their well-characterized nonradioactive [$^{185/187}$Re]rhenium counterparts as references. The RP-HPLC retention times of compounds **4a**, **4b**, **5a**, **5b**, [$^{185/187}$Re]CpTR-PA, and [^{99m}Tc]CpTT-PA are summarized in Table 1. ^{99m}Tc-labeled compounds and their [$^{185/187}$Re]CpTR counterparts exhibited similar changes in their retention times after each reaction. Slight differences in RP-HPLC retention times between [^{99m}Tc]CpTT derivatives and their [$^{185/187}$Re]CpTR counterparts were observed, and [^{99m}Tc]CpTT derivatives showed slightly longer retention times than did their [$^{185/187}$Re]CpTR counterparts, as shown in Table 1. Similar differences in RP-HPLC retention times were observed between [^{99m}Tc]CpTT derivatives and their [$^{185/187}$Re]CpTR counterparts.[23,24] From these results, we concluded that the chemical structure of [^{99m}Tc]-CpTT-PA would be identical to its rhenium counterpart, [$^{185/187}$Re]CpTR-PA.

The Langendorff perfusion study was performed according to the procedure of Yamamichi et al.[25] and Mori et al.[26] with slight modifications. The majority of perfused [^{125}I]IPPA was incorporated in the myocardium and detected in lipid fractions as 13-(p-[^{125}I]iodophenyl)tridecanoic acid (Figures 2 and 3). On the other hand, p-[^{125}I]iodobenzoic acid was observed as the major radiometabolite in the perfusate (Figure 3). Previous studies of radiolabeled and non-radioactive IPPA in perfused heart showed the presence of a variety of metabolites including p-iodobenzoic acid and 11-(p-iodophenyl)undecanoic acid along with a small amount of IPPA.[27,28] A variety of radiometabolites were also observed in the hydrolyzed heart extracts of perfused heart following injection of [^{123}I]BMIPP.[25,29,30] There were some differences in the components of perfusate between present and previous studies, which would have affected the metabolism of [^{125}I]IPPA in the heart. These results indicated that the present Langendorff perfusion model would be appropriate for estimating fatty acid metabolism of the heart.

[^{99m}Tc]CpTT-PA exhibited the highest myocardial accumulation of 3.85 %ID/g at 1 min postinjection with a maximum heart-to-blood ratio of 4.60 at 10 min postinjection (Table 2). In the Langendorff perfusion study, approximately 34% of perfused [^{99m}Tc]CpTT-PA was retained in the heart, and approximately 33% in the perfusates was present as metabolites (Figures 2 and 3). Since high in vivo stability of CpTM (M = ^{99m}Tc or $^{186/188}$Re) structure has been well documented,[17,20,23,24,31] the multiple radioactivity peaks on RP-HPLC between [^{99m}Tc]-CpTT-PA and [^{99m}Tc]CpTT-propionic acid suggested the presence of multiple radiometabolites, as also observed in the metabolic studies of radioiodinated fatty acid analogues.[25,27−30] The gathered findings demonstrated that [^{99m}Tc]CpTT-PA was incorporated into myocardium, recognized as a fatty acid and metabolized as such.

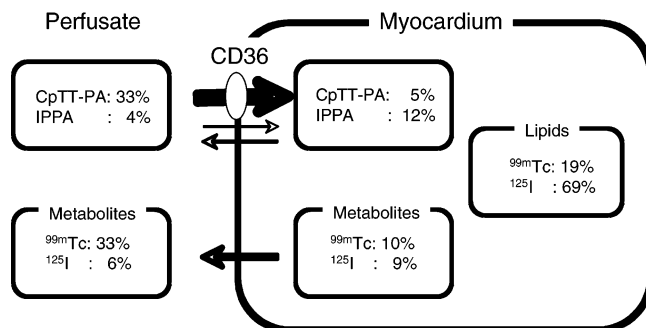


Figure 4. Fate of [^{99m}Tc]CpTT-PA and [^{125}I]IPPA in perfused rat hearts after 2 h recirculation of perfusate.

Figure 4 summarizes the fate of [^{99m}Tc]CpTT-PA and [^{125}I]-IPPA in perfused rat heart after 2 h recirculation of perfusate. The proportion of [^{99m}Tc]CpTT-PA incorporated into myocardium during perfusion was lower than that of [^{125}I]IPPA (approximately 67% for [^{99m}Tc]CpTT-PA and 96% for [^{125}I]-IPPA). Similar results were observed in a biodistribution study where myocardial uptake of [^{99m}Tc]CpTT-PA was significantly lower than that of [^{125}I]IPPA (Table 2). Besides passive diffusion, long chain fatty acids are incorporated into myocardium via protein-mediated system such as translocase/CD36 and fatty acid transport protein.[1,32−34] [^{99m}Tc]CpTT-PA is a lipophilic compound with a retention time slightly shorter than [^{125}I]IPPA on RP-HPLC. Thus, the lower myocardial uptake of [^{99m}Tc]-CpTT-PA would be attributable to the low affinity of [^{99m}Tc]-CpTT-PA for the fatty acid transporter(s). This may also account for the slower elimination rate of radioactivity from the blood at earlier postinjection time of [^{99m}Tc]CpTT-PA.

Once taken up in the myocardium, more than 92% of [^{99m}Tc]-CpTT-PA was metabolized to [^{99m}Tc]CpTT-propionic acid or converted to lipids. Similarly, approximately 88% of perfused [^{125}I]IPPA was metabolized or converted to lipids. The majority of [^{125}I]IPPA was stored in lipids after one cycle of β-oxidation. In contrast, the majority of [^{99m}Tc]CpTT-PA was metabolized to [^{99m}Tc]CpTT-propionic acid, a metabolite after six cycles of β-oxidation of [^{99m}Tc]CpTT-PA, and excreted from the myocardium although [^{99m}Tc]CpTT-COOH was expected as the final radiometabolite. These results suggest that an introduction of [^{99m}Tc]CpTT moiety at the ω-position of pentadecanoic acid may have hindered further recognition of [^{99m}Tc]CpTT-propionic acid by the enzymes involved in β-oxidation and may have affected metabolic pathway during β-oxidation. Similar results were observed in the metabolic studies of [^{99m}Tc]MAMA-HA in the liver and [^{99m}Tc]MAMA-HDA in the body, where [^{99m}Tc]-MAMA-butyric acid was observed as the final radiometabolite of the two compounds.[15,16] [^{99m}Tc]CpTT-8-oxooctanoic acid also generated [^{99m}Tc]CpTT-4-oxobutyric acid in the body.[23] This suggests that an introduction of [^{99m}Tc]CpTT group at the ω-position of a fatty acid would impair enzyme recognition less than that of [^{99m}Tc]MAMA or [^{99m}Tc]CpTT-oxo group.

Conclusion

At the initial stage of ^{99m}Tc radiopharmaceutical development, it was thought that Tc is a foreign substance and should be recognized as such by the body. However, the development of ^{99m}Tc-labeled compounds that cross the intact blood−brain barrier[35−37] stimulated the development of currently available ^{99m}Tc-labeled perfusion agents of the brain.[38,39] The findings in this study demonstrated for the first time that a long chain ^{99m}Tc-labeled fatty acid analogue, [^{99m}Tc]CpTT-PA, was transported and metabolized as a substrate for the energy production of the heart. Thus, the present study may pave the way for developing ^{99m}Tc-labeled fatty acid analogues that provide myocardial fatty acid metabolism by external imaging in clinical studies.

Experimental Section

General Information. [^{99m}Tc]Pertechnetate ($^{99m}TcO_4^-$) was eluted in saline solution on a daily basis from Daiichi Radioisotopes Labs generator (Chiba, Japan). Reversed phase HPLC (RP-HPLC) was performed with a Cosmosil 5C_{18}-AR-300 column (4.6 × 150 mm, Nacalai Tesque, Kyoto, Japan) at a flow rate of 1 mL/min with a gradient mobile phase starting from 30% A (0.1% aqueous trifluoroacetic acid (TFA)) and 70% B (acetonitrile with 0.1% TFA) to 0% A and 100% B at 30 min (system 1) or from 50% A and 50% B to 0% A and 100% B at 30 min (system 2). Each eluent was collected with a fraction collector (RediFlac, GE Healthcare Bioscience, Tokyo, Japan) at 30 s intervals, and the radioactivity counts in each fraction (500 μL) were determined with an auto well γ counter (ARC-380M, Aloka, Tokyo). The radioactivity of the eluent was measured immediately for ^{99m}Tc radioactivity (120 −150 keV) and 5 days later for ^{125}I radioactivity (20 − 40 keV) to reduce the crossover of the ^{99m}Tc radioactivity to the ^{125}I channel. The crossover of ^{125}I activity to the ^{99m}Tc channel was negligible. TLC analyses were performed with silica plates (Silica gel 60 F_{254}, Merck, Tokyo) developed with chloroform. SepPak Plus (C_{18} short body, 360 mg/cartridge, Waters, Tokyo) was activated with 6 mL each of ethanol and water prior to use. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-ALPHA 400 spectrometer (JEOL Ltd., Tokyo) with tetramethylsilane as an internal standard. Fast-atom bombardment mass spectra (FAB-MS) were taken on a JEOL JMS-HX-110A mass spectrometer (JEOL Ltd.). Two masses were reported for rhenium-containing fragments to indicate the significant isotopic abundances of both ^{185}Re and ^{187}Re. Each peak was observed to have the proper relative abundances. Elemental analyses were performed by PE-2400 (Perkin-Elmer Japan, Tokyo). 15-(*p*-[^{125}I]Iodophenyl)pentadecanoic acid ([^{125}I]IPPA) and 13-(*p*-iodophenyl)tridecanoic acid were prepared according to the procedure described previously.[16,40] Other reagents were of reagent grade and used as received.

Pentadecanedioic Acid Monomethyl Ester (2). Thionyl chloride (4 mL, 55 mmol) was added dropwise to methanol (40 mL) at −10 °C. After the solution stood for 10 min at the same temperature, pentadecanedioic acid (**1**) (6.0 g, 22 mmol) was added. The temperature of the solution was gradually increased to the boiling point, and the solution was refluxed for 5 h. After the solution was cooled to room temperature, the solvent was evaporated in vacuo, and the residue was dissolved in ether (40 mL). The organic layer was washed with saturated aqueous NaCl (40 mL × 3) and then dried over anhydrous $CaSO_4$. After the solvent was removed, dimethyl ester of compound **1** was obtained as a white solid (6.3 g, 95.0%). This compound was used without further purification.

A solution of barium hydroxide (1.48 g, 8.5 mmol) in dry methanol (100 mL) was added dropwise to a solution of the dimethyl ester of compound **1** (5.25 g, 17 mmol) in dry methanol (120 mL). After the mixture stood for 17 h at room temperature, the precipitate was collected by suction filtration and washed with methanol (20 mL). The barium salt was shaken for a few minutes in a separatory funnel with a mixture of 4 N HCl (100 mL) and ether (100 mL). The aqueous layer, together with any precipitated barium chloride was removed and extracted again with ether (100 mL). The combined ether extracts were washed with water and dried over anhydrous $CaSO_4$. After the solvent was removed, the residue was purified by open column chromatography using silica gel and subsequent elution with a mixture of ethyl acetate−hexane (1:2) to produce compound **2** (3.80 g, 78.2%). ^{1}H-NMR ($CDCl_3$) δ: 3.84 [s, 3H, CH_3], 2.84 [m, 4H, -CH_2-CO-], 1.79 [m, 4H, -*CH_2*-CH_2-CO-], 1.49 [s, 18H, -CH_2-]. FAB-MS: *m/z* 287 (M + H)$^+$. Found: 287.

15-Ferrocenoyl-15-oxopentadecanoic Acid Methyl Ester (3). This compound was synthesized according to the procedure of

Vogel et al. with slight modification as follows.[21] Compound **2** (2.0 g, 6.9 mmol) was dissolved in thionyl chloride (5 mL, 69 mmol) and refluxed for 3 h. After residual thionyl chloride was removed in vacuo, the crude 1-methyl pentadecanedioic acid chloride was dissolved in dichloromethane (10 mL) containing anhydrous aluminum chloride (1.3 g, 9.8 mmol), and then it was added dropwise to a solution of ferrocene (1.3 g, 6.9 mmol) in dry dichloromethane (10 mL). The mixed solution was kept stirring overnight and then poured into ice-cold water (30 mL). Ethyl acetate (30 mL) was added to the solution, and the organic phase was extracted and washed with brine (30 mL × 3) and then dried over anhydrous $CaSO_4$. After the solution was removed, the residue was purified by open column chromatography using silica gel and subsequent elution with a mixture of chloroform−hexane (5:2) to produce compound **3** (1.5 g, 49%). ^{1}H-NMR ($CDCl_3$) δ: 4.75 [s, 2H, ferrocene], 4.47 [s, 2H, ferrocene], 4.18 [s, 5H, ferrocene], 3.65 [s, 3H, COO-CH_3], 2.68 [t, 2H, -CH_2-CO-], 2.29 [t, 3H, -CH_2-COO-], 1.59−1.70 [m, 4H -CH_2-CH_2-CO-], 1.24 [s, 18H, -CH_2-]. FAB-MS: *m/z* 455 $(M + H)^+$. Found: 455.

Tricarbonyl(15-cyclopentadienyl-15-oxopentadecanoic acid methyl ester)rhenium (4a). This compound was synthesized according to the procedure of Spradau et al.[20] with slight modification as follows. To a mixture of compound **3** (472 mg, 1.0 mmol), ammonium perrhenate (89 mg, 0.33 mmol), chromium hexacarbonyl (410 mg, 1.9 mmol), and chromium(III) chloride anhydrous (110 mg, 0.67 mmol) in a pressure tube (0.8 × 8.5 cm, Taiatsu glass kogyo, Tokyo) was added dry methanol. After the tube was inserted in silicon oil at 180 °C for 45 min, the reaction mixture was cooled at room temperature. After the filtration through Celite, the filtrate was removed in vacuo, and the residue was purified with open column chromatography using silica gel and subsequent elution using a mixture of ethyl acetate and hexane (1:4) to produce compound **4a** (54.5 mg, 27.3%) as a white powder. ^{1}H-NMR ($CDCl_3$) δ: 5.96 [s, 2H, Cp], 5.37 [s, 2H, Cp], 3.64 [s, 3H, CH_3], 2.55 [t, 2H, -CH_2-CO-], 2.28 [t, 3H, -CH_2-COO-], 1.55−1.68 [m, 4H -CH_2-CH_2-CO-], 1.23 [s, 18H, -CH_2-]. FAB-MS: *m/z* 603/605 $(M + H)^+$. Found: 603/605.

Tricarbonyl(15-cyclopentadienyl pentadecanoic acid methyl ester)rhenium (5a). This compound was synthesized according to the procedure of Bhattacharyya[22] as follows. Compound **4a** (47 mg, 78 μmol) was dissolved in dichloromethane (1 mL), and titanium(IV) chloride (14.7 mg, 78 μmol) dissolved in dichloromethane (1 mL) was added to the solution. A solution of triethylsilane (36.3 mg, 312 μmol) in dichloromethane (1 mL) was then added to the mixture with stirring. After the mixture was stirred for 14 h at room temperature, the organic layer was washed with 5% sodium carbonate (5 mL) and dried over anhydrous $CaSO_4$. After the solvent was removed in vacuo, the residue was purified by open column chromatography using silica gel and subsequent elution using a mixture of chloroform-hexane (5:2) to produce the compound **5a** as a white powder (23 mg, 50%). ^{1}H-NMR ($CDCl_3$) δ: 5.20 [s, 4H, Cp], 3.62 [s, 3H, CH_3], 2.37 [t, 2H, -CH_2-Cp-], 2.29 [t, 2H, -CH_2-CO-], 1.43−1.71 [m, 4H, -CH_2-CH_2-Cp, -CH_2-CH_2-CO-], 1.23 [s, 20H, -CH_2-]. FAB-MS: *m/z* 589/591 $(M + H)^+$. Found: 589/591.

Tricarbonyl(15-cyclopentadienyl pentadecanoic acid)rhenium ([$^{185/187}$Re]CpTR-PA). Compound **5a** (11 mg, 19 μmol) was dissolved in ethanol (600 μL) and mixed with aqueous sodium hydroxide (2 N, 200 μL) for 8 h at room temperature. After being acidified with concentrated HCl (ca. 120 μL), ethyl acetate (5 mL) was added to the solution and it was washed with 1% HCl solution (5 mL × 3). After the solution was dried over anhydrous $CaSO_4$, the solution was evaporated in vacuo to obtain [$^{185/187}$Re]CpTR-PA as a white solid (8.3 mg, 77.4%). ^{1}H-NMR ($CDCl_3$) δ: 5.21 [s, 4H, Cp], 2.35 [m, 4H, -CH_2-Cp-, -CH_2-CO-], 1.42−1.69 [m, 4H, -CH_2-CH_2-Cp, -CH_2-CH_2-CO-], 1.21 [s, 20H, -CH_2-]. FAB-MS: *m/z* 575/577 $(M + H)^+$. Found: 575/577. Anal. ($C_{23}H_{33}O_5$-Re) C, H, N.

Tricarbonyl(3-cyclopentadienyl propionic acid)rhenium ([$^{185/187}$Re]-CpTR-Propionic Acid). This compound was synthesized by the reaction of ferrocene and malonic acid monomethylester and subsequent reduction of the carbonyl group, according to the procedure described above in 5% yield. This compound showed a single peak at a retention time of 5.0 min on RP-HPLC (system 2). ^{1}H-NMR ($CDCl_3$) δ: 5.23 [s, 4H, Cp], 2.35 [t, 2H, -CH_2-Cp-], 2.30 [t, 2H, -CH_2-CO-], 1.43−1.50 [m, 2H, -CH_2-]. FAB-MS: *m/z* 407/408 $(M + H)^+$. Found: 406/408. Anal. ($C_{11}H_9O_5Re$) C, H, N.

[^{99m}Tc]Tricarbonyl(15-cyclopentadienyl-15-oxopentadecanoic acid methyl ester)technetium (4b). This compound was synthesized according to the procedure of Spradau et al.[20] with slight modification as follows. A solution of [^{99m}Tc]$NaTcO_4$ in dry methanol (500 μL) was added to the mixture of compound **3** (10 mg, 22 μmol), chromium hexacarbonyl (14 mg, 64 μmol), and chromium(III) chloride (11 mg, 58 μmol) in the pressure tube (0.8 × 8.5 cm, Taiatsu glass kogyo). The tube was inserted in silicon oil at 180 °C for 45 min. After being cooled to room temperature, the solvent was evaporated in vacuo. The residue was dissolved in chloroform and purified by open column chromatography using silica gel and subsequent elution with chloroform as an eluent to produce the compound **4b** in radiochemical yield of 80%.

[^{99m}Tc]Tricarbonyl(15-cyclopentadieyl pentadecanoic acid methyl ester)technetium (5b). Titanium(IV) chloride (8 μL) dissolved in dichloromethane (0.5 mL) was added to compound **4b**. Triethylsilane (50.5 μL) dissolved in dichloromethane (0.5 mL) was added to the mixture with stirring. After the mixture was stirred for 1 h at room temperature, ether (2 mL) and water (2 mL) were added to the mixture. The organic solvent was extracted from the mixture and evaporated in vacuo to produce the compound **5b** in radiochemical yield of 55%.

[^{99m}Tc]Tricarbonyl(15-cyclopentadieyl pentadecanoic acid)-technetium ([^{99m}Tc]CpTT-PA). Compound **5b** was dissolved in ethanol (600 μL) and mixed with 2 N aqueous sodium hydroxide (200 μL) at 95 °C for 10 min. After being cooled to room temperature, the mixture was neutralized with 2 N HCl and loaded onto a SepPak plus cartridge. The cartridge was successively washed with water (5 mL) and eluted with ethanol (3 mL). The first eluted ethanol fraction (100 μL) was discarded, and the combined eluents were evaporated in vacuo. The residue was purified by RP-HPLC (system 1) to produce [^{99m}Tc]CpTT-PA in radiochemical yield of 49%.

[^{99m}Tc]Tricarbonyl(3-cyclopentadieyl propionic acid)technetium ([^{99m}Tc]CpTT-Propionic Acid). This compound was synthesized by the reaction of ferrocene and malonic acid monomethyl ester according to the procedure described above in radiochemical yield of 21%. [^{99m}Tc]CpTT-propionic acid showed a single radioactivity peak at a retention time of 5.0 min on RP-HPLC (system 2).

Biodistribution Study. Animal studies were conducted in accordance with our institutional guidelines and were approved by the Chiba University Animal Care Committee. [^{99m}Tc]CpTT-PA (1.85 MBq) and [^{125}I]IPPA (1.85 MBq) were dissolved in ethanol (750 μL) and added dropwise to a stirred solution of 1% bovine serum albumin (BSA, 14.25 mL) in saline. The solution was then filtered through a 0.22 μm polycarbonate filter. The solution (37 kBq each of [^{99m}Tc]CpTT-PA and [^{125}I]IPPA) was administered to Wistar rats (male, 200 g) from tail vein. At appropriate time points after the injection, rats were sacrificed by decapitation. Tissues of interest were removed and weighed, and the radioactivity counts were determined using an auto well γ counter.

Isolated Rat Heart Studies. This study was performed according to the procedure of Yamamichi et al.[25] and Mori et al.[26] with slight modifications. The hearts were rapidly removed from the male Wistar rats (200−300 g) anesthetized with pentobarbital (50 mg/kg) and mounted on a Langendorff perfusion system. The perfusate used was 5 mM HEPES buffer (pH 7.4) containing 123 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 5 mM AcONa, 5 mM $CaCl_2$, and 6 mM glucose. The hearts were perfused at a steady rate of 8−10 mL/min by a peristaltic pump (Pump P-1, GE Healthcare Biosciences), and oxygenation of the perfusate was achieved with a mixture of 95% O_2 and 5% CO_2. The hearts had a steady rate of contraction (180−200 times/min). After stabilizing the hearts for 10 min, a mixture of [^{99m}Tc]CpTT-PA (74 kBq) and [^{125}I]IPPA

(74 kBq) in 0.3 mL of saline containing 1% BSA were loaded into the perfusate (30 mL). After the perfusate was recirculated for 2 h, the perfusate (5 mL) was acidified with 1 N HCl to pH 1.0 and the radioactive fractions were extracted by passage through a SepPak cartridge that was then eluted with methanol (5 mL, extract efficiency of 92.6%). These methanol solutions were analyzed using RP-HPLC (system 2). After the completion of the experiments, the hearts were dismounted, minced, and homogenized. The lipids were extracted using a modified Folch technique[29,41] in which the myocardial homogenates were mixed with 5 mL of 2:1 chloroform−methanol and acidified with 50% H_2SO_4 (pH 1) (extraction efficiency of 93.2%). After the precipitate was removed, the filtrates were analyzed by TLC. The filtrates were then hydrolyzed with 10 M KOH (1 mL) at 60 °C for 1 h. After bringing acidified with 50% H_2SO_4 (pH 1), the final products were extracted with chloroform (3 mL, extraction efficiency: 92.7%). The extracts were evaporated in vacuo and analyzed by RP-HPLC (system 2).

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Supporting Information Available: Elemental analyses data for tricarbonyl(15-cyclopentadienyl pentadecanoic acid)rhenium ([$^{185/187}$Re]CpTR-PA) and tricarbonyl(3-cyclopentadienoyl propionic acid)rhenium ([$^{185/187}$Re]CpTR-propionic acid). This material is available free of charge via the Internet at http://pubs.acs.org.

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