Comparative Evaluation of Fatty Acids Labeled with C-11, Cl-34m, Br-77, and I-123 for Metabolic Studies of the Myocardium: Concise Communication

H.-J. Machulla, G. Stöcklin, Ch. Kupfernagel, Ch. Freundlieb, A. Höck, K. Vyska, and L. E. Feinendegen

Institut für Chemie 1 and Institut für Medizin der KFA, D-517, Jülich GmbH, Federal Republic of Germany

Various long-chain fatty acids have been labeled with C-11, Cl-34m, Br-77, and I-123 and evaluated for their potential application in measuring myocardial metabolism in vivo. Comparative studies of the kinetics of accumulation and clearance from the heart muscle of mice indicate that the extraction of ω-halofatty acids is more efficient than that of α-halofatty acids. Among the ω-halofatty acids, the highest uptake is observed for the 17-iodoheptadecanoic acid, which shows an extraction behavior almost identical to that of [1-^{11}C] palmitic acid, although with a higher radioactivity level in blood due to the release of free iodide.

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Long-chain free fatty acids are a major energy source for the myocardium. Several investigations (e.g., *1, 2*) have used fatty acids labelled with C-14 and I-131 to study cardiac metabolism. In vivo application for potential diagnostic purposes, however, demands more suitable radionuclides such as C-11 ($T_{1/2}$ = 20.3 min) or I-123 ($T_{1/2}$ = 13.3 hr) (for a recent review see *3* and *4*). Myocardial extraction rates have been measured for the first time by Weiss et al. (*5*) using C-11 and by Poe and Robinson (*6,7*) using I-123. We decided to study the extraction rate and kinetics of utilization for various labeled fatty acids in the myocardium of mice in order to extend the application of these tracers to the measurement of human myocardial metabolism.

Preparation methods resulting in practically carrier-free products were to be preferred, since in metabolic function studies possible biochemical equilibria thus remain undisturbed. Carrier-free preparations, however, may lead to unexpected difficulties and therefore even simple, well-known reactions have to be carefully worked out. In the case of short-lived radionuclides—in particular C-11 and Cl-34m—the preparation, including final chromatographic purification, has to be completed fast. As we have recently pointed out in a review article (*8*), the radioanalytical control of the identity and purity of a product is most effectively accomplished by high-pressure liquid chromatography.

MATERIALS AND METHODS

Production of radionuclides. At the Isochronous Cyclotron (JULIC) and the Compact Cyclotron CV 28 of the KFA Jülich the radionuclides were produced by the following reactions: $^{14}N(p,\alpha)\,^{11}C$ (*9*), $^{35}Cl(p,pn)^{34m}Cl$ (*10*), $^{79,81}Br(d,xn)^{76,77}Kr(\beta^+,EC)^{76,77}Br$ (*10,11*), and $^{127}I(d,6n)^{123}Xe(\beta^+,EC)^{123}I$ (*4,12*). The experimental conditions have been described in detail elsewhere (*9–12*). In the case of Cl-34m, Szilard-Chalmers separation was applied using K_2ReCl_6 as target material (*13*). The yield of carrier-free $^{34m}Cl^-$ was 15%.

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Fol reprints contact: G. Stöcklin, Institut fur Chemie 1, Nuklearchemie der KFA, Jülich GmbH, Federal Republic of Germany.

TABLE 1. PREPARATION AND PURIFICATION OF LABELED FATTY ACIDS

Fatty acid	Radionuclide	Labeling reaction	Reaction time (min)	Radiochem. yield (%)	High-pressure liquid chromatography	Flow (ml/min)	k' *	Net retention (min)
Palmitic acid	C-11 ($T_{1/2}$ = 20.3 min)	R-MgBr + $^{11}CO_2$	5	50–60	Latek Spherisorb 55 W 10 μm, 20 cm long, 1 cm i.d. n-heptane + 0.2% acetic acid	9.9	14.7	8.0
α-Cl-stearic acid	Cl-34m ($T_{1/2}$ = 32.0 min)	Cl-for-Br exchange in DMSO	20	30	Waters μ-Bondapak NH_2, 30 cm long, 0.4 cm i.d. n-heptane + 2% acetic acid	10.0	13.4	4.0
α-Br-stearic acid	Br-77 ($T_{1/2}$ = 56.0 h)	Br-for-I exchange in $CHCl_3$	20	40	Li Chrosorb Si60 acetic acid 10 μm, 15 cm long, 1 cm i.d. n-heptane + 0.35% acetic acid	9.0	17	8.5
α-I-stearic acid	I-123 ($T_{1/2}$ = 13.3 h)	I-for-Br exchange in $CHCl_3$	30	95			14	6.8
17-Br-heptadecanoic acid	Br-77 ($T_{1/2}$ = 56 h)	Br-for-I exchange in acetone	180	62	Latek Spherisorb 55 W 5 μm, 50 cm long, 0.4 cm i.d., n-heptane + 1% acetic acid	2.0	7.6	15.2
17-I-heptadecanoic acid	I-123 ($T_{1/2}$ = 13.3 h)	I-for-Br exchange in acetone	120	50			6.5	13.0

* Column capacity factor k' is defined by equation $k' = (v - v_o)/v_o$, where v and v_o denote the elution and void volumes.

Labeling procedure and quality control. Alpha-halogenated stearic acid and ω-halogenated heptadecanoic acid were labeled by nucleophilic substitution in dimethyl sulfoxide DMSO, $CHCl_3$, or acetone. The details are given in Table 1. All halofatty acids were synthesized by nonisotopic halogen exchange similar to the procedure used by Robinson and Lee for the preparation of 16-I-hexadecenoic acid (*14*). Nonradioactive materials used as parent compounds and for chromatographic tests (such as α-bromo- and α-iodostearic acids) were also prepared by nonisotopic nucleophilic exchange, whereas ω-bromo- and ω-iodoheptadecanoic acid were obtained commercially. The [1-^{11}C] palmitic acid was synthesized by reaction of the corresponding Grignard compound with $^{11}CO_2$ in radiochemical yields of 50–60%. Traces of oxygen gave rise to nonradioactive impurities of a long-chain alcohol, and this had to be separated, since it prevented solution of the [^{11}C] palmitic acid in albumin solution.

The conditions for high-pressure liquid chromatography applied to all compounds prepared are also listed in Table 1. The technical details have been published previously (*8*). Preparations of 17-I-heptadecanoic acid showed unexpected radioactive impurities of the order of 10%, which demonstrated the need for careful chromatographic separation. In the case of α-halogenated fatty acids, the product was completely separated from the analogous parent compound—i.e., the product was carrier free. The ω-halogenated fatty acids were not separated from the parent and thus contained nonradioactive carrier (see below). Isotopic carriers were not added.

Animal experiments. After chromatographic purification, the eluent containing the product was evaporated in vacuo at room temperature and the radioactive residue dissolved in a 20% solution of human serum albumin, which was then diluted to 6%. After sterilization by Millipore filtration 0.2 ml of the solution was intravenously administered to NMRI mice. Before administration of the labeled fatty acids, their radiochemical purity and stability in the HSA solution were examined. In the case of the α-halofatty acids, which are most sensitive to deiodination, immediate analysis of the HSA solution showed that less than 2% iodide and less than 0.6% bromide were present. Less than 2% free iodide was observed under identical conditions for the ω-iodo-heptadecanoic acid. The free iodide content of all halofatty acids did not change significantly during a further storage period of two days. Only after five days was 50% free iodide observed in the case of α-halofatty acid. All animal experiments were carried out with freshly prepared HSA solutions immediately after

purification of the fatty acid by high-pressure liquid chromatography. The mice were killed between 15 sec and 10 min after injection. Radioactivity in blood and heart (blotted dry of blood) was measured. Six mice were used for each time interval. The measured radioactivity was related to the total amount of injected dose.

Whereas the α-halofatty acids were carrier-free, the ω-halogenated compounds contained 30 μg or less of the nonisotopic parent carrier per single injection (≤ 1.0 mg/kg_{mouse}). In the case of the 17-^{123}I-heptadecanoic acid, a possible influence of the co-injected unlabeled homologous bromofatty acid was evaluated by comparing the extraction of the labeled fatty acid containing ≤ 0.7 μg (≦ 0.03 mg/kg_{mouse}) unlabeled parent, and also without any starting material, with that obtained in a typical experiment with 30 μg of nonisotopic carrier. Within the experimental error we did not observe any difference.

We assume, therefore, that under the experimental conditions the co-injected nonlabeled fatty acids are not affecting the results.

Analysis of free halide in blood. We also determined, as a function of time, the amounts of free ionic iodine-123 and bromine-77 in blood after administration of the correspondingly labeled fatty acids, by high-pressure liquid chromatography, using a column (11 cm × 0.18 cm) filled with Aminex A-27 in the NO_3^- form. Iodide and bromide were eluted with 0.5 N and 0.05 N $NaNO_3$ solution, respectively. For the analysis, 10–20 μl of blood were directly injected on the column (*15*).

RESULTS AND DISCUSSION

In Fig. 1 we have compared the kinetics of myocardial accumulation and clearance of radioactively labeled α-halofatty acids with that of [1-^{11}C] palmitic acid, since an altered physiological behavior caused by the inductive and/or steric effect of the α-halogen was expected. It can be seen that, within the first 3 min after injection, the radioactivity is concentrated in the heart muscle, with the rate of accumulation and the height of the maximum depending on the type of acid under investigation. Highest uptake is observed in the case of the [1-^{11}C] palmitic acid (43 ± 8%/g), whereas all the α-halogenated acids show considerably less accumulation. The maximum for α-^{34m}Cl-stearic acid was 25%/g, whereas α-^{123}I- and α-^{77}Br-stearic acid exhibit even smaller maxima (14 ± 2.3%/g and 15 ± 5.4%/g, respectively). The radioactivity retained in the heart after 4–10 min levels off between about 10%/g and 5%/g.

The observed behavior of the α-halofatty acids may be explained by steric hindrance and/or inductive

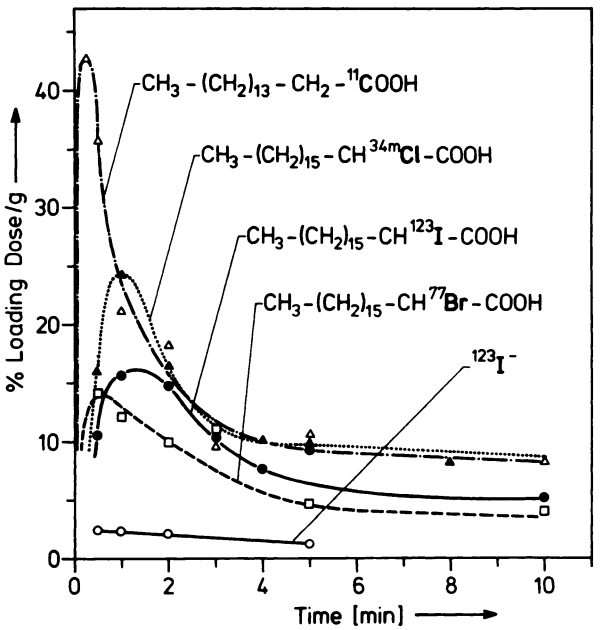

FIG. 1. Time course of radioactivity in mouse heart muscle after i.v. injection of α-^{34m}Cl-, α-^{77}Br-, α-^{123}I-stearic acid, [1-^{11}C] palmitic acid and ionic I-123 (200 μl of a 6% HSA solution containing about 100 nCi of practically carrier-free product). Average deviations are within ±20% of indicated values.

effects that influence esterification with coenzyme A and carnitin and thereby inhibit passage through the mitochondrial membrane. It had been shown (*16*)

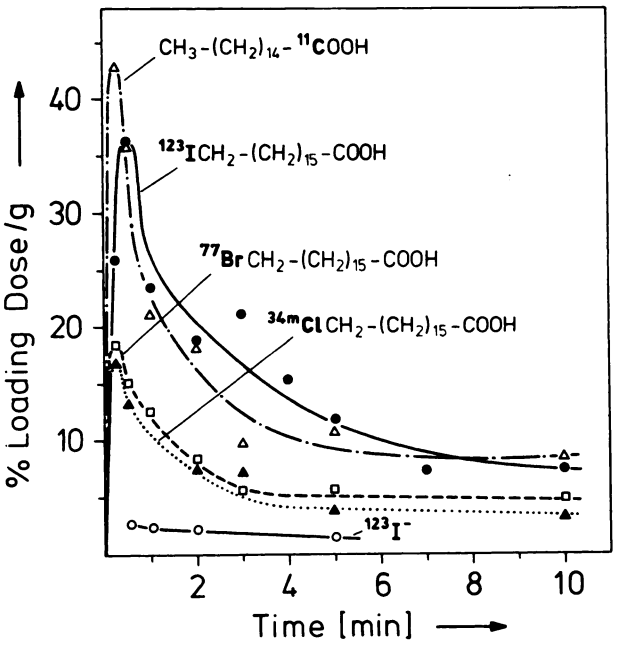

FIG. 2. Time course of radioactivity in heart muscle after i.v. injection of 17-^{34m}Cl-, 17-^{77}Br-, 17-^{123}I-heptadecanoic acid, [1-^{11}C] palmitic acid and ionic I-123 (200 μl of a 6% HSA solution containing about 100 nCi. All ω-halofatty acids contained <30 μg of homologous nonradioactive starting material as carrier). Average deviations are within ±22% of indicated values.

that the presence of α-bromopalmitate leads to an inhibition of palmitate oxidation by the heart, and that the selective inhibitory effect of the α-bromofatty acid on fatty-acid oxidation in intact cells may involve inhibition of activation (*17*), in addition to the inhibition of carnitine palmitoyltransferase by CoA esters of α-bromo long-chain acids. This explanation stimulated experiments with ω-halofatty acids, where the halogen substitutes the methyl group of stearic acid, and in which case such steric or inductive effects should not be observed. Therefore 17-^{34m}Cl-, 17-^{77}Br-, and 17-^{123}I-heptadecanoic acids were administered to mice and similarly compared with [1-^{11}C] palmitic acid (Fig. 2). The highest accumulation among the ω-halofatty acids is found for the ω-^{123}I-heptadecanoic acid (36 ± 7%/g), which, within experimental error, matches that of [1-^{11}C] palmitic acid. In contrast, the ω-^{77}Br- and ω-^{34m}Cl-heptadecanoic acids concentrate only to around 17 ± 4.0%/g. In any case the observed dependencies show basically similar types of curves differing by a factor of two or less. This may lead to the assumption that we are dealing with similar uptake and clearance mechanisms. In the case of the α-chloro- and α-iodostearic acids, on the other hand, the broader peaks of accumulation indicate slower uptake and slower release; the α-chlorofatty acid, in particular, concentrates up to 24% and still remains at 10% between 3 and 10 min. The obviously more hindered uptake and metabolism is also confirmed by analysis of the activity in blood. One minute after injection of α-bromo- and α-iodostearic acid, 43% of the total blood activity is present as free bromide, and 57% as free iodide (see insert in Fig. 3). The corresponding ω-bromo- and ω-iodofatty acids, however, already show 100% of the total blood activity as free halide after 1 min, indicating the faster uptake and metabolism in contrast to those of the α-halogenated acids.

With respect to in vivo application, the radioactivity level in the blood is also of interest, since in external counting techniques the sum of blood and myocardium is generally measured and a low blood background is desirable. Figure 3 shows the activity in blood within the first 10 min for α-^{123}I- and α-^{77}Br-stearic acids, 17-^{123}I- and 17-^{77}Br-heptadecanoic acids, and for [1-^{11}C] palmitic acid. Three minutes after administration the lowest blood activity is found for [1-^{11}C] palmitic acid (0.5%/g). This is understandable, since the carboxyl C-11 label ends up in the citrous cycle and is eventually released as $^{11}CO_2$, which rapidly leaves the blood. This had been demonstrated by Kong and Friedberg with [^{14}C] palmitic acid, injected into the left coronary artery of dogs (*18*).

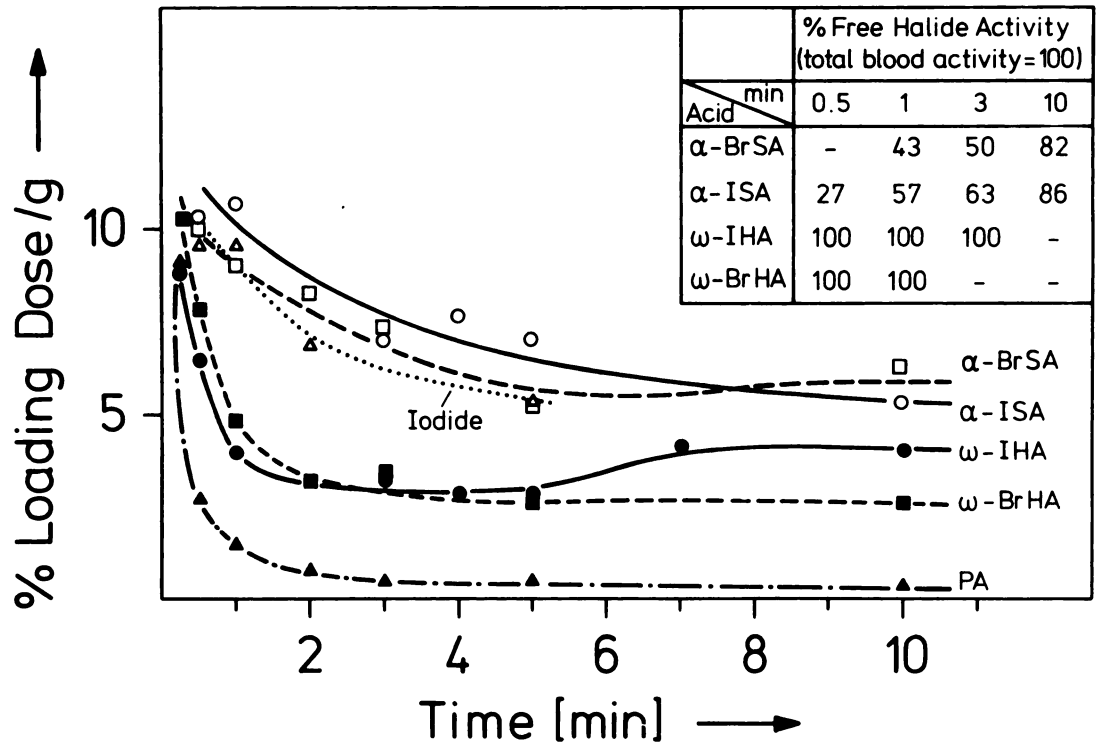

FIG. 3. Time course of radioactivity in blood after i.v. injection of α-^{77}Br-, α-^{123}I-stearic acid (α-BrSA, α-ISA), 17-^{77}Br-, 17-^{123}I-heptadecanoic acid (ω-BrHA, ω-IHA), [1-^{11}C] palmitic acid (PA), and ionic I-123 (200 μl of a 6% HSA solution containing about 100 nCi. All ω-halofatty acids contained <30 μg of homologous nonradioactive starting material as carrier). Average deviations are within ±49% for PA and ±23% for the other compounds; those for the values in the insert table are within ±17%.

In the case of the ω-^{77}Br- and ω-^{123}I-heptadecanoic acids the blood activity decreases to 3%/g within 2 min after injection and does not show any significant change thereafter.

To prove that in the case of the halogenated acids the activity accumulation in the myocardium is really due to the metabolism of the fatty acids and not to accumulation of free halide resulting from dehalogenation of the halofatty acid in the blood, free iodide was also injected into mice under identical conditions. Less than 3%/g was found in the myocardial tissue within the first 5 min, indicating that the accumulation of the halogenated fatty acids is indeed the result of metabolism in the heart muscle.

In vivo experiments have been carried out with 17-iodo-heptadecanoic acid in rabbits (*19*), and they in principle confirm the in vitro findings with mice. Indium-113m transferrin was used to subtract the blood background. These findings in rabbits will be reported separately.

CONCLUSION

In summary it can be stated that 17-iodo-heptadecanoic acid may be a useful scanning tracer, particularly for dynamic metabolic studies of the myocardium. In view of the release of iodide, it would require prior blocking of the thyroid. In contrast to carbon-11, the relatively long half life of iodine-123 would allow shipment to distant hospitals, and the 159-keV photon can be used with present-day gamma cameras. On the other hand, C-11-labeled fatty acids, even though useful only on site, are advantageous considering the low blood background and the possibility of use with a positron tomograph.

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