

# Pharmacokinetics and Metabolism of the Methyl-Branched Fatty Acid (BMIPP) in Animals and Humans

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The aim of this study was to further characterize the major metabolite of 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP). **Methods:** Radioactive components of  $^{131}\text{I}$ -BMIPP were evaluated in Langendorff-perfused rat hearts, as well as in blood samples from 20 patients after injection of  $^{123}\text{I}$ -BMIPP. Rat hearts were perfused with pH 7.4 Krebs-Henseleit buffer with or without 0.4 mmol/L bovine serum albumin (BSA) or 0.4 mmol/L palmitate. Lipids were Folch extracted and hydrolyzed from samples of the outflow, as well as from homogenized hearts. Radioactive components were determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analyses. The major metabolite of BMIPP was then further characterized by electrospray mass spectrometry. **Results:** The rat heart perfusate showed one major polar metabolite observed by TLC ( $R_f = 0.35$ ; solvent = benzene-dioxane-acetic acid 80:18:2). The addition of BSA/palmitate to the perfusate buffer significantly increased backdiffusion of BMIPP ( $R_f = 0.55$ ), as well as reduced BMIPP uptake and metabolism. The major metabolite was identified by mass spectral analysis as 2-(p-iodophenyl)acetic acid ( $\text{IPC}_2$ ). From TLC and HPLC analyses of the serum lipids obtained from patients, the same metabolite could be identified with levels increasing over time (0%, 5.2% and 11.8% of the injected dose; 3 min, 20 min and 3 h postinjection, respectively). In addition to the identification of unmetabolized BMIPP (53.9%), the rat heart lipid hydrolysate also contained  $\alpha$ -methyl-14-(p-iodophenyl)tetradecanoic acid (20.8%), 12-(p-iodophenyl)-substituted-dodecanoic (17.1%), -hexanoic acid (5.2%) and  $\text{IPC}_2$  (1.1%). **Conclusion:** The animal results show the complexity of uptake, metabolism and release of BMIPP from which a part is metabolized through  $\alpha$ - and subsequent  $\beta$ -oxidation to the final  $\text{IPC}_2$  metabolite as confirmed by mass spectral analysis. The results from patient studies suggest that the slow myocardial washout observed in vivo after intravenous administration of BMIPP may represent a similar process, because both unmetabolized BMIPP and the final metabolite were also identified in serum samples.

**Key Words:** BMIPP; fatty acid metabolism; mass spectrometry; Langendorff hearts

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**E**valuation of the regional myocardial uptake of  $^{123}\text{I}$ -labeled fatty acids by SPECT requires special approaches to

reduce the rate of metabolism of the compound and obtain a stable tracer concentration for the required acquisition periods ( $>15$  min). As one approach, introduction of a  $\beta$ -methyl group in fatty acid analogs was expected to interfere with  $\beta$ -oxidation at the level of formation of the 3-ketoacyl-CoA intermediate. An agent that was first described in 1984 (1,2), 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP), shows the expected slow washout (3,4) and has been shown to be a useful agent for SPECT studies in humans (5-7). Although myocardial washout of radioactivity is much slower for BMIPP than the 15-(p-phenyl)pentadecanoic acid (IPPA) straight-chain analog, the BMIPP agent does not have the irreversible retention that was expected. A further understanding of the identity of the radioactive component(s) in the myocardial outflow is important to the interpretation of differential washout rates in ischemic myocardium.

There is increasing interest in the use of  $^{123}\text{I}$ -BMIPP as a tool for the SPECT evaluation of regional myocardial fatty acid uptake in humans (8-11), especially since this tracer became commercially available in Japan (Cardiodine; Nihon Medi-Physics Inc., Nishinomiya, Japan). It is therefore important to understand the factors affecting the slow myocardial washout of BMIPP and to determine whether backdiffusion of unmetabolized BMIPP is a significant factor in the regional retention of this agent. This backdiffusion phenomenon must also be addressed as a function of the carrier protein albumin concentration and competing fatty acids, such as palmitate, that may play an important role in the analysis of BMIPP SPECT slices, especially in ischemic, stunned or hibernating myocardial regions (12).

The goals of the current study were to evaluate the effects of the presence of 0.4 mmol/L bovine serum albumin (BSA) with or without 0.4 mmol/L palmitate in the traditional Krebs-Henseleit buffer on the relative contribution of the release of the metabolites and backdiffusion of the unmetabolized BMIPP, to identify the metabolite(s) by mass spectrometry and to correlate these results with data obtained from analysis of blood samples from in vivo patient investigations.

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## MATERIALS AND METHODS

### Labeling

<sup>123</sup>I (Cygne, Eindhoven, The Netherlands) and <sup>131</sup>I (Amersham, Braunschweig, Germany) were obtained as 0.02 mol/L NaOH solutions, and the radiiodinated BMIPP tracer was prepared by the thallation-iodide displacement of the BMPP substrate as described in detail previously (1,13), with slight modifications. The average chemical yield was 87.6% (range 75%–90%). Purification was achieved either by silica gel column chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub> elution, radiochemical purity > 95% by thin-layer chromatography [TLC] analysis: SiO<sub>2</sub>-GF, MeOH-CHCl<sub>3</sub>, 6:94) or by high-performance liquid chromatography (HPLC). After vacuum evaporation of the purified compound and solubilization in ethanol (Nucleosil 100–5 C<sub>18</sub> [Machery & Nagel, Dueven, Germany], 250 × 4 mm; solvent A: 0.2% HCOOH in H<sub>2</sub>O, B: methanol 80%–100% B within 90 min; 1.5 mL/min; ultraviolet [UV] at 206 nm; detection of radioactivity by a flow-through detector), BMIPP was obtained carrier free.

### Isolated Rat Heart Studies

Hearts were removed from thiopental sodium-anesthetized (25 mg) Fischer or Sprague-Dawley rats and mounted on the perfusion apparatus as previously described (14–16). The hearts had a steady rate of contraction (180–210 times/min), and perfusate blood gases and pH values (7.4) were monitored throughout the studies with an Instrumentation Laboratory Model 1304 pH/blood gas analyzer (Micro, IL). The Krebs-Henseleit buffer was prepared in the usual manner (16,17), and BSA (0.4 mmol/L) and potassium palmitate (0.4 mmol/L) were added if needed. Hearts were perfused at a steady rate of 8–10 mL/min, and oxygenation of the perfusate was achieved by a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>.

### Tracer Administration, Sampling and Lipid Analysis

**Method I.** After stabilization of the hearts for 5 min, the labeled <sup>131</sup>I-BMIPP dissolved in 0.2–0.3 mL BSA was injected over a 15-s interval. Serial samples were then taken each minute for a 15-min period. After completion of the perfusion, the hearts were dismantled, minced and homogenized and the lipids extracted by a modified Folch technique (18) in which the myocardial homogenates and the 1, 3, 5, 7, 10 and 15 min perfusate samples were mixed with 5 mL of 2:1 chloroform-methanol, acidified with H<sub>2</sub>SO<sub>4</sub> (pH 2) and centrifuged. The methanol layer contained the organic compounds for further analysis. More than 95% of the radioactivity was extracted in the chloroform layer. Ten hearts were investigated per time point. The final products were evaporated and dissolved in ethanol. TLC analyses were performed on 0.25-mm-thick layers of silica gel GF-254 coated on aluminium foil (Analtech, Inc., Newark, DE), using both petroleum ether-ethyl ether-HOAc (70:30:1) and benzene-dioxane-HOAc (80:18:2) solvent systems. Standards were also spotted on the plate as reference points. After development, the plates were sprayed with a 1:1 (volume/volume) mixture of saturated potassium chromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) and sulfuric acid, dried for 20 min at 180°C to make the standards visible and then cut into 20 segments and counted. The data were expressed as a 20-segment histogram as the percentage of the total radioactivity in each segment.

**Method II.** In two Langendorff rat heart experiments, 5 mL BSA solution (140 mg BSA/mL) containing 5 mg cold iodinated BMIPP and 18.9 MBq <sup>131</sup>I-BMIPP were added to 4.5 mL of a Krebs-Henseleit solution. The labeled <sup>131</sup>I-BMIPP was administered at a steady rate (0.2 mL/min) over the perfusion period of 60 min. After

completion of the perfusion period, the collected perfusate was acidified with HCl to pH 1.0 and the resulting metabolites were extracted by passage through SepPak (C<sub>18</sub>) cartridges (Waters, Milford, MA) that were then eluted with methanol. After completion of the experiments, the hearts were dismantled, minced and homogenized and the lipids extracted as described in method I. Heart lipids were hydrolyzed with 10 mol/L KOH at 60°C for 1 h. The final products were extracted, and the extracts were evaporated and redissolved in methanol for further analysis.

HPLC analysis (perfusate and heart lipids) was achieved with standard reverse phase methods with a LATEK system (Latek, Heidelberg, Germany). The column was Nucleosil C<sub>18</sub> 5 μm (270 × 4 mm). Solvent A was methanol and solvent B was 0.2% HCOOH. For an efficient separation of the BMIPP metabolites, the following solvent gradients were used: gradient I was 80%–100% A within 90 min at 0.8 mL/min, and gradient II consisted of 60%–100% A within 90 min at 0.8 mL/min. UV detection was set to 206 nm (Latek). The radioactivity was measured at 365 keV with a 20% symmetrical window (Berthold, Bad Wildbad, Germany). The detectors were equipped with a dual-channel integrator C-R5A (Shimadzu, Kyoto, Japan). All solvents were of HPLC grade. All UV peaks were compared with synthesized standards (Nihon Medi-Physics, Kyoto, Japan), and the data were processed with CLASS-UniPac software (Shimadzu).

The mass spectral analyses were performed with a triple quadrupole instrument model TSQ 7000 instrument (Finnigan, San Jose, CA) equipped with a Finnigan ESI source. The detector was operated at 1.4 kV, and the transfer capillary was maintained at 200°C. Argon was used as collision gas at a nominal pressure of 2 mm Hg. The samples were infused at a rate of 3 μL/min in methanol.

**Patient Studies.** Governmental permission and approval of this study by the local ethical committee was obtained, and all fully informed patients gave their written consent. We investigated 20 patients (5 women, 15 men; mean age 54.7 y) with coronary artery disease referred to differentiate ischemic, viable and nonviable myocardial areas (detailed description of the myocardial results in [4]). All patients were assessed by coronary angiography, and 2 patients had no coronary artery disease.

After labeling the BMPP substrate with <sup>123</sup>I, the compound was dissolved in a small volume of absolute ethanol (100 μL), mixed with 10% human albumin solution (5–10 mL) and then filtered through a 0.22-μm Millipore filter (Millipore Inc., Bedford, MA). The product was sterile and pyrogen free, and no adverse reactions were observed in any patient. After intravenous injection of 200 MBq (5 mCi) of the final <sup>123</sup>I-BMIPP radiopharmaceutical, blood samples were withdrawn at 3 and 20 min and at 3 h after injection. Lipids were extracted from the serum by the modified Folch technique, and an aliquot was analyzed by TLC in two different solvent systems (see method I). Standards (p-iodobenzoic acid, cold iodinated BMIPP and tripalmitin) were also spotted on the plates as reference points. After analysis, the TLC plates were cut into 15 segments that were counted. The measured activities were expanded to the blood volume that was calculated from weight and height (19) of the patient, and this value was compared with the injected activity. This analysis provided an estimate of the percentage injected dose of each radioactive peak expanded to the blood volume.

**Statistics.** In method I and in the patient investigations, mean values and the error of the mean were calculated. Differences of the mean values were evaluated by the Student *t* test for unpaired

**TABLE 1**  
Relative Thin-Layer Chromatography Mobilities ( $R_f$ )  
for Standard Lipids

Lipid	$R_f$ value	
	System A	
	petroleum ether-ether-HOAc (70:30:1)	System B benzene-dioxane-HOAc (80:18:12)
Free fatty acids	0.41	0.62
Monoglycerides	0.08	0.11
Diglycerides	0.21	0.30
Triglycerides	0.84	0.95
Phospholipids	0.01	0.15
p-iodobenzoic acid	0.24	0.42

values between two groups. Significant differences were considered to be present when  $P$  was  $<0.05$ . In method II, all values are expressed as an average of the two experiments.

## RESULTS

### Method I

The results of the readings of our standards are summarized in Table 1, giving an estimate of the performance of the TLC systems. Table 2 shows the amounts of the original tracer BMIPP and its final metabolite 2-(p-iodophenyl)acetic acid ( $IPC_2$ ) at various time points and different compositions of the perfusate buffer. The total counts of each TLC plate were read and normalized to the highest value that was set to 100%. The remainder of the radioactivity on the TLC plates consisted of background distributed on the TLC plates.

### Method II

In the HPLC separation of labeled BMIPP, the UV and count profiles showed one single peak of either BMIPP (i.e., noniodinated substrate used for preparation of BMIPP) or BMIPP, respectively, indicating a carrier-free radiopharma-

**TABLE 3**  
Results of Analysis of Blood Lipids of Patients

Time postinjection	% Injected dose	
	BMIPP	$IPC_2$
3 min	$26.5 \pm 4.8$	0
30 min	$2.8 \pm 0.5$	$5.2 \pm 1.8$
3 h	0	$7.7 \pm 2.1$ $11.8 \pm 2.5^*$

\*Urine corrected.

$n = 20$ ;  $IPC_2 = 2$ -(p-iodophenyl)acetic acid.

Values given as mean  $\pm$  SD.

ceutical that was administered to the rats. The perfusates contained 92.4% of the administered activity, whereas in the heart an average of 6.4% of the administered activity was found.

The nonradioactive carrier 0.01 mol/L BMIPP solution (5  $\mu$ L) was added to an aliquot of the extracted lipids of the perfusate to identify the retention time of the original tracer (69.7 min) by HPLC analysis with gradient system I. The major component was  $^{131}I$ -BMIPP, which was present as 65.9% of the relative activity of the whole chromatogram or 56.5% of the injected dose, which was measured before injection. All the metabolites we could detect were four polar components that were observed with retention time values of 4.9, 7.9, 9.1 and 62.8 min, which contained 10.7%, 9.9%, 5.6% and 0.5% of the injected dose, respectively. In addition, a less polar fraction compared with BMIPP of unknown origin was detectable (86.6 min retention time, 5.8% injected dose) that represented the ethyl ester of BMIPP. This is supported by the absence of this component in the hydrolyzed sample, although this is the only evidence to identify this peak. A synthesized standard was not available for this peak. A comparison of the retention time values of the  $IPC_2$ , 4-(p-iodophenyl)butyric acid ( $IPC_4$ ), 6-(p-

**TABLE 2**  
Relative Percentage Composition of Folch Extracted Components in Outflow Determined by TLC Analysis in 10 Rats

Perfusate (min)	KH only		KH + BSA		KH + BSA + Pal	
	BMIPP	$IPC_2$	BMIPP	$IPC_2$	BMIPP	$IPC_2$
1	$80 \pm 15^*$	$4 \pm 0.6\ddagger$	$69 \pm 5.4^*$	$12 \pm 1.3\ddagger$	$82 \pm 18^*$	$4 \pm 0.5\ddagger$
3	$12 \pm 3.0\ddagger$	$48 \pm 9.1\ddagger$	$33 \pm 6.1\ddagger$	$44 \pm 9.1^*$	$76 \pm 12\ddagger$	$9 \pm 1.4\ddagger$
5	$3 \pm 0.5\ddagger$	$78 \pm 8.0\§$	$10 \pm 0.9^*$	$70 \pm 7.2^*$	$41 \pm 9.3\ddagger$	$27 \pm 4.1\§$
7	$2 \pm 0.4\ddagger$	$78 \pm 9.2\ddagger$	$7 \pm 0.6^*$	$77 \pm 11^*$	$35 \pm 6.0\ddagger$	$34 \pm 8.3\ddagger$
10	$3 \pm 0.4\ddagger$	$68 \pm 11\ $	$10 \pm 1.2^*$	$64 \pm 13^*$	$34 \pm 9.1\ddagger$	$33 \pm 5.0\ $
15	$4 \pm 0.3\ddagger$	$67 \pm 7.0\ddagger$	$7 \pm 1.0^*$	$80 \pm 10^*$	$31 \pm 11\ddagger$	$29 \pm 6.1\ddagger$

\*Not significant.

$\ddagger P < 0.005$ .

$\ddagger P < 0.01$ .

$\§ P < 0.001$ .

$\| P < 0.02$ .

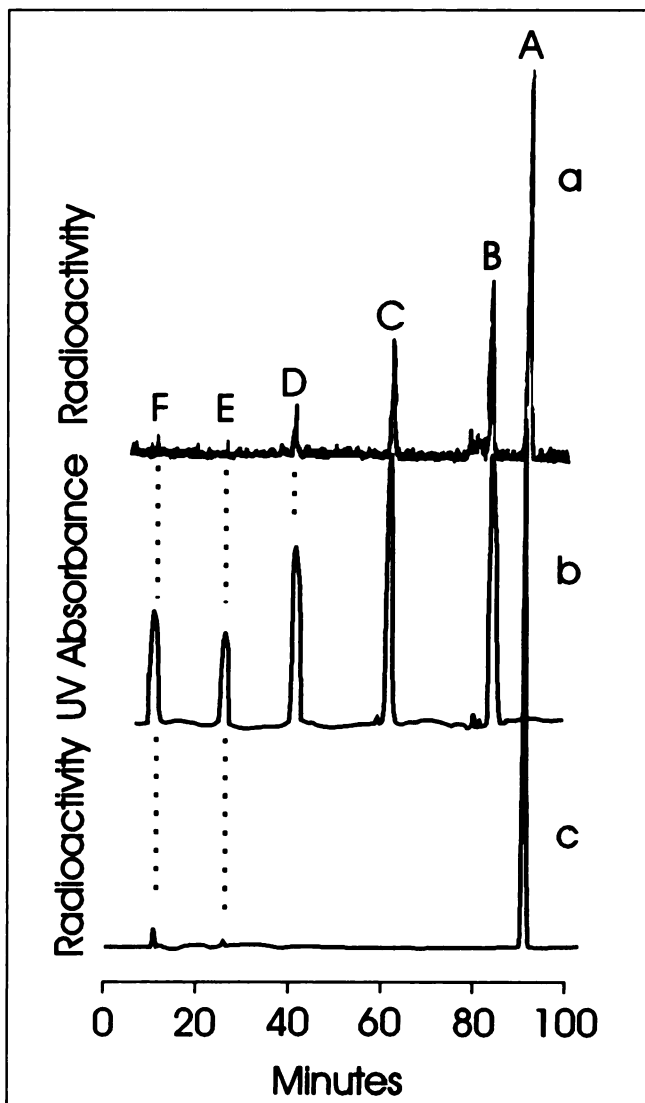
TLC = thin-layer chromatography; KH = Krebs-Henseleit buffer; BSA = bovine serum albumin; Pal = palmitate; BMIPP = 15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid;  $IPC_2 = 2$ -(p-iodophenyl)acetic acid.

Values given as mean  $\pm$  SD.

iodophenyl)hexanoic (IPC<sub>6</sub>), 12-(p-iodophenyl) dodecanoic (IPC<sub>12</sub>) and  $\alpha$ -methyl-14-(p-iodophenyl)tetradecanoic acid (AMIPC<sub>14</sub>) standards revealed that the polar components could be identified as IPC<sub>2</sub>, IPC<sub>4</sub> and AMIPC<sub>14</sub> for the retention times 4.9, 9.1 and 62.8 min, respectively. The polar component with a retention time of 7.9 min could not be matched with any of the known standards.

HPLC analysis of the hydrolyzed heart lipids revealed that IPC<sub>2</sub>, IPC<sub>6</sub>, IPC<sub>12</sub>, AMIPC<sub>14</sub> and <sup>131</sup>I-BMIPP were detected in percentages of 1.1%, 5.2%, 17.1%, 20.8% and 53.9% of the relative activity or 0.08%, 0.4%, 1.2%, 1.4% and 3.6% of the injected dose, respectively (Fig. 1). Analysis of the remaining perfusate after SepPak extraction showed no <sup>131</sup>I-labeled metabolites.

For better separation of the polar metabolites, the HPLC



**FIGURE 1.** HPLC analysis of lipid fractions after injection of <sup>131</sup>I-BMIPP to isolated, Langendorff perfused rat heart. (a) radioactive compounds in hydrolyzed rat heart lipids. (b) UV profile of synthesized standards. (c) radioactive compounds in rat heart perfusate. A = BMIPP; B = AMIPC<sub>14</sub>; C = IPC<sub>12</sub>; D = IPC<sub>6</sub>; E = IPC<sub>4</sub>; F = IPC<sub>2</sub>.

gradient system II was used for analysis of the remainder of the extracted perfusate lipids. In this gradient, IPC<sub>2</sub> was identified at 12.4 min retention time. This fraction was collected again, purified by HPLC and analyzed by negative ESI-MS/MS (Fig. 2A). Using the [iodide] – fragment at mass-to-charge ratio (*m/z*) 126.9 and the parent scan mode, the specific signal of the metabolite could be selectively detected without contributions from other contaminants in the sample. Figure 2B illustrates this feature of ESI-MS/MS showing only the parent-ion peak of 4-iodophenylacetic acid (*m/z* = 261), iodide (*m/z* = 127) and a fragment at *m/z* = 217. Reference measurement of IPC<sub>2</sub> standard revealed the same fragment at *m/z* = 217, indicating decarboxylation of the parent ion.

#### Patient Data

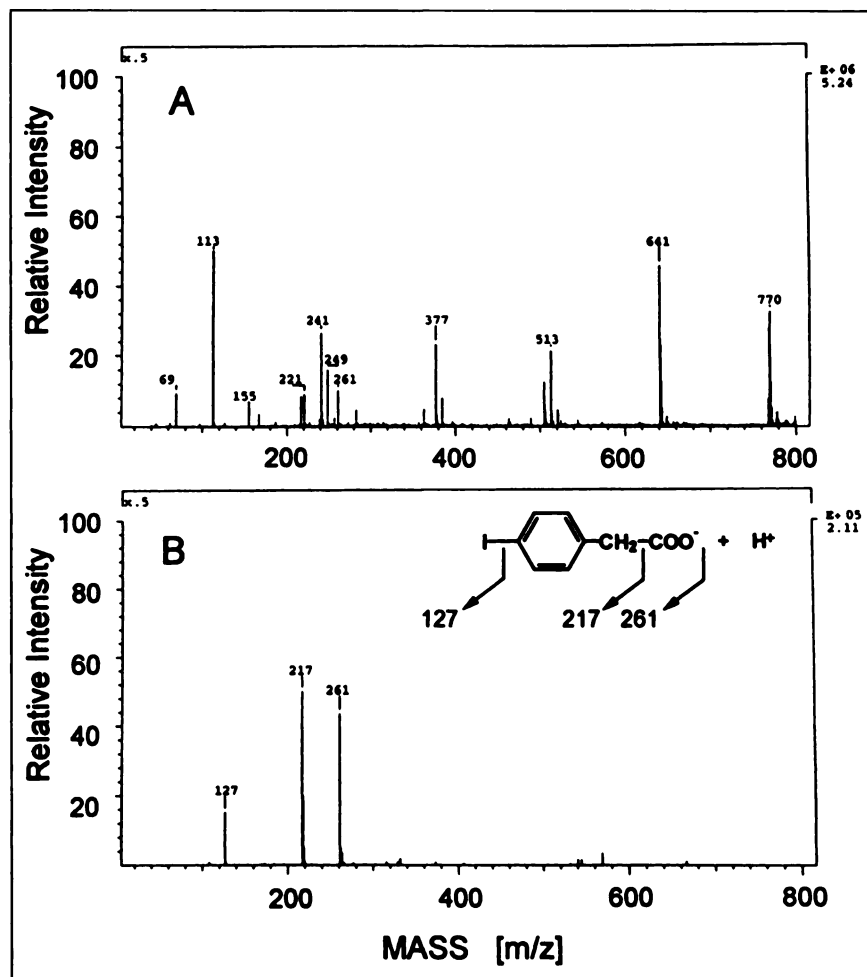
In the TLC analysis of the serum lipids of the patients, the same major metabolite as in the isolated rat heart studies (Fig. 3) was identified in increasing amounts over time (0%  $\pm$  1.4%, 5.2%  $\pm$  2.5% and 7.7%  $\pm$  2.1% of the injected dose at 3 min, 20 min and 3 h, respectively) and the identity of this metabolite could be substantiated by HPLC analysis. In the whole-body scan we found 4.1%  $\pm$  2.4% of the injected dose in the urinary bladder 3 h after injection, which is reasonably assigned as activity associated with the final metabolite, so that the total amount of IPC<sub>2</sub> 3 h after injection is 11.8%  $\pm$  2.2% of the injected dose.

#### DISCUSSION

Our data demonstrate that the final major metabolite of <sup>123</sup>I- and <sup>131</sup>I-BMIPP is IPC<sub>2</sub> in animal and humans to an amount of 10.7% of the injected dose within a 60-min perfusion period in the isolated rat heart model and 11.8%  $\pm$  2.2% of the injected dose 3 h after injection in *in vivo* studies in humans. These results illustrate that the slow release of radioactivity of BMIPP in the human myocardium detected by sequential scintigraphy is the result of initial  $\alpha$ -oxidation of the compound in the cytosol, followed by subsequent  $\beta$ -oxidation in the mitochondria, as had been hypothesized previously (20).

#### Animal Studies

Our earlier studies clearly demonstrated the unexpected release of a polar metabolite as the principal radioactive component present in the outflow of Langendorff-perfused rat hearts (16,21) administered radioiodinated BMIPP, which represents a convenient tool to evaluate the metabolism and release kinetics of radiolabeled fatty acids. This metabolite was detected experimentally in isolated rat hearts perfused with the traditional Krebs-Henseleit buffer (16) and was also detectable in patients (4). Because an evaluation of the relative contribution of metabolite release and backdiffusion of unmetabolized BMIPP is relevant to an understanding of the behavior of this agent *in vivo*, the current studies were conducted with modified Krebs-Henseleit buffers containing 0.4 mmol/L BSA or 0.4 mmol/L potassium palmitate. On the other hand, experiments were also performed to confirm the



**FIGURE 2.** (A) Negative ESS-mass spectrum of peak F (IPC<sub>2</sub>) in (c) of Figure 1 after HPLC separation and purification. (B) Same sample using [iodide] – fragment at m/z 126.9 and parent scan mode. Specific signal of metabolite could be selectively detected without contributions from other contaminants in sample, thus, showing only parent-ion peak of 2-(p-iodophenyl)acetic acid (m/z = 261), iodide (m/z = 127) and metabolite at m/z = 217. Chemical structure of IPC<sub>2</sub> is also displayed along with corresponding masses.

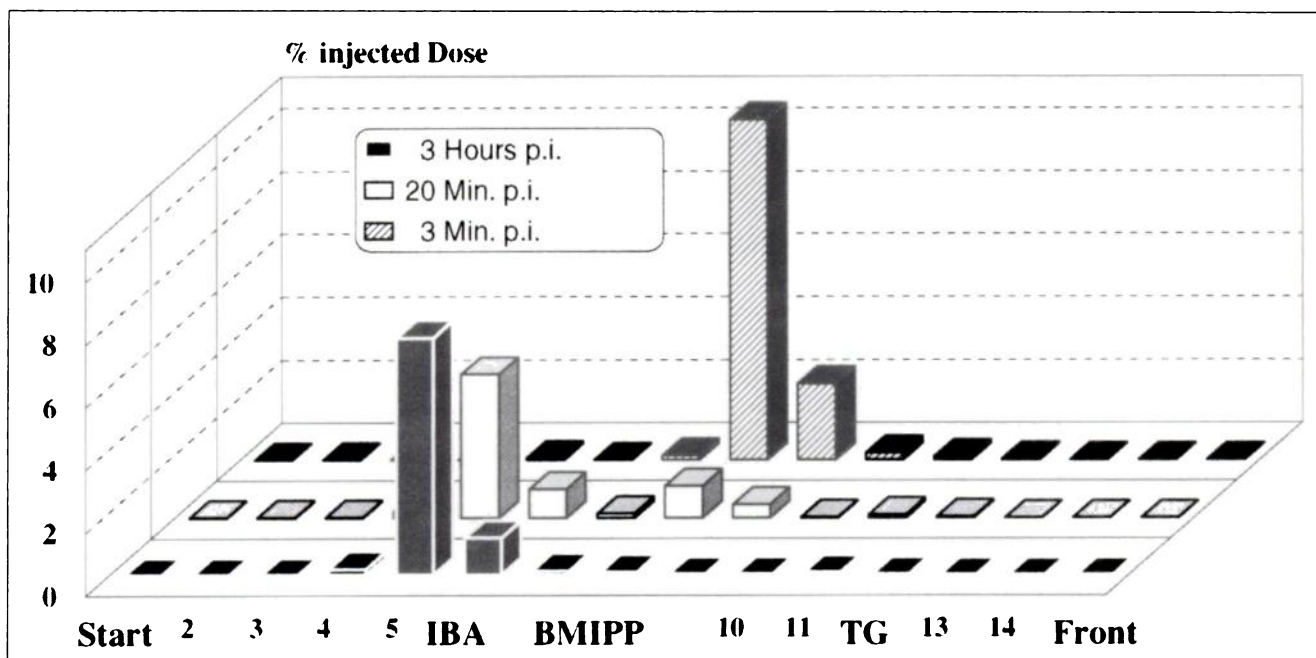
identity of the metabolite by its mass, which has been identified by its chromatographic behavior in comparison with synthesized standards (22).

The rat hearts were perfused retrograde in the usual manner (16) for 15 or 60 min, and effluent samples were collected and lipids obtained by the Folch technique for either TLC or HPLC analysis. Analysis by TLC (benzene-dioxane-AcOH, 80:18:2) provided an evaluation of the distribution of radioactivity between BMIPP ( $R_f = 0.55$ ) and the metabolite ( $R_f = 0.35$ ). By comparing the effects of either 0.4 mmol/L BSA or 0.4 mmol/L BSA plus 0.4 mmol/L palmitate on the relative amounts of metabolite(s) and BMIPP in the outflow of the isolated hearts, an estimation of the potential contribution of backdiffusion of BMIPP *in vivo* could be obtained. Table 2 shows that, if the experimental set-up is approaching more physiologic conditions by adding BSA or palmitate, the amount of unmetabolized BMIPP in the outflow significantly increases, especially at late time points and the metabolite decreases. These results clearly illustrate the contribution of backdiffusion of BMIPP in the isolated rat heart system in the presence of albumin and the changes in metabolism when palmitate is present in the perfusate.

Discussions in the literature of the clinical implications of

the magnitude and significance of backdiffusion of radiolabeled palmitate have focused on the interpretation of the <sup>11</sup>C-palmitate time-activity curves of ischemic regions (23,24). Studies with <sup>11</sup>C-palmitate usually assume that information extracted from an analysis of the time-activity curves, which measure the radioactive content of the myocardium over time with PET, principally reflects loss of the radiolabeled CO<sub>2</sub> catabolite from complete oxidation of palmitate (25). Other investigators have shown, however, that significant levels of unmetabolized palmitate are lost from ischemic regions of the canine myocardium and that a quantification of the efflux of this unmetabolized material may be important in quantifying regional myocardial fatty acid metabolism by PET (24). In fact, studies in open-chest canine studies have shown that about 16% of radiolabeled palmitate backdiffuses from the myocardium in control dogs over a 30-min period (23). An important observation was obtained in other studies using open-chest dogs with an ischemia-like situation. Backdiffusion increased to 42% of the administered <sup>123</sup>I-BMIPP in the situation of ischemia compared with 25% in controls, which was statistically significant (26). Thus, in ischemia, the backdiffusion of unmetabolized fatty acid is a very important factor.

Although the clinical use of <sup>123</sup>I-BMIPP focuses on the



**FIGURE 3.** TLC analysis of radioactive lipid fractions in serum of one patient 3 and 20 min and 3 h after injection of  $^{123}\text{I}$ -BMIPP along with cold standards. Over time there is increasing amount of polar metabolite (even more polar than IBA) of BMIPP identified by HPLC analysis as  $\text{IPC}_2$ . p.i. = postinjection; IBA = p-iodobenzoic acid; BMIPP = cold iodinated BMIPP; TG = tripalmitin.

SPECT evaluation of regional fatty acid uptake patterns in various myocardial diseases (7,10,27,28) and not on evaluation of time-activity curves, the current studies with normoxic rat hearts may advance the understanding and interpretation of the washout of radioiodinated BMIPP detected in vivo by either planar imaging (3) or successive SPECT studies (4,12,29).

Our TLC results have been confirmed by HPLC and demonstrate that backdiffusion is influenced not only by the presence of albumin but also by the availability of alternative energy substrates, because the traditional Krebs-Henseleit buffer contains glucose as the principal energy source. Confirmation of these results by in vivo studies will require sampling in the coronary sinus in larger animals.

In the perfusates, we could not identify significant amounts of metabolites originating from the first steps of  $\alpha$ - or  $\beta$ -oxidation of the labeled BMIPP compound, which is in contrast to previous reports (22). This might be due to the perfusion protocol that was used in those studies, including right ventricular pacing and a forced perfusion period over 4 h. In such a protocol much more cell damage may have occurred, releasing the initial metabolites into the perfusate. Another reason might be the recirculation mode used in those studies that would concentrate metabolites in the perfusate. In this study, the metabolites  $\text{AMIPC}_{14}$ ,  $\text{IPC}_{12}$  and even  $\text{IPC}_6$  were detectable after a 60-min perfusion period only in the heart lipid hydrolysate (Fig. 1), indicating that these metabolites are not released into the perfusate, which is in agreement with a published study (30).

Analysis of the major polar metabolite in the perfusate  $\text{IPC}_2$  by an advanced method of mass spectrometry gave

further evidence that the chemical identity of this metabolite is  $\text{IPC}_2$  (Fig. 2), as has been suggested elsewhere (22,30). The mass peaks shown in Figure 2A cannot be assigned to defined substances. They represent unspecific background peaks that are suppressing the mass signals of the low concentrated BMIPP metabolite  $\text{IPC}_2$  barely visible at 261 atomic mass unit (amu). The value of ESI-MS/MS running in "parent ion scan mode" becomes obvious in Figure 2B. The fragmentation pattern of this method is explained in the formula. Because substances producing background signals do not form the 127 amu fragment, they do not show up in Figure 2B.

#### Patient Data

The average activity loss in the normally perfused heart over 3 h is  $18.7\% \pm 5.1\%$ , as measured by the time-activity curve data (4). Because the usual  $\beta$ -oxidative process is apparently inhibited by the methyl group, an alternative metabolic pathway has been suggested (31) that might involve  $\alpha$ -oxidation, as discussed by several authors (32–36), or an alternative process that has not yet been elucidated.

Our data clearly demonstrate that BMIPP is metabolized either by the heart or other organs, because there is activity detectable in the urinary bladder that must be due to excretion of the hydrophilic metabolite by the kidneys. This is supported by the analysis of the urine of patients after administration of BMIPP (21,37). After lipid extraction, a significant amount of the radioactivity was assigned as the organic phase, and TLC and HPLC analyses revealed a polar component that was addressed as a metabolite of BMIPP.

Analysis of blood lipids by TLC and HPLC showed a metabolite more polar than the BMIPP standard and even more polar than p-iodobenzoic acid, which was proposed as the principal metabolite of IPPA (38). The polar fraction from BMIPP metabolism increased slowly with time, implying a low rate of metabolic formation. This polar compound appears to correspond with the same metabolite of BMIPP found earlier in isolated heart experiments (16), because both compounds had the same  $R_f$  value of 0.35. This metabolite might be a product of  $\alpha$ -oxidation, which is known to occur in ischemic conditions (35). Because this metabolite was also detected in patients without coronary artery disease, as evidenced by normal BMIPP scans, this metabolic pathway might be at least partly an alternative to  $\beta$ -oxidation in the normal perfused nonischemic heart.

Experimental data (22) as well as analyses of the metabolic products of BMIPP in the blood and urine of patients (37) have suggested that the major metabolite is IPC<sub>2</sub>. We have substantiated these results by HPLC analysis of the extracted lipids of blood samples and subsequent identification by mass spectrometry in the rat heart experiments. From similar experiments dealing with the final metabolite of the straight chain fatty acid IPPA, one would expect 1-(p-phenyl)benzoic acid as the final metabolite (38). Other investigators have used more sophisticated techniques, avoiding esterification or other derivatization techniques that prevent further degradation or even decomposition of the compounds. In addition, the newer technique is performed under much milder conditions and has provided evidence that 3-(p-iodophenyl)propenoic acid is the major metabolite of IPPA (39). This result is in agreement with our finding, if removal of the first carbon atom of BMIPP by  $\alpha$ -oxidation is considered.

In addition, the results of this study dramatically show that the presence of albumin, as well of palmitate, influence the pharmacokinetics of this fatty acid and that degradation of BMIPP occurs in vivo. Up to 3 h after injection of <sup>123</sup>I-BMIPP, there is an increasing amount of the final IPC<sub>2</sub> metabolite.

## CONCLUSION

The animal results demonstrate the complexity of release of radioactivity from the myocardium after injection of BMIPP. These results indicate a portion of the accumulated dose is metabolized through  $\alpha$ - and subsequent  $\beta$ -oxidation, and results in humans suggest that the slow washout observed in vivo may represent a similar process. In physiologic conditions in which albumin, palmitate and lactate are present, especially in ischemic areas, a major component of radioactivity release may be backdiffusion of the unmetabolized BMIPP tracer, which might influence the interpretation of SPECT images.

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