

Pharmacokinetics and Metabolism of ^{123}I -BMIPP Fatty Acid Analog in Healthy and CD36-Deficient Subjects

Tohru Yoshizumi, Shuichi Nozaki, Kazuki Fukuchi, Koichi Yamasaki, Takahiro Fukuchi, Takao Maruyama, Yoshiaki Tomiyama, Shizuya Yamashita, Tsunehiko Nishimura, and Yuji Matsuzawa

Division of Radiology, Minoh City Hospital, Minoh; and Second Department of Internal Medicine and Division of Tracer Kinetics, Biomedical Research Center, Osaka University Medical School, Suita, Japan

Some have suggested that CD36, which is a multifunctional receptor with a molecular weight of 88 kDa, functions as a long-chain fatty acid (LCFA) transporter. We recently reported on a complete myocardial accumulation defect of the radiolabeled LCFA analog ^{123}I -15-(p-iodophenyl)-(R,S)-methylpentadecanoic acid (BMIPP) in patients with CD36 deficiency. In this study, we investigated the pharmacokinetics of BMIPP in patients with a myocardial accumulation defect of BMIPP accompanied by CD36 deficiency. **Methods:** Five patients (3 men, 2 women) with CD36 deficiency and 3 healthy men were investigated. Serial myocardial images were obtained every 70 s for 20 min (dynamic acquisition) and at 30, 60, 120, 180, and 240 min (static acquisition) after an intravenous bolus injection of 148 MBq BMIPP. Whole-body imaging was performed 60 min after injection. Plasma levels of BMIPP and its final metabolite, p-iodophenylacetic acid, at 2, 5, 10, 30, 60, 120, and 240 min after administration were determined. **Results:** In the CD36-deficient patients, myocardial images could not be obtained for up to 240 min after administration, and cardiac pool images showing only the cardiac chambers were obtained. The heart-to-mediastinum ratio was significantly lower in the CD36-deficient patients than in the healthy volunteers (1.71 ± 0.11 versus 2.95 ± 0.22 , $P < 0.05$). Hepatic uptake of BMIPP was nearly double in CD36-deficient patients. The elimination of BMIPP from the circulation was retarded in the CD36-deficient patients. **Conclusion:** We suggest that CD36 deficiency leads to decreased myocardial accumulation of BMIPP and retardation of BMIPP elimination from the circulation. The accumulation defect is probably caused by a defect in LCFA uptake into the myocardium through CD36.

Key Words: CD36 deficiency; ^{123}I -BMIPP; long-chain fatty acids; myocardium

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As a glycoprotein with a molecular weight of 88 kDa, CD36 is assumed to have a double-membrane penetrating structure (1). Its expression has been reported in myocytes, platelets, monocytes, macrophages, and capillary endothelial cells as well as adipocytes. It is a multifunctional

receptor that acts as a collagen and thrombospondin receptor in platelets, as an oxidized low-density lipoprotein receptor (2), and as a long-chain fatty acid (LCFA) transport protein in adipocytes (3). Our coresearchers identified patients with CD36 deficiency and reported their genetic abnormalities (4–6). However, the pathophysiologic outcome of this defect has not yet been clarified.

The radiolabeled LCFA analog ^{123}I -15-(p-iodophenyl)-(R,S)-methylpentadecanoic acid (BMIPP) has been used in nuclear cardiology to study myocardial fatty acid metabolism in patients with coronary artery disease and hypertrophic cardiomyopathy (7,8). Some cases with a myocardial accumulation defect have been reported (9,10). Recently, Kudoh et al. (10) reported that PET revealed a distinct deficiency in ^{11}C -palmitate uptake in patients with a BMIPP accumulation defect and suggested that such patients may lack LCFA uptake, although the molecular basis for the lack of LCFA uptake was not clarified. In this study, we examined sequential changes in myocardial images as well as the pharmacokinetic behaviors of both BMIPP and its metabolites in the plasma in CD36-deficient patients.

MATERIALS AND METHODS

Subjects

We investigated 5 outpatients (3 men, 2 women) who were examined in the Second Department of Internal Medicine, Osaka University Hospital, and found to have a CD36 deficiency in both monocytes and platelets (11). Three patients had coronary artery disease, 1 had hypertrophic cardiomyopathy (HCM), and 1 had hyperlipidemia (Table 1). Three healthy volunteers were also enrolled as a control group and underwent the same examinations as the patients. Informed consent was obtained from all subjects. The investigation conformed with the principles outlined in the Declaration of Helsinki.

Imaging and Analytic Protocol

The BMIPP used in this study was a commercially available product. Its radiochemical purity was greater than 98%, and 148 MBq BMIPP (2 mL) contained 0.8 mg carrier BMIPP.

The subjects fasted for 6 h or longer before the examination. To examine the myocardial uptake of BMIPP, we obtained serial myocardial images every 70 s for 20 min (dynamic acquisition) and

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For correspondence or reprints contact: Tohru Yoshizumi, Division of Radiology, Minoh City Hospital, Kayano 5-7-1, Minoh City, Osaka, 562-8562, Japan.

TABLE 1
Subject Profile

Subject no.	Age (y)	Sex	Disease	CD36
1	56	M	HCM	Deficiency +
2	51	F	Hypertlipidemia	Deficiency +
3	63	F	CAD	Deficiency +
4	65	M	CAD	Deficiency +
5	72	M	CAD	Deficiency +
6	40	M	Control	Normal
7	37	M	Control	Normal
8	25	M	Control	Normal

HCM = hypertrophic cardiomyopathy; CAD = coronary artery disease; + = positive.

at 30, 60, 120, 180, and 240 min (static acquisition) after an intravenous bolus injection of 148 MBq BMIPP. Whole-body imaging was performed 60 min after injection (Fig. 1).

The heart-to-mediastinum ratio (12) was measured for the 30-min image. Regions of interest were manually drawn over the entire left ventricle and mediastinum (background) with reference to images from a ²⁰¹Tl perfusion scan. The heart-to-mediastinum ratio was determined as a ratio of count or pixel values in the 2 regions of interest. From whole-body images, BMIPP uptake ratios, relative to the whole-body counts, in the heart, liver, and thigh (average of left and right) were calculated.

Pharmacokinetics of BMIPP and its Metabolites

To determine the radioactivity of BMIPP and its metabolites, 5 mL venous blood were sampled in each subject before and 2, 5, 10,

30, 60, 120, and 240 min after injection (Fig. 1). After measuring total radioactivity concentration in the plasma, we extracted each plasma sample with a 2:1 mixture of chloroform and methanol (13). The organic layer was developed by thin-layer chromatography with a solvent consisting of a 60:40:1 mixture of n-hexane: diethylether:acetic acid. R_f values for BMIPP and the final metabolite, p-iodophenylacetic acid (PIPA), were approximately 0.53 and 0.25, respectively (14). A component with an R_f value of 0.9 was regarded as PIPA-ester, because alkaline hydrolysis of this component gave an R_f value similar to that of PIPA. A bioimaging analyzer was used to measure the fractional ratios of BMIPP, PIPA, PIPA-ester, and intermediate and other (unknown) metabolites. The fractional ratios were multiplied by the total radioactivity concentration at each sampling time with a correction for the subject's body surface area to obtain the plasma concentrations of each component.

RESULTS

All the healthy volunteers exhibited clear myocardial images by 10 min after BMIPP administration. In the CD36-deficient patients, myocardial images could not be obtained until 240 min after administration, and cardiac blood-pool images showing only the cardiac chambers were obtained (Fig. 2). The heart-to-mediastinum ratio at 30 min was significantly lower in the CD36-deficient patients than in the healthy volunteers (1.71 ± 0.11 versus 2.95 ± 0.22 , $P < 0.05$).

Whole-body imaging performed 60 min after the administration of BMIPP also failed to yield myocardial images in the CD36-deficient patients. In contrast, uptake of BMIPP

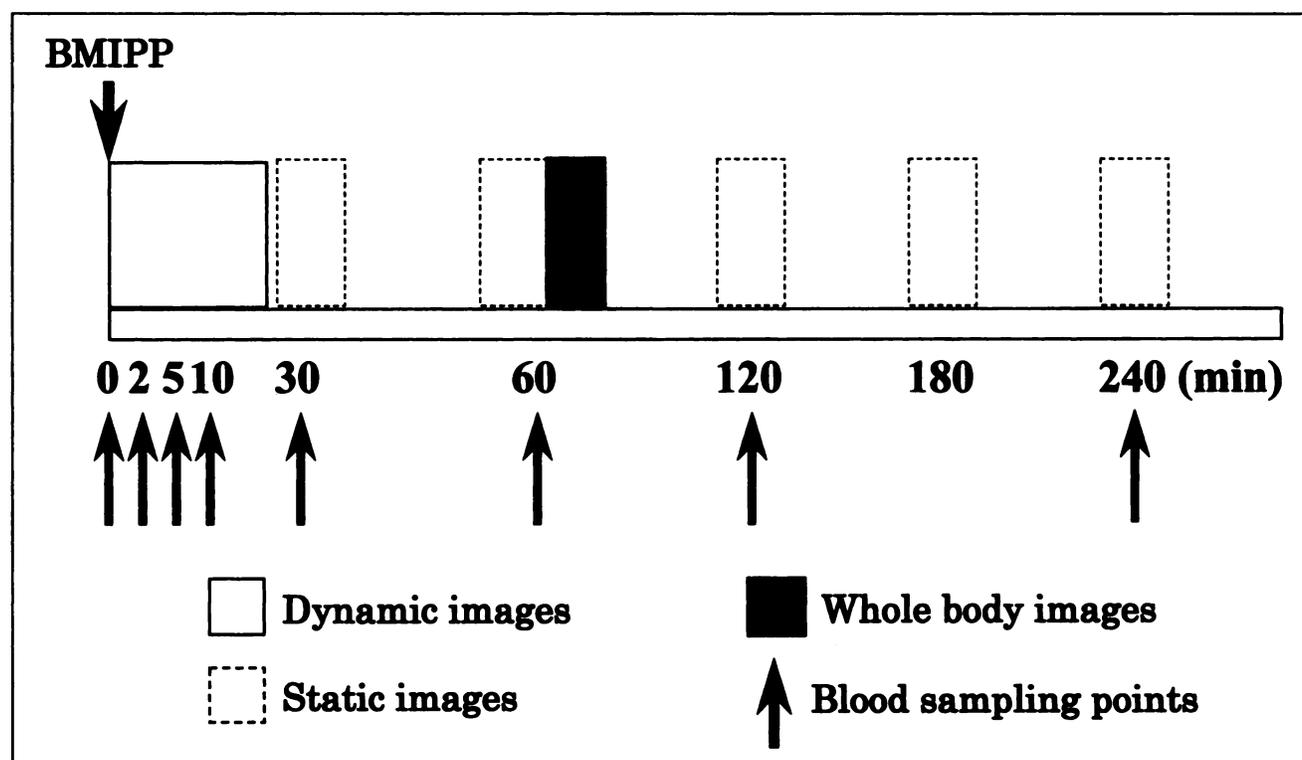


FIGURE 1. Study protocol. After bolus injection of BMIPP, dynamic (0–20 min) and static (30, 60, 120, 180, and 240 min) myocardial imaging, whole-body imaging (60 min), and blood sampling (0, 2, 5, 10, 30, 60, 120, and 240 min) were performed.

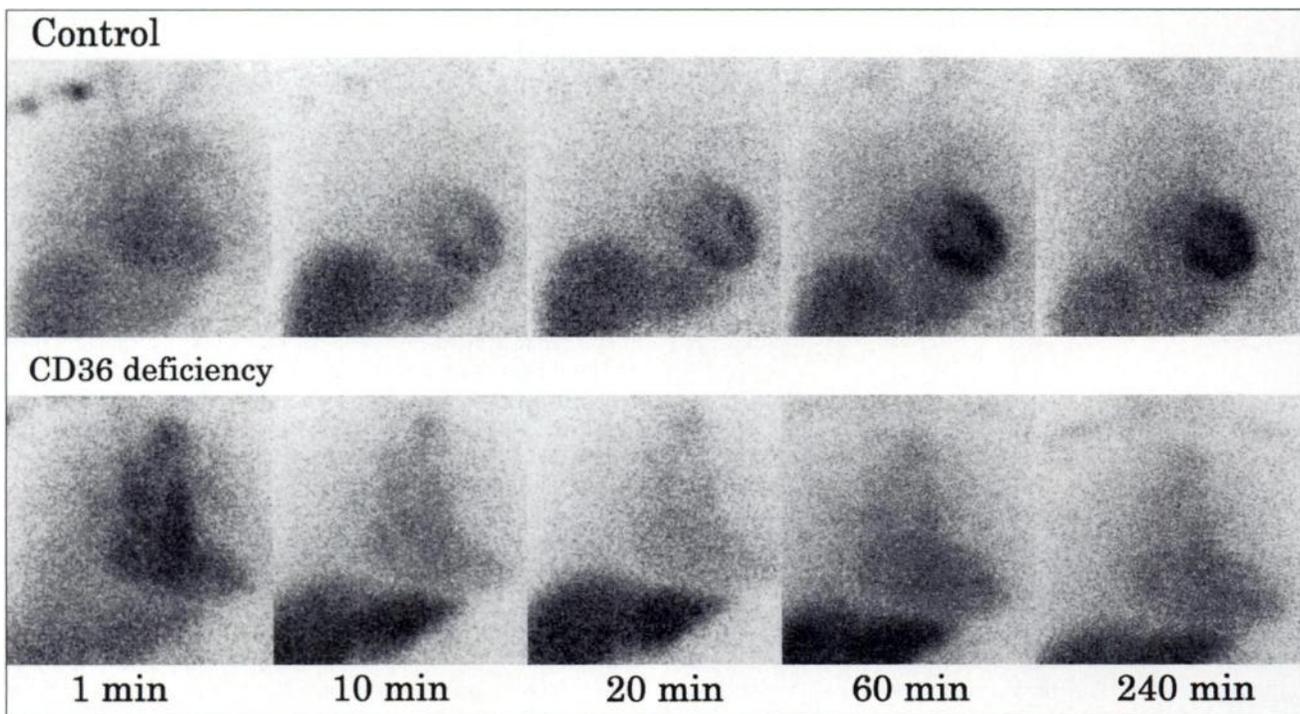


FIGURE 2. Serial BMIPP myocardial scintigrams in healthy volunteer and CD36-deficient patient. Myocardial uptake of BMIPP was observed within 10 min in volunteer but was not observed until 240 min in patient.

was shown clearly in the liver of these patients (Fig. 3). The myocardial uptake ratio was significantly lower in the CD36-deficient patients than in the healthy volunteers (3.48 ± 0.37 versus 5.32 ± 0.37 , $P < 0.05$). However, uptake of BMIPP in the liver was significantly higher in the patients than in the healthy volunteers ($24.9\% \pm 2.65\%$ versus $13.2\% \pm 0.63\%$, $P < 0.05$). Uptake in the thigh was insignificantly lower in the CD36-deficient patients (Table 2).

The total radioactivity in plasma (which contains both BMIPP and its metabolites) was higher in CD36-deficient patients than in healthy volunteers at all measurement points. In particular, the difference between the 2 groups was most significant up to 60 min after injection. At later times, the difference was reduced (Fig. 4).

Elimination of BMIPP from the circulation was significantly less in the CD36-deficient patients than in the healthy volunteers up to 120 min after administration (Fig. 5). The concentration of PIPA was not different between the 2 groups (Fig. 6).

DISCUSSION

CD36 deficiency was originally identified in a patient who was refractory to platelet transfusion (15,16). Platelet CD36 deficiency has been identified in approximately 3% and 0.3% of the populations of Japan (15) and the United States (17), respectively. Analysis of CD36 cDNA revealed an abnormality of the CD36 molecule that was homozygous for the substitution of $^{478}C \rightarrow T$ in patients with CD36 deficiency (18,19). However, these patients appear to be healthy and suffer no obvious hemostatic disease (20), and the

pathophysiologic consequences of this deficiency have not been defined. The CD36-deficient patients in this study were suffering from hyperlipidemia, coronary artery disease, or HCM. Tanaka et al. (21) recently suggested an association between CD36 deficiency and cardiomyopathy, and a patient with HCM was also included in this study.

We investigated the pharmacokinetics of BMIPP in patients with a myocardial accumulation defect of this tracer accompanying CD36 deficiency. Myocardial uptake of BMIPP was absent for up to 240 min in the CD36-deficient patients. These data suggest that BMIPP does not enter CD36-deficient cardiomyocytes, probably owing to lack of the cell membrane LCFA transporter CD36, because we showed by immunochemistry that the myocardium obtained from a CD36-deficient patient suffering from HCM (patient 1) did not express CD36 protein on the cell surface. This mechanism differs distinctly from that in the patients previously reported (22), in whom accelerated backdiffusion caused by a decrease in the retention capacity of BMIPP in the myocardium was suggested. The current data further suggest that CD36 is a major transporter of LCFAs in myocardium. Although Schaffer and Lodish (23) reported such a transporter other than CD36 in rats, this protein may not have a major role in humans.

Whole-body images revealed that uptake of BMIPP in the liver was increased in the CD36-deficient patients. These results suggest that transporters other than CD36 may participate in fatty acid uptake in the liver. Stremmel et al. (24) reported a fatty acid transport protein other than CD36 in the liver using animal tissues and cells. Because high

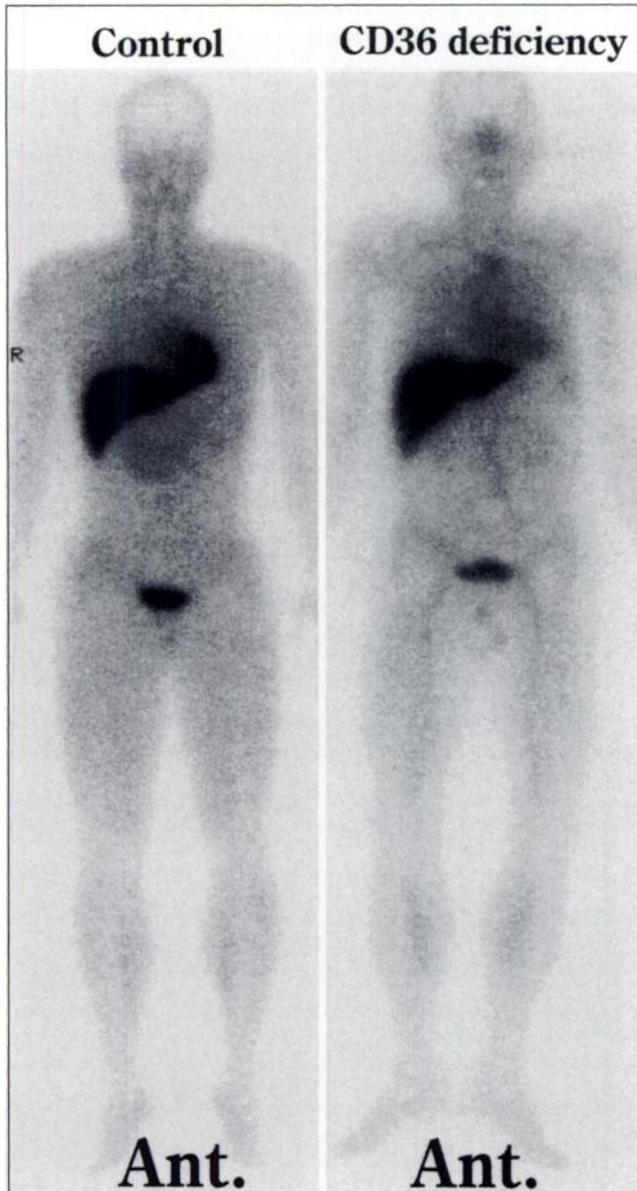


FIGURE 3. Whole-body images of BMIPP in healthy volunteer and CD36-deficient patient 60 min after administration. Myocardial uptake of BMIPP was not seen in patient. Liver uptake was high in both volunteer and patient. Ant. = anterior.

TABLE 2
Uptake Ratio from Whole-Body Imaging (60-Minute)

Site	Control	CD36 deficiency	P
Liver	13.2 ± 0.63	24.9 ± 2.65	<0.05
Myocardium	5.32 ± 0.37	3.48 ± 0.37	<0.05
Thigh	1.28 ± 0.63	0.98 ± 0.08	NS

NS = not statistically significant.
Values are percentage injected dose.

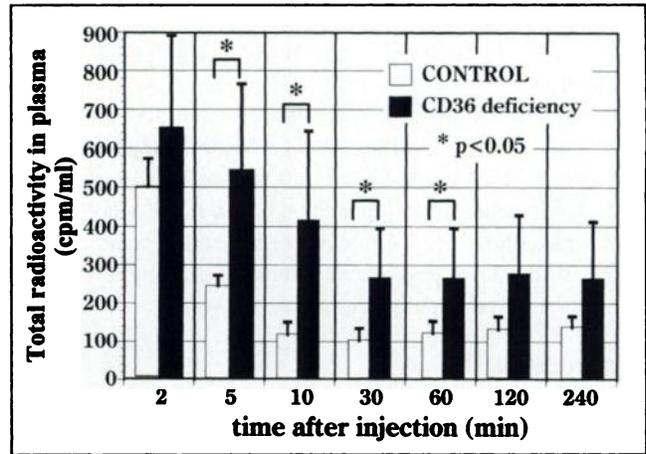


FIGURE 4. Time course of total radioactivity concentration in plasma after administration of BMIPP. Elimination of plasma radioactivity was delayed in CD36-deficient patients.

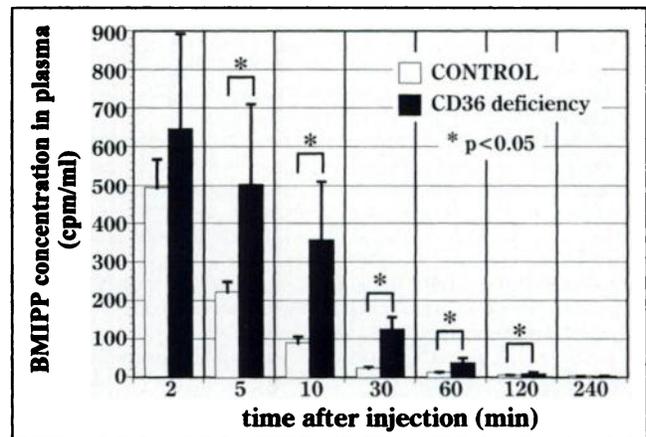


FIGURE 5. Time course of unmetabolized BMIPP concentration in plasma after administration of BMIPP. Elimination of BMIPP from circulation was delayed in CD36-deficient patients.

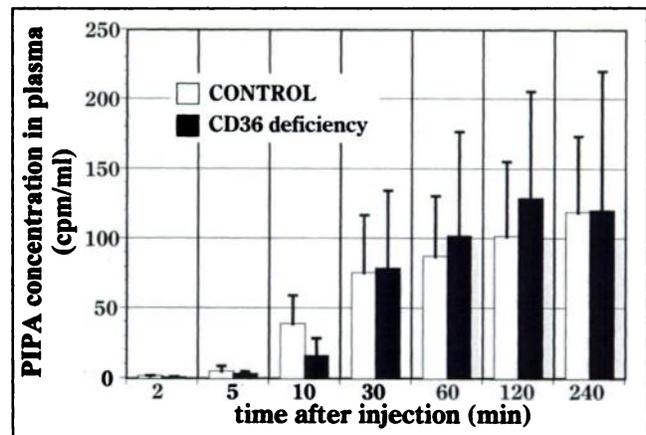


FIGURE 6. Time course of PIPA concentration in plasma after administration of BMIPP. CD36-deficient patients and healthy volunteers did not differ.

uptake of BMIPP was found in the liver of CD36-deficient patients, this protein also appeared to participate in the human liver.

When we followed the plasma radioactivity of BMIPP and its metabolites, elimination of BMIPP from the circulation was delayed in the CD36-deficient patients, suggesting that LCFA metabolism depends on CD36 in the whole body. The retardation of LCFA metabolism and high uptake of LCFA in the liver may cause attenuation of lipid metabolism, such as the hyperlipidemia seen in patient 2.

CONCLUSION

CD36 is an important protein for LCFA transport in the myocardium. Deficiency of this protein leads to decreased myocardial accumulation of BMIPP. The deficiency of CD36-mediated LCFA uptake is a novel mechanism for an accumulation defect of BMIPP in myocardium.

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