CD36 mediates long-chain fatty acid transport in human myocardium: Complete myocardial accumulation defect of radiolabeled long-chain fatty acid analog in subjects with CD36 deficiency

Shuichi Nozaki,1 Takao Tanaka,2 Shizuya Yamashita,1 Koichi Sohmiya,1 Tohru Yoshizumi,3 Fumio Okamoto,2 Yasushi Kitaura,2 Chikao Kotake,4 Hiroyuki Nishida,4 Atsuyuki Nakata,1 Tsutomu Nakagawa,1 Kengo Matsumoto,1 Kaoru Kameda-Takemura,1 Seiji Tadokoro,1 Yoshiyuki Kurata,5 Yoshiaki Tomiyama,1 Keishiro Kawamura2 and Yuji Matsuzawa1

1The Second Department of Internal Medicine, Osaka University Medical School; 2The Third Division, Department of Internal Medicine, Osaka Medical College; 3Minoo City Hospital; 4Saiseikai Nakatsu Hospital; 5Department of Blood Transfusion, Osaka University Hospital, Osaka, Japan

Abstract

Long-chain fatty acids (LCFA) are the major energy substrate for heart and their oxidation is important for achieving maximal cardiac work. However, the mechanism of uptake of LCFA by myocardium has not been clarified. We previously reported that bovine myocardial LCFA transporter has a sequence homology to human CD36. Clinically, total defect of myocardial uptake of radiolabeled long-chain fatty acid analog [123I-BMIPP: Iodine-123 15-(p-iodophenyl)-(R,S)-methylpentadecanoic acid] has been reported in some restricted cases, but the etiology has not been clarified. In the present study, we analyzed CD36 expression and CD36 gene in subjects who showed total lack of myocardial 123I-BMIPP accumulation, and, vice versa, evaluated myocardial 123I-BMIPP uptake in subjects with CD36 deficiency. Four unrelated subjects were evaluated; Two were found to have negative myocardial LCFA accumulation by 123I-BMIPP scintigraphy, after which the expression of CD36 on their platelets and monocytes was analyzed. Remaining two subjects were identified as CD36 deficiency by screening, then 123I-BMIPP scintigraphy was performed. Expression of CD36 on platelets and monocytes was measured by flow cytometric analysis. The molecular defects responsible for CD36 deficiency was detected by allele-specific restriction enzyme analysis. CD36 expression was totally deficient in all 4 subjects on both platelets and monocytes. Two subjects were homozygous for a 478C→T mutation. One was heterozygous for the dinucleotide deletion of exon V and single nucleotide insertion of exon X, and remaining one was considered to be heterozygous for the dinucleotide deletion of exon V and an unknown gene abnormality. All cases demonstrated a completely negative accumulation of myocardial LCFA despite of normal myocardial perfusion, which was evaluated by thallium scintigraphy. In addition, all cases demonstrated apparently normal hepatic LCFA accumulation Thus, these findings suggested that CD36 acts as a major myocardial specific LCFA transporter in humans. (Mol Cell Biochem 192: 129–135, 1999)

Key words: CD36 deficiency, myocardial long-chain fatty acid uptake, mutation of CD36 gene

Address for offprints: S. Nozaki, The Second Department of Internal Medicine, Osaka University Medical School, 2-2, Yamadaoka, Suita, Osaka 565 Japan.
**Introduction**

Long-chain fatty acids (LCFA) are the major energy substrate for heart and their oxidation is important for achieving maximal cardiac work. Although LCFA is thought to be taken up via specific transporter, the myocardial LCFA uptake mechanism(s) has not yet been clarified. Abumrad et al. cloned fatty acid transporter (FAT) in rats which has high homology with CD36 in humans [1]. We also previously isolated a myocardial LCFA transporter from bovine heart [2], which showed a sequence homology to human CD36.

CD36 is a glycoprotein with a molecular weight of 88 kDa and is expressed on platelets, monocytes/macrophages, capillary endothelial cells and adipocytes [1]. It has been proposed that CD36 is a multifunctional molecule [3], including receptors for thrombospondin [4], collagen [5], oxidized LDL [6], or a membrane bound fatty acids transporter [1, 2].

A lack of CD36 expression on platelets was first identified in a thrombocytopenic patient with refractoriness to HLA-matched platelet transfusion [7]. The patient had anti-platelet antibody, Naka antibody. Tomiyama et al. revealed that Naka antigen was identical to CD36 [8]. Since then, we found several cases with CD36 deficiency and have clarified the molecular defects responsible for CD36 deficiency [9–12]. We and others also identified two types of CD36 deficiency [13, 14]. In type I CD36 deficiency, neither platelets nor monocytes express CD36; in type II CD36 deficiency, monocytes express CD36, but platelets do not. The frequency of type I CD36 deficiency is much rarer than that of type II CD36 deficiency [13]. However, proposed roles of CD36 have not been clarified, especially for LCFA uptake in human.

The clinical availability of radioisotope LCFA analogues has made it possible to investigate myocardial LCFA metabolism in patients with heart disease. Among them, Iodine-123 15-(p-iodophenyl)-(R,S)-methylpentadecanoic acid (123I-BMIPP) has the character to show normal myocardial extraction and no readily catabolism through the oxidative pathway [15]. In clinical studies, many researches have described the clinical usefulness of LCFA analog scintigraphy in ischemic heart disease [16, 17] and hypertrophic cardiomyopathy [18, 19]. Recently total lack of accumulation has been reported in very restricted cases, in whom the etiology has not yet been identified [20, 21]. We also experienced a case with CD36 deficiency [22] with hypertrophic cardiomyopathy (HCM) who showed absence of myocardial LCFA accumulation.

Based on these background, in this study we investigated the relationship between the abnormalities of CD36 molecule and myocardial LCFA uptake. CD36 expression levels and CD36 gene were analyzed in two individuals who showed no LCFA accumulation by myocardial 123I-BMIPP scintigraphy. In addition, we also studied the myocardial LCFA accumulation using 123I-BMIPP scintigraphy in two individuals who were identified as CD36 deficiency at the screening of normal volunteers and outpatient clinics. The present study shows that all these 4 individuals were type I CD36 deficiency with a complete absence of myocardial LCFA accumulation despite an apparently normal hepatic LCFA accumulation. These findings suggest that abnormalities of CD36 molecule might cause the total myocardial accumulation deficiency of 123I-BMIPP and that CD36 might be a myocardial specific LCFA transporter.

**Materials and methods**

**Subjects**

Four unrelated subjects were enrolled in this study. In two subjects (Cases 1 and 2), who were found to have absence of myocardial LCFA accumulation by 123I-BMIPP scintigraphy, the expression of CD36 on their platelets and monocytes was analyzed. The remaining two subjects were identified to be CD36 deficiency by screening. The expression of CD36 on platelets was screened in 629 normal volunteers and 285 outpatients by flow cytometric analysis. Two out of 4 type I CD36-deficient subjects found in the screening were investigated in this study. The subjects’ clinical profiles are as follows:

**Case 1:** K.S. (63-year-old male) was diagnosed as having HCM at the age of 39 years, and had been followed up at the outpatient clinic of Osaka Medical College Hospital. At the age of 61 years, his myocardial scintigram demonstrated a negative myocardial LCFA accumulation in spite of an apparently normal myocardial perfusion by thallium scintigraphy.

**Case 2:** H.Y. (56-year-old male) visited Saiseikai Nakatsu Hospital because of chest pain. Coronary angiography demonstrated 90% stenosis at the left anterior descending coronary artery and myocardial scintigraphy revealed the absence of myocardial accumulation of 123I-BMIPP.

**Case 3:** A.T. (51-year-old female) visited Osaka Red Cross Blood Center at the age of 48 years for donating blood. Flow cytometric analysis on screening revealed that both her platelets and monocytes lacked CD36 expression on the cell surface. She suffered from hypertension, hyperlipidemia and obesity, hence she was referred to the outpatient clinic of Osaka University Hospital.

**Case 4:** S.A. (63-year-old female) was admitted for the examination of exertional chest pain. Flow cytometric analysis revealed that both her platelets and monocytes lacked CD36 expression on the cell surface. Thallium (T1)-scintigraphy at rest showed no defect in myocardium although cardiac catheterization revealed 90% stenosis in the left anterior descending coronary artery and 90% stenosis in the right coronary artery.