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종 설

장쇄 수산화 아세틸코에이 탈수소효소 결핍증에 대한 고찰

계명대학교 의과대학 소아청소년과학교실

강 석 진

Very Long Chain Acyl-coenzyme A Dehydrogenase Deficiency: A Review of Pathophysiology, Clinical Manifestations, Diagnosis, and Treatment

Seokjin Kang, MD

Department of Pediatrics Keimyung University School of Medicine, Daegu, Republic of Korea

Very long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency (VLCADD) leads to a defective β-oxidation, specifically during prolonged fasting, infection, or exercise. Patients with VLCADD usually suffer from cardiomyopathy, hypoketotic hypoglycemia, hepatic dysfunction, exercise intolerance, muscle pain, and rhabdomyolysis, and sometimes succumb to sudden death. VLCADD is generally classified into three phenotypes: severe early-onset cardiac and multiorgan failure, hypoketotic hypoglycemia, and later-onset episodic myopathy. Diagnostic evaluation comprises acylcarnitine analysis, genetic analysis, and VLCAD activity assay. In the acylcarnitine analysis, the key metabolites are C14:1, C14:2, C14, and C12:1. A C14:1 level 〉1 mmol/L strongly suggests VLCADD. Various treatment recommendations are available for this condition. Dietary management includes decreasing fat content, increasing medium-chain triglyceride levels, and decreasing fasting periods. Supplementation with L-carnitine is controversial. Triheptanoin (a seven-carbon fatty acid triglyceride) treatment demonstrates improvement of cardiac functions. Bezafibrate may improve the quality of life of patients with VLCAD.

Key words: Acy-CoA dehydrogenase, Cardiomyopathies, Hypoglycemia, Bezafibrate, Tetradecanoycarnitine, Triheptanoin

Introduction

Very long-chain acyl-CoA dehydrogenase (VLCAD) (OMIM 201475, EC1.3.99.13) deficiency is an inborn error of mitochondrial β-oxidation. VLCAD is an enzyme present in the inner mitochondrial membrane and catalyzes the dehydrogenation of long-chain fatty acids with a chain length of 14 to 20 carbons, which is the first step of β-oxidation[1]. β-oxidation can occur in both mitochondria and peroxisomes, in which the acyl-CoA esters undergo dehydrogenation, hydration, another dehydrogenation, and thiolytic cleavage[2]. The enzymes involved in β-oxidation include acyl-CoA dehydrogenase, trifunctional protein, carnitine palmitoyltransferase I and II, carnitine-acylcarnitine translocase, and carnitine transporter. Electron transfer proteins and electron transferring flavoprotein dehydrogenases are also involved in this process[3]. Fatty acids are important fuels for the liver, myocardium, and skeletal muscles, specifically during fasting. Prolonged fasting, vomiting,

Corresponding: Seokjin Kang, MD, Ph.D
Department of Pediatrics Keimyung University School of Medicine, 1035, Dalgubeol-daero, Dalseo-gu, Daegu, Republic of Korea
Phone: +82-53-258-4852, Fax: +82-53-258-4875
Email address: pedjin625@gmail.com

diarrhea, infectious illnesses, or heavy physical exercise trigger the malfunction of these organs, presenting with hypoglycemia, rhabdomyolysis, cardiomyopathy, arrhythmia, or liver dysfunction, and occasionally encephalopathy or unexpected sudden death in individuals with defective enzymes of β-oxidation[4]. This review covers the pathophysiology, clinical manifestations, diagnosis, and treatment of VLCADD.

Pathophysiology

Mitochondrial fatty acid β-oxidation (FAO) is a physiological response to tissue energy depletion during fasting, increased muscular activity, and fever. The heart requires very high energy and continuous ATP production to maintain contraction[5]. FAO provides up to 80% of the energy required for heart function[6]. Thus, defect in FAO results in cardiomyopathy. Excess lipid storage in the liver due to the accumulation of unoxidized fatty acid leads to hepatomegaly and elevation of transaminases[7]. During considerably long fasting periods, the liver uses acetyl CoA to generate ketone bodies rather than glucose to maintain brain function, which is associated with hypoketotic hypoglycemia[8].

With exercise, particularly prolonged exercise (>60 min), slow skeletal muscles with a high mitochondrial density use glucose and long-chain fatty acids to generate energy. After the cessation of exercise and during the recovery period, glucose utilization is spared by converting glucose to glycogen, and lipid oxidation is elevated to meet fuel requirements[9]. In addition, VLCAD controls a critical point in supplying electrons to the respiratory chain and provides a pathway permissive to ketone production. Thereafter, a significant reduction in VLCAD activity results in impairment of maintenance and adaptation to long fasting periods, and generation of energy for exercise.

Clinical characteristics

Severe early-onset cardiac and multiorgan failure owing to VLCAD deficiency generally emerges during the starting months of life with dilated or hypertrophic cardiomyopathy, pericardial effusion, and arrhythmias, as well as hypotonia, hepatomegaly, and intermittent hypoketotic hypoglycemia [10,11] Pena et al. reported the clinical features and results of genetic analysis of 52 VLCAD patients diagnosed using newborn screening programs. Among them, three patients had abnormal results of cardiac evaluations at the initial evaluation. Abnormal findings of electrocardiogram and echocardiogram were following: atrial flutter with aberrant conduction, prolonged QT interval (QTc, 478 msec), moderate right ventricular/pulmonary arterial hypertension, and mild concentric left ventricular hypertrophy. Fatal cardiomyopathy is associated with frameshift mutations[12].

Hypoketotic hypoglycemia with hepatomegaly, hypoactivity, and hyporeflexia can occur in neonates and early childhood[12,13]. Zhang et al. reported clinical feature of seven patients with VLCAD deficiency. Five of seven patients presented recurrent hypoketotic hypoglycemia and hepatic dysfunction shortly after birth or at the age of 1 year.

The most common phenotype is the late-onset episodic myopathy, including exercise intolerance, muscle cramps, muscle pain, myoglobinuria, and rhabdomyolysis provoked by exercise[14]. An elevated creatinine kinase level is noted during 0.3-13 years. Muscle pain or muscle cramps generally occur after 1-2 h of hard exercise lasting up to 48 h. Rhabdomyolysis, usually defined as Creati-

nine kinase (CK) ≥1,000 μM, can be triggered by fasting, cold, and intensive activities, such as swimming, cycling, playing tennis, and skiing. However, acute renal failure induced by myoglobinuria is rare[12,15].

Diagnosis

VLCAD deficiency may be prevalent in individuals with suggestive findings, including cardiac abnormalities, hypoketotic hypoglycemia, elevated transaminase levels, exercise intolerance, muscle cramps, and rhabdomyolysis.

1. Acylcarnitine analysis using tandem mass spectrometry

For biochemical analysis, a confirmatory acylcarnitine test should be performed. The important metabolites are C14:1, C14:2, C14, C12:1, and their ratios[16,17]. Merinero B et al. reported that C14:1 level was between 0.36-3.01 mM/L (cut-off <0.17 mM/L) in VLCADD patients. Levels of C14:1/C2, C14:1/C16, and C14:1/C12 were 0.05-0.74 (cutoff <0.012-0.023), 2.0~11.3 (cutoff < 1.27-2.39), and 3.6-39.5 (cutoff <2.65-5.69) in those, respectively. Patients with pathogenic homozygous or compound heterozygous mutations showed a plasma C14:1 level >1 mM/L, usually[18]. However, carriers and some healthy individuals showed increased C14:1 level above the cut-off limit. If a patient has been fed or has received a glucose infusion, abnormalities in plasma acylcarnitine profiles may not be detected[1]. The plasma acylcarnitine test suffers from the drawback of not being able to predict the severity of the VLCAD deficiency phenotype. Normalized acylcarnitine levels on follow-up analysis could not exclude this disorder[19]. Therefore, further confirmatory evaluation is required for a precise diagnosis.

2. Molecular genetic analysis

Very long-chain acyl-CoA dehydrogenase is encoded by *ACADVL* located at 17p13.1, comprising 20 exons and spanning approximately 5.4 kb. The most common pathogenic allele is c.848T>C (p.Val283Ala), which was identified in symptomatic compound heterozygotes and homozygotes. It accounts for approximately 10%[1] to 29%[2] of all pathogenic alleles identified by newborn screening. Hesse et al. reported that the majority of pathogenic variants in patients with residual enzyme activity ranging 0-23% were identified through sequence analysis[20]. Miller et al. reported that array comparative genomic hybridization analysis failed to identify copy number variations within *ACADVL* in patients referred for abnormal results of newborn screening tests. In a few case reports, deletion or duplication of *ACADVL* has been identified[21]. Therefore, sequence analysis followed by gene-targeted deletion/duplication analysis are generally recommended to identify such mutations. In case of novel mutations or variants of unknown significance with no functional information, diagnosis may still be inconclusive. When genetic analysis fails to definitely diagnose the mutations, enzyme activity assay using cultured lymphocytes or fibroblasts, fatty acid oxidation flux, immunoblot analysis, and in vitro probe assays may be helpful.

3. Enzyme activity assay

Enzyme activity testing based on the oxidation of palmitoyl-CoA in leukocytes, cultured fibroblasts, liver, heart, skeletal muscle, and amniocytes by electron transfer flavoprotein or ferricenium

reduction is a well-described method[16,22]. An increased specificity is noticed when the products are separated and quantitated by high-performance liquid chromatography or tandem mass spectrometry. The clinical availability of this assay varies with time. Testing the expression of immunoreactive VLCAD protein antigen (immunoblot) uses polyclonal or specific antibodies to make a semi-quantitative assessment of the levels of expressed VLCAD antigen in protein extracts derived from cultured fibroblasts. Levels lower than 10% of the control level are consistent with VLCAD deficiency[23]. FAO flux is verified by measuring the production of radiolabeled H_2O from [9,10-3H(N)]-oleic acid using fibroblasts as a measure of β-oxidation capacity[24,25]. Diekman et al. reported that FAO flux is more strongly correlated with clinical severity score than VLCAD activity and is a useful tool for predicting the risk of developing symptoms, including cardiomyopathy, myopathy, and hypoglycemia[23].

Management

1. Initial evaluation

Screen-positive infants with arrythmia, hepatomegaly, poor feeding, hypotonia are recommended to evaluating the following: echocardiography, electrocardiogram, ammonia, lactate, glucose, liver transaminases, plasma CK and carnitine.

2. Dietary management

In symptomatic patients, the suggested fat content of the diet is 25-30% of the total energy, and the diet must be enriched in Medium Chain Triglyceride (MCT) to provide 20% of the total energy in order to bypass long chain fatty acid oxidation for energy production. The suggested maximum fasting periods are 3 h in neonates and 10 h after 12 months when in stable conditions[26]. If an infant has a moderate to severe phenotype or develops clinical symptoms, regular formula and breastfeeding should be discontinued. For infants with a moderate and a severe phenotype, recommended energy % from MCT of total energy are 10-30% and 25-45%, respectively. For individuals over one year age with a severe phenotype, recommended percent of energy from long chain fatty acid and MCT are 10% and 10-30%, respectively[27]. 1) MCT supply just before extensive exercise benefits patients with exercise intolerance. Behrend et al. reported supplementation of 0.5 g MCT per 1 kg of lean body mass significantly lowered cardiac workload by offering ketone bodies to cardiac muscle[28]. Bleeker et al. suggested individualized dietary strategies based on the FAO flux score in fibroblasts. Patients with FAO flux score <10% should receive a strict dietary treatment[29].

3. Triheptanoin

Triheptanoin is a synthetic and medium odd-chain (C7) triglyceride, which produces acetyl-CoA, propionyl-CoA, succinyl-CoA, and ketone bodies for generating ATP via gluconeogenesis. In a double-blind, randomized controlled trial, patients with long-chain fatty oxidation disorders over seven years of age were treated with triheptanoin (C7) or trioctanoin (C8) containing 20% of the total daily energy requirement for four months, and showed an increase in ejection fraction and a decrease in wall mass of the left ventricles. However, musculoskeletal symptoms were not significantly different between the two groups, suggesting that C7 treatment alone may be insuf-

ficient to prevent musculoskeletal symptoms[30].

4. L-carnitine

Carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is an amino acid derivative, which plays a key role in the transport of long-chain fatty acids into the inner mitochondrial matrix, where β-oxidation takes place[31]. In patients with VLCADD, the accumulation of long-chain acylcarnitine has been associated with muscle pathology[10]. Carnitine is also required to form acylcarnitine esters to release long-chain acyl-CoA from the mitochondria[32]. However, the effect of carnitine supplementation remains controversial. In mice, carnitine supplementation is associated with a reduction in endogenous carnitine biosynthesis and significant accumulation of acylcarnitine in the liver. In addition, carnitine supplementation does not prevent a decrease in free carnitine levels after exercise in mice[32]. Until human studies are available, carnitine administration should be performed with awareness of the potential underlying risks.

5. Bezafibrate

Bezafibrate [2-(p-(2-(p-chlorobenzamido) ethyl)-phenoxy)-2-methyl propionic acid] is an agonist of peroxisome proliferator-activated receptor and decreases the levels of human serum lipids by enhancing the transcription of several enzymes related to β-oxidation. A Japanese study included six patients with VLCADD and two with carnitine palmitoyl transferase-II deficiency (CPT-II). They were administered bezafibrate for 24 weeks (200, 300, and 600 mg/day for patients aged 3-7.5, 7.5-12, and >12 years, respectively). The frequency of myopathic attack increased in three, decreased in three, and showed no change in two patients. However, the quality of life, revealed by the 36-Item Short Form Health Survey, was significantly elevated after bezafibrate treatment, without adverse drug reactions[3]. In a French study including six adult patients with childhood-onset muscular form CPT-II deficiency, 600 mg/d bezafibrate administration for 6 months decreased the frequency of rhabdomyolysis and plasma CK levels without significant adverse effects. The level of palmitate oxidation and quality of life using revealed by the 36-Item Short Form Health Survey increased[33]. In contrast, bezafibrate treatment did not significantly improve clinical symptoms or fatty acid oxidation capacity during exercise in patients with VLCADD and CPT-II deficiency[34].

Conclusion

An increasing number of patients are diagnosed with VLCADD. Individual diet management according to age, severity of VLCAD and symptoms is of primary importance. Supplementation with L-carnitine is controversial, while triheptanoin improves cardiac functions and bezafibrate decreases frequencies of the rhabdomyolysis and improves the quality of life.

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