Legal Medicine 22 (2016) 13–17

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Short Communication

A case of sudden unexpected infant death involving a homozygotic twin with the thermolabile *CPT2* variant, accompanied by rotavirus infection and treatment with an antibiotic containing pivalic acid

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ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 20 June 2016
Accepted 24 July 2016
Available online 25 July 2016

Keywords:
Sudden unexpected infant death
Fatty acid oxidation
CPT2
Pivalic acid

ABSTRACT

We investigated a case of sudden unexpected death involving a 22-month-old male homozygotic twin infant. After both of the twins had suffered from gastroenteritis, one was found dead in his bed, but his brother survived and has since been healthy. Notably, only the deceased had been treated with an antibiotic containing pivalic acid, which may sometimes cause hypocarnitinemia. Postmortem computed tomography and medicolegal autopsy demonstrated severe liver steatosis, and subsequent genetic analysis revealed that the twin had the thermolabile variant of *carnitine palmitoyl transferase 2* (*CPT2*). On the basis of these facts, we concluded that the cause of death had been fatty acid oxidation deficiency accelerated by an antibiotic containing pivalic acid and virus infection in this infant harboring the thermolabile genetic variant of *CPT2*. Although each factor alone was not fatal, their combination appeared to have resulted in sudden unexpected infant death.

1. Introduction

A significant proportion of sudden unexpected infant death has been reported to be associated with fatty acid oxidation (FAO) deficiency [1–3]. Among the enzymes and cofactors involved in FAO, carnitine palmitoyl transferase II (CPTII) is an enzyme localized on the mitochondrial inner membrane, and converts long-chain acylcarnitines to long-chain acyl-CoAs, maintaining the normal metabolism of mitochondrial β-oxidation [4–6]. There are several pathogenic variants of *carnitine palmitoyl transferase 2* (*CPT2*) accounting for CPTII deficiency, which is described as impaired mitochondrial β-oxidation accompanied by fatty acid accumulation. Affected patients are vulnerable to energy crisis, although significant symptoms may not appear during daily living activities because there is sufficient energy production from basic glycolysis. However, hypoglycemia due to fasting or gastrointestinal infection can trigger acute energy failure, including sudden unexpected death in infants.

Among the pathogenic variants of *CPT2*, the thermolabile polymorphism p.Phe352Cys is found only in East Asians, and reduces enzyme activity during periods of high temperature [7–9]. The allelic frequency of the polymorphism is 0.21 in Japan. The polymorphism p.Val368Ile is also associated with thermal instability of the enzyme, with a frequency of 0.70 in Japan and 0.51 in southern European populations. Although these polymorphisms are assumed to be risk factors for influenza-associated encephalopathy, their association with sudden unexpected infant death has also been investigated [8–11]. Antibiotics containing pivalic acid cause carnitine depletion through excretion of pivaloyl-carnitine into urine [12]. Although several studies have reported pivalic acid-associated encephalopathy [13,14], few have investigated pivalic acid-associated FAO deficiency.

Here we investigated a case of sudden unexpected death with severe liver steatosis in a 1-year-old monozygotic twin harboring the thermolabile variant of *CPT2*. Notably, of the two twins, only the deceased had been given an antibiotic containing pivalic acid, even though both twins had shown similar symptoms of gastroenteritis due to rotavirus infection. In the case, it was considered that the genetic background and history of drug treatment might have synergistically contributed to impairment of FAO, leading to death. A combination of these factors might be considered to pose a risk of unexpected sudden death in Japanese infants.

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http://dx.doi.org/10.1016/j.legalmed.2016.07.005
1344-6223/

2. Case report

The deceased was a 22-month-old male infant, who had been born as one of homozygotic twins by cesarean section after 35 weeks of gestation. Although the birth weights were low at 2012 g and 2114 g, the brothers had not shown any particular history of illness or developmental disorders. Neonatal mass spectrometry screening of a blood sample from the deceased had not indicated FAO deficiency. Four days before death, when the decedent's brother had developed diarrhea, vomiting and a fever of 38.4 °C, both of the twins were seen at a clinic, and the deceased had shown no significant symptoms. Three days before death, both were again seen at the same clinic, because the brother's symptoms had persisted. Two days before death, only the deceased was seen at another clinic because he had developed similar symptoms such as diarrhea, vomiting and high fever. One day before death, both brothers were seen at the former clinic because the symptoms of the deceased had persisted. In all, the twins were seen at the clinics three and four times, respectively, and were treated with several different drugs for presumed viral gastroenteritis, as shown in Table 1. Notably, only the deceased was treated with cefditoren pivoxil, an antibiotic containing pivalic acid.

The deceased was found in a state of cardiac arrest in bed in the morning, whereas his brother recovered and has since been healthy. The deceased was transferred to a hospital, but resuscitation was unsuccessful. His stools showed a positive reaction for rotavirus, although no other laboratory tests were carried out. Postmortem CT scan demonstrated fatty liver (Fig. 1), but no fatal change including brain edema. To clarify the cause of the sudden unexpected death, a medicolegal autopsy was performed 10 h after death.

Table 1
A list of prescribed drugs for the deceased, his brother, or both of them.

Only the deceased	Both of monozygotic twin	Only his brother
Acetaminophen	Albumin tannate	Amoxicillin hydrate
Alimemazine tartrate	Bifidobacterium	
Ambroxol hydrochloride	Cyproheptadine hydrochloride hydrate	
Cefditoren pivoxil	Metoclopramide hydrochloride	
Diazepam	Natural aluminum silicate	
Domperidone	Tipepidine Bifidobacterium	
Fosfomycin Sodium		
L-Carbocisteine		
Lysozyme Hydrochloride		

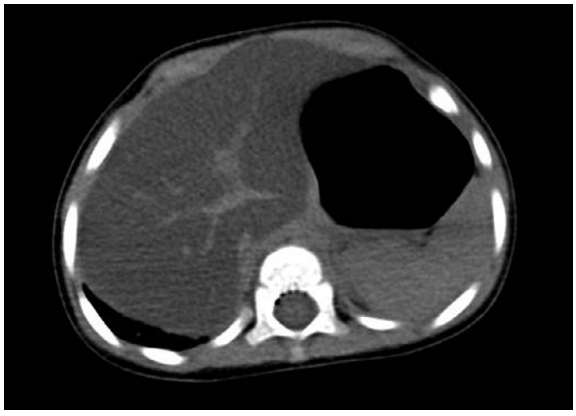

Fig. 1. Postmortem computed tomography image of the abdomen. Postmortem computed tomography was performed at the hospital to which the deceased had been transferred. The image demonstrates low absorbance in the whole liver, suggesting severe fatty liver.

3. Autopsy findings

At autopsy, the body measured 82 cm and weighed 9.7 kg. External examination did not reveal any significant changes other than traces of medical treatment and attempted resuscitation. Internal examination indicated fatty liver and congestion of organs such as the lungs, spleen and kidneys. Histologically, the liver showed diffuse and severe steatosis (Fig. 2A, B). There was no evidence of hepatitis, hepatocellular necrosis, fibrosis, or ductular reaction. Periodic acid-Schiff staining with diastase digestion showed no glycogen accumulation in hepatocytes. Macroscopic examination or Hematoxylin-Eosin staining of the heart demonstrated no morphological changes including necrosis, fibrosis or arterial sclerosis, though Oil Red O staining clarified mild fatty deposition (Fig. 2C, D). On the other hand, we were unable to find any evidence of encephalitis or brain edema. No mass spectrometry analysis for toxicological screening or acyl-carnitine analyses was performed, and no laboratory test for viral infections was conducted. A toxicological screening test using Triage (Biosite Inc., San Diego, CA) gave a negative result except for benzodiazepine. Although the test was semi-quantitative, the amount demonstrated seemed relatively low, consistent with the history of treatment. In addition, benzodiazepine poisoning has never been reported to be a cause of liver steatosis. Therefore, the final diagnosis was sudden death with severe liver steatosis, possibly caused by FAO disorder.

4. Genetic analysis

To test for potential congenital FAO disorders, we carried out genetic analyses of *acyl-CoA dehydrogenase, very long chain* (*ACADVL*), *carnitine-acylcarnitine translocase* (*CACT*) and *CPT2*, whose dysfunction is one of the main causes of congenital FAO disorders in Japan, although the precise frequency has not been clarified [15]. Genomic DNA was prepared from cadaveric blood obtained at autopsy. PCR with the DNA and direct sequencing were carried out as follows. Genomic DNA (100 ng) was mixed in a 50-μl final volume containing 1 × PrimeSTAR® GXL buffer with PrimeSTAR® GXL DNA Polymerase (TaKaRa, Shiga, Japan), 0.2 mmol/L dNTP, and 0.2 μmol/L each primer. The nucleotide sequences of the amplified fragment were determined with a BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with specific primers for the targets. The sequencing run was performed on a Genetic Analyzer (model 310, Applied Biosystems). PCR primers and conditions used for *ACADVL* were described previously [16], and those for *CACT* and *CPT2* are shown in Supplement Tables 1 and 2, respectively.

Direct sequencing of *CPT2* exons 1–5 revealed heterozygosity for c.1055T > G [p.Phe352Cys] and homozygosity for c.1102G > A [p.Val368Ile] (Fig. 3). In other words, *CPT2* consisted of the heterozygous alleles [p.Phe352Cys:p.Val368Ile] and [p.Phe352:p.Val368Ile], while the allele [p.Phe352Cys:p.Val368Ile] was a thermolabile variant of *CPT2* [8]. In contrast, sequence determination of all exons of *ACADVL* and *CACT* did not reveal any substitutions associated with FAO deficiency. Genetic analysis of a blood sample from the surviving brother gave the same results.