Bezaﬁbrate can be a new treatment option for mitochondrial fatty acid oxidation disorders: Evaluation by in vitro probe acylcarnitine assay

Seiji Yamaguchi a,⁎, Hong Li a,b, Jamiyan Purevsuren a, Kenji Yamada a, Midori Furui a, Tomoo Takahashi a, Yuichi Mushimoto a, Hironori Kobayashi a, Yuki Hasegawa a, Takeshi Taketani a, Toshiyuki Fukao c, Seiji Fukuda a

a Department of Pediatrics, Shimane University School of Medicine, Izumo, Shimane 693-8501, Japan
b Department of Pediatrics, the Afiliated Hospital of Ningxia Medical University, Yinchuan 750004, China
c Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, 501-1194, Japan

A R T I C L E   I N F O

Article history:
Received 29 May 2012
Received in revised form 5 July 2012
Accepted 5 July 2012
Available online 14 July 2012

Keywords:
Mitochondrial fatty acid oxidation disorder
Bezaﬁbrate
New treatment
Hypolipidemic drug
In vitro probe acylcarnitine assay
Peroxisome proliferation activator receptor

A B S T R A C T

Background: The number of patients with mitochondrial fatty acid oxidation (FAO) disorders is recently becoming larger with the spread of newborn mass screening. Despite the advances in metabolic and molecular characterization of FAO disorders, the therapeutic studies are still limited. It was reported recently that bezafibrate (BEZ), an agonist of peroxisome proliferating activator receptor (PPAR), can restore FAO activity in cells from carnitine palmitoyltransferase-2 (CPT2) and very-long-chain acyl-CoA dehydrogenase (VLCAD) deﬁciencies as well as clinical symptoms in the adult patients.

Methods: In this study, the therapeutic effect of BEZ was determined by in vitro probe acylcarnitine (IVP) assay using cultured ﬁbroblasts and tandem mass spectrometry on various FAO disorders. The clinical trial of BEZ treatment for a boy with the intermediate form of glutaric acidemia type 2 (GA2) was also performed.

Results: The effect of BEZ was proven in cells from various FAO disorders including GA2, deficiencies of VLCAD, medium-chain acyl-CoA dehydrogenase, CPT2, carnitine acylcarnitine translocase and triﬁunctional protein, by the IVP assay. The aberrantly elevated long- or medium-chain acylcarnitines that are characteristic for each FAO disorder were clearly corrected by the presence of BEZ (0.4 mmol/L) in culture medium. Moreover, daily administration of BEZ in a 2-year-old boy with GA2 dramatically improved his motor and cognitive skills, accompanied by sustained reduction of C4, C8, C10 and C12 acylcarnitines in blood, and normalized the urinary organic acid proﬁle. No major adverse effects have been observed.

Conclusion: These results indicate that BEZ could be a new treatment option for FAO disorders.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Mitochondrial β-oxidation (FAO) is an essential energy producing pathway, particularly during the reduced energy supply from carbohydrate due to prolonged starvation or low caloric intake during infection, diarrhea or febrile illness. A number of FAO disorders have been recognized with the spread of tandem mass spectrometry (MS/MS) in the field of study of inborn metabolic disease as well as neonatal mass screening [1,2]. Many of them show episodic attacks like lethargy, acute encephalopathy or even sudden death due to energy production insufﬁciency.

It is considered that the FAO system consists of the following four groups: 1) carnitine cycle, which activates long-chain fatty acids for undergoing β-oxidation, including carnitine transporter (OCTN2), carnitine palmitoyltransferase-1 or -2 (CPT1 or CPT2, respectively, EC 2.3.1.21, or carnitine acylcarnitine translocase (CACT, EC 2.3.1.21); 2) long-chain FAO, whose enzymes are connected to the mitochondrial inner membrane, including very-long-chain acyl-CoA dehydrogenase (VLCAD, EC 1.3.99.13) deficiency, and triﬁunctional protein (TPP, EC 1.1.1.211 and EC 2.3.1.16); 3) medium-chain FAO, whose enzymes are located in the mitochondrial matrix, including medium- and short-chain acyl-CoA dehydrogenases (MCAD, EC 1.3.99.3 and SCAD, EC 1.3.8.1) respectively, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, or medium- and short-chain 3-ketothiolase (MCKAT and SCKAT, respectively); and 4) electron transfer system, from the dehydrogenases to respiratory chain, including electron transferring flavoprotein (ETF, EC 1.5.8.2) and ETF dehydrogenase (ETFDH, EC 1.5.5.1) [3–5].

Clinical features of FAO disorders can be roughly divided into the following three types: 1) severe form (neonatal form): patients present life-threatening illness with profound hypoglycemia, liver failure or hyperammonemia, and are often fatal in early infancy; 2) intermediate...
form (juvenile form); patients have intermittent episodic attacks like lethargy, encephalopathy, or even sudden death often onset in infancy or young childhood; 3) mild form (myopathic form); the patients may often show late onset after school ages or adulthood with episodes of hypotonia, myalgia, lethargy, myopathy-like symptoms, or liver dysfunction [6].

In vitro probe acylcarnitine profiling (IVP) assay was developed to evaluate FAO disorders recently [7,8]. Acylcarnitine (AC) profiles in the special culture medium as below after incubating with fatty acids as substrates are determined by MS/MS. Bezafibrate (BEZ) is a hypolipidemic drug, which is an agonist of peroxisome proliferating activator receptor (PPAR), and is claimed to act for induction of several FAO enzymes [9–11].

In this study, the effect of BEZ on various FAO disorders was evaluated using the IVP assay. Furthermore, we report an in vivo trial of BEZ on a boy with the intermediate form of GA2, presenting dramatic improvement with BEZ.

2. Materials and methods

2.1. Subjects and skin fibroblasts

Fibroblasts from 10 Japanese children with FAO disorders, one each of severe and intermediate forms of GA2, 2 each of severe and myopathic (mild) forms of VLCAD deficiency, one each of deficiencies of MCAD, CPT2, CACT, and TFP as well as 6 controls (healthy volunteers, passages 3 to 16) were used. The clinical types and genotypes are shown in Table 1. The child with MCAD deficiency was detected in a newborn mass screening and non-symptomatic, while one with the intermediate form of CPT2 deficiency had liver dysfunction in infancy. The child with the intermediate form of CACT deficiency had two life-threatening episodes in infancy, and after that no episodes were noted with normal development [12]. The child with TFP deficiency had an episode of liver failure in infancy, and then intermittent episodes of myalgia or hypotonia particularly following infection.

The clinical types and genotypes are shown in Table 1. In all cases, at least one allele has missense mutation, although the other alleles had missense or truncated mutations. In CACT deficiency (case 9), a missense mutation in an initiation codon (c.3G>A) in SLC25A29 was detected, but this could harbor a residual activity (Fukao et al., unpublished data).

2.2. In vitro probe assay with BEZ

Fibroblasts were cultured in 75 cm² flasks (Iwaki, Tokyo, Japan) containing modified Eagle’s minimal essential medium (MEM; Nissui, Tokyo, Japan) supplemented with 2 mmol/L of l-glutamine (Nacalai Tesque, Kyoto, Japan), 10% FBS (Sigma, St Louis, MO, USA) and 1% penicillin/streptomycin (Sigma) at 37 °C in a humidified 5% CO₂/95% air incubator [13].

Fibroblasts harvested by trypsinization were seeded onto 6-well microplates (35 mm i.d., Iwaki, Japan) with the fresh above medium (2 mL/per well) until they reached confluence. Thereafter, the cells were washed twice with Dulbecco’s phosphate buffered saline (DPBS; Invitrogen, Carlsbad, CA, USA) and cultured for 96 h in 1 mL of experimental substrate (experimental medium). The experimental medium is MEM containing bovine serum albumin (0.4% essential fatty acid-free BSA; Sigma), l-carnitine (0.4 mmol/L; Sigma), unlabeled palmitic acid (0.2 mmol/L; Nacalai Tesque) and 1% penicillin/streptomycin without l-glutamine, in the presence or absence of BEZ (0.4 mmol/L; Sigma). AC profiles in the culture medium were analyzed after 96 h. The experiments for each case were performed in triplicate.

2.3. Quantitative acylcarnitine analysis

ACs in culture medium supernatants were analyzed using MS/MS (API 3000; Applied Biosystems, Foster City, CA, USA) as described previously [13]. Briefly, methanol (200 μL) including an isotopically-labeled internal standard (Cambridge Isotope Laboratories, Kit NSK-A/B, Cambridge, UK) was added to 10 μL of the supernatant from culture medium. The portions were placed on ice for 30 min, and centrifuged at 1000 × g for 10 min. Then, 150 μL of the supernatant was dried under a nitrogen stream, and butyl-derivatized with 50 μL of 3N n-butanol-HCl at 65 °C for 15 min. The dried butylated sample was dissolved in 100 μL of 80% acetonitrile:water (4:1 v/v). The ACs in 10 μL of the resultant aliquots were analyzed using MS/MS and quantified using ChemoView™ software (Applied Biosystems/MDS SCIEX, Toronto, Canada).

Protein concentrations were measured by a modification of the Bradford method using the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA), according to the manufacturer’s instruction. The AC concentrations are expressed as nmol/mg protein.

2.4. Organic acid analysis using GC/MS

Urinary organic acids were analyzed according to the previous method [14]. Briefly, 40 μg of tropate (IS-2) and 20 μg each of heptadecanoate (IS-1) and tetracosane (C24) as internal standards were added to a urine specimen containing 0.2 mg creatinine. The samples were oxime-derivatized, and solvent extracted with ethylacetate, and trimethylsilylated (TMS-derivatization). The resultant aliquots were subjected to GC/MS (Shimadzu GC/MS QP2010 Plus, Kyoto, Japan), with a DB-5 column of 0.25 mm ID × 30 m, 1 μm film thickness (J&W, Folsom, CA). The temperature program was from 100 °C to 290 °C at a rate of 4 °C/min.

Table 1 Clinical types and genotypes of patients with mitochondrial fatty acid oxidation disorders investigated.

<table>
<thead>
<tr>
<th>Disease &amp; case No.</th>
<th>Phenotype</th>
<th>Gene</th>
<th>Genotype, nucleotides (amino acids)</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA2</td>
<td>Severe</td>
<td>ETFB</td>
<td>c.799G&gt;A (G267R)</td>
<td>c.7C&gt;T</td>
<td>(R3X)</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>ETFDH</td>
<td>c.1217G&gt;A (S406N)</td>
<td>c.1675C&gt;T</td>
<td>(R559X)</td>
</tr>
<tr>
<td>VLCAD deficiency</td>
<td>Severe</td>
<td>ACADV</td>
<td>c.553G&gt;A (G185S)</td>
<td>IVS9+1g&gt;c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>ACADV</td>
<td>c.454G&gt;A (G152S)</td>
<td>c.997insT</td>
<td>(A333fsX338)</td>
</tr>
<tr>
<td></td>
<td>Myopathic</td>
<td>ACADV</td>
<td>c.900A&gt;G (K264E)</td>
<td>c.997insT</td>
<td>(A333fsX338)</td>
</tr>
<tr>
<td></td>
<td>Myopathic</td>
<td>ACADV</td>
<td>c.1144A&gt;C (I382Q)</td>
<td>c.1339G&gt;A</td>
<td>(G447R)</td>
</tr>
<tr>
<td>MCAD deficiency</td>
<td>Non-symptomatic</td>
<td>ACADM</td>
<td>c.134A&gt;G (Q45R)</td>
<td>c.448delCTGA</td>
<td>(T150fsX153)</td>
</tr>
<tr>
<td>CPT2 deficiency</td>
<td>Intermediate</td>
<td>CPT2</td>
<td>c.151A&gt;G (R51G)</td>
<td>c.520G&gt;A</td>
<td>(E174K)</td>
</tr>
<tr>
<td>CACT deficiency</td>
<td>Intermediate</td>
<td>SLC25A29</td>
<td>c.3G&gt;A (M11)</td>
<td>IVS4+1g&gt;t</td>
<td></td>
</tr>
<tr>
<td>TFP deficiency</td>
<td>Intermediate</td>
<td>HADHB</td>
<td>c.739C&gt;T (R247C)</td>
<td>c.817delG</td>
<td>(D273X292)</td>
</tr>
</tbody>
</table>

Abbreviations: MCAD, medium-chain acyl-CoA dehydrogenase; GA2, glutaric acidemia type 2; VLCAD, very-long-chain acyl-CoA dehydrogenase; CPT2, carnitine palmitoyltransferase-2; TFP, mitochondrial trifunctional protein; CACT, carnitine acylcarnitine translocase. Case 2 (C) is a boy with GA2 who underwent the clinical trial of BEZ. Non-symptomatic case 7 (H) was detected in the newborn mass screening. Severe, Intermediate, and myopathic forms are mentioned in the text. (B) to (K) correspond to those of Fig. 1.

S. Yamaguchi et al. / Molecular Genetics and Metabolism 107 (2012) 87–91
2.5. BEZ trial on a child with the intermittent form of GA2

A Japanese boy with GA2 was detected in the newborn mass screening using MS/MS, and had no special symptoms in infancy with therapies of special formula and carnitine (approximately 100 mg/kg/day, div. 3). After 1 year of age, however, he sometimes experienced episodes of hypotonia or lethargy following infection, and muscle weakness, often falling. At the age 2 years and 1 month, he was hospitalized for 2 and a half months, because of infection and lethargy, receiving treatments including artificial respiration to repeated aspiration pneumonia and unconsciousness in intensive care unit (ICU). At discharge, he could not walk alone, and could speak only a few words. So, his family consulted us, and strongly expressed a desire for any new therapies that might help their son.

Thereafter, under the approval by the ethical committee of Shimane University, we started a clinical trial of BEZ, continuing the dietary and carnitine therapies as before, since 2 years and 9 months of his age. His body weight ranged from 12 to 14 kg during the treatment, and 200 to 300 mg/day (approximately 17 to 25 mg/kg/day, div. 3) of BEZ was used in the trial. BEZ was purchased from Kissei Co Ltd, Tokyo, Japan. The study had no potential conflicts of interest (COI) to the authors.

3. Results

3.1. Effects of BEZ on FAO disorders by IVP assay

The AC profiles in the culture medium of fibroblasts from various FAO disorders in the presence and absence of bezafibrate are illustrated in Fig. 1. In control cells, C2 (acetylcarnitine) is the only prominent peak, and many of ACs further decreased in the presence of BEZ (Fig. 1A).

In the severe form of GA2 (Fig. 1B/S), C16 was apparently decreased, and C2 increased in the presence of BEZ, while C16 was extremely high before BEZ addition. The increase of C2 may indicate the acceleration of FAO, namely an increase of acetyl-CoA production. In the intermediate form of GA2 (Fig. 1C/I), all elevated ACs clearly decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.

In 2 cases of the severe form of VLCAD deficiency (Figs. 1D/S1, and 1E/S2), elevation of C14 and C16 was larger, compared with that in 2 cases of the mild form (Figs. 1F/M1, and 1G/M2). The elevated ACs decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.

In 2 cases of the severe form of VLCAD deficiency (Figs. 1D/S1, and 1E/S2), elevation of C14 and C16 was larger, compared with that in 2 cases of the mild form (Figs. 1F/M1, and 1G/M2). The elevated ACs decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.

In 2 cases of the severe form of VLCAD deficiency (Figs. 1D/S1, and 1E/S2), elevation of C14 and C16 was larger, compared with that in 2 cases of the mild form (Figs. 1F/M1, and 1G/M2). The elevated ACs decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.

In 2 cases of the severe form of VLCAD deficiency (Figs. 1D/S1, and 1E/S2), elevation of C14 and C16 was larger, compared with that in 2 cases of the mild form (Figs. 1F/M1, and 1G/M2). The elevated ACs decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.

In 2 cases of the severe form of VLCAD deficiency (Figs. 1D/S1, and 1E/S2), elevation of C14 and C16 was larger, compared with that in 2 cases of the mild form (Figs. 1F/M1, and 1G/M2). The elevated ACs decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.

In 2 cases of the severe form of VLCAD deficiency (Figs. 1D/S1, and 1E/S2), elevation of C14 and C16 was larger, compared with that in 2 cases of the mild form (Figs. 1F/M1, and 1G/M2). The elevated ACs decreased and normalized in the presence of BEZ, although broad ranges of ACs from C4 to C16 were extremely high before adding BEZ. This patient is the case 3 in Table 1, who underwent the clinical trial of BEZ treatment as illustrated in Fig. 2.
such as C10, C12, C14, or C16 in both the severe and mild forms apparently decreased in the presence of BEZ.

In MCAD deficiency (Fig. 1H), the AC peaks of C4 to C10 were significant, but in the presence of BEZ, these AC peaks were almost normalized. In cases of CPT2 deficiency (Fig. 1I), CACT deficiency (Fig. 1J) and TFP deficiency (Fig. 1K), the extremely high AC peaks of C16 and/or C12 apparently decreased to an almost normal level, in the presence of BEZ.

3.2. Clinical trial of BEZ to a GA2 patient

Since the start of BEZ treatment, his motor and social development, and languages remarkably improved, and no metabolic episodes were noted. He became able to walk alone, showed improved muscle strength, and could speak markedly more words in a few weeks. Furthermore, several months later, he could ride a kid’s tricycle by himself, although his intellectual ability was on the borderline for entrance into a kindergarten. For at least 1 year of the administration, no adverse effects of BEZ such as hypolipidemia or rhabdomyolysis have been observed.

The routine laboratory data such as blood AST, ALT, LDH or CK were in normal or subnormal ranges as shown in Table 2, showing stable levels of each test, although these laboratory data had sometimes fluctuated, in particular, when his condition was unstable before the initiation of BEZ. For example, during the stay in the ICU at the age of 2 years, the maximum levels of AST, ALT, LDH or CK were 1450 IU/L, 825 IU/L, 5200 IU/L, or 10,750 IU/L, respectively. The maximum level of blood ammonia at the ICU was 126 μg/dL, while no significant elevation was observed after that. Hypoglycemic attacks have not been noted.

BEZ is a hypolipidemic drug, and we have paid attention to the blood level of Cholesterol (TChol), because of the potential adverse effects. The dose of BEZ was 100 mg/day for the first 3 days, 200 mg/day for 4 days, and 300 mg/day for 2 months, respectively, as shown in Fig. 2A. At 2 months after starting BEZ of 300 mg/day, TChol level was a bit low, 117 mg/dL. Since then the dose has been lowered to 200 or 250 mg/day, and the TChol level has ranged between around 130 to 150 mg/mL, as shown in Table 2.

The changes in the AC levels of C4, C8, C10, and C12 are illustrated in Figs. 2B and C, respectively. All the increased ACs returned to approximately normal levels with the administration of BEZ after several months. In particular, C4 decreased to the normal range within a few weeks. Urinary organic acid analysis showed remarkable increases of ethylmalonate, methylsuccinate, adipate, 2-hydroxyglutarate, hexanoylglycine, suberate, and suberylglycine, before the BEZ treatment as shown in Fig. 3. The abnormalities in urinary organic acids were markedly corrected as early as 2 weeks after the initiation of BEZ therapy. The profile was almost normal but for a slight increase of ethylmalonate, and/or hexanoylglycine as illustrated in Fig. 3B.

4. Discussion

The treatments for FAO disorders have generally been described as follows: 1) avoiding a “long fasting”: it prevents the increased requirement of fuel from FAO; 2) early infusion of glucose: it should be performed during the metabolic stress resulting from infection, diarrhea or overexercise, to prevent hypercatabolism; 3) carnitine therapy: it may be effective in many cases, although controversy

---

Table 2

<table>
<thead>
<tr>
<th>(Unit)</th>
<th>Before</th>
<th>After the start of BEZ treatment</th>
<th>Reference value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1w</td>
<td>2m</td>
<td>6m</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>47</td>
<td>35</td>
<td>44</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>27</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>448</td>
<td>426</td>
<td>392</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>496</td>
<td>185</td>
<td>187</td>
</tr>
<tr>
<td>TChol (mg/dL)</td>
<td>161</td>
<td>127</td>
<td>117</td>
</tr>
</tbody>
</table>

*: used in Shimane University Hospital. Abbreviations: AST, aspartate amino transferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; CK, creatine kinase; and TChol, total cholesterol.

---

Fig. 3. Urinary organic acid profiles before and after bezafibrate administration. A, The total ion chromatogram (GC/MS) of urinary organic acids just before the start of BEZ; B, One year after the treatment; C, Normal control. Abbreviations: IS-2, IS-1 and C24 are tropate, heptadecanoate, and tetracosane, respectively, as internal standards; EM, ethylmalonate; SA, succinate; MS, methylsuccinate; Ad, adipate; 2HG, 2-hydroxyglutarate; 2KG, 2-ketoglutarate; HG, hexanoylglycine; SB, suberate; CA, citrate; SG, suberylglycine. Metabolites judged as abnormal are shown in bold letters underlined.
remains in some cases; and 4) dietary therapy, including high carbohydrate/low lipid diet: Dietary restriction in FAO disorders may be less strict [15–18]. In this study, we demonstrated the effect of BEZ on various FAO disorders at both in vitro and in vivo levels. It was indicated by the IVP assay that FAO capacity was corrected by BEZ in various FAO disorders, and a clinical trial of BEZ in a boy with the intermediate form of GA2 showed a favorable consequence. Bastin, Djordui and their colleagues reported the potential effect of BEZ for FAO disorders showing the increase of enzyme activity and mRNA production in several FAO enzymes from normal individuals, or reduced ACs in cells from VLCAD deficiency by the IVP assay using stable isotope-labeled palmitate [19]. Furthermore, they are performing a clinical trial on adult cases of mild form of CPT2 deficiency [20,21]. We should continue to pay attention to potential adverse effects of BEZ, including hypolipidemia or rhabdomyolysis, although such signs have never seen up to now.

We used the IVP assay to investigate the effect of BEZ in the other FAO disorders including GA2, deficiencies of MCAD, CACT, and TFP as well as CPT2 or VLCAD deficiencies. The beneficial effect of BEZ was clearly demonstrated in all these cases tested in this study, which included the clinically intermediate or severe forms as well as the mild form, having missense mutation of at least one allele. However, it is not yet clear whether the effect of BEZ is due to induction of mutant enzyme itself, or due to stimulation of the other FAO enzymes. If the effect is due to the latter mechanism, BEZ could potentially induce a “high pressure” on the FAO pathway, even resulting in devastating outcomes. We should further investigate the effect on the other severe forms of FAO disorders, the relation with the genotypes, or the dose dependency.

BEZ is an agonist of PPAR, which facilitates transcription of genes encoding FAO enzymes, and subsequently induces FAO enzyme production. Eventually, it can be considered to correct the FAO capacity in FAO disorders. Recently, it was reported that resveratrol which is a natural polyphenol and an activator of Sirtuin 1, is also expected to be a novel treatment option for FAO disorders [22]. The effect of resveratrol on FAO capacity can also be evaluated by the IVP assay like this study.

In conclusion, BEZ could be a new promising treatment option for FAO disorders. Many of patients with FAO disorders, particularly children with the milder form or adult cases, are intellectually normal, and their life prognosis is favorable if they can be prevented from severe episodes like encephalopathy. Symptoms or severity of FAO disorders are very heterogeneous depending on the disease, genetic background or lifestyle. Additional clinical studies of BEZ treatment will be essential for confirmation of its safety and practical utility.

Acknowledgments

The authors thank Dr. M. Ito, Kagawa Children’s Hospital, Japan, for kindly providing clinical data before the BEZ treatment of this patient, Dr. T. Hashimoto, professor emeritus of Shinshu University, for helpful comments on our study, and also thank MS, M. Hattori, Y. Ito, E. Mizuno, N. Tomita and T. Esumi, for their technical assistance. Finally, the authors thank Dr. Paul Langman, Iwate University, Japan for his kind assistance with English usage. This study was supported by grants from the Ministry of Science, Culture, and Sports (S.Y. and J.P.), and from the Ministry of Health, Labour and Welfare (S.Y.), of Japan. The authors had no potential conflicts of interest (COI) associated with this work. This study was approved by the ethics committee of Shimane University.

References
