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Leucine Loading Test is Only Discriminative for 3-Methylglutaconic Aciduria Due to AUH Defect

Saskia B. Wortmann • Leo A.J. Kluijtmans • Silvia Sequeira • Ron A. Wevers • Eva Morava

Abstract

Currently, six inborn errors of metabolism with 3-methylglutaconic aciduria as discriminative feature are known. The “Primary 3-methylglutaconic aciduria,” 3-methylglutaconyl-CoA hydratase deficiency or AUH defect, is a disorder of leucine catabolism. For all other subtypes, also denoted “Secondary 3-methylglutaconic acidurias” (TAZ defect or Barth syndrome, SERAC1 defect or MEGDEL syndrome, OPA3 defect or Costeff syndrome, DNAJC19 defect or DCMA syndrome, TMEM70 defect, “not otherwise specified (NOS) 3-MGA-uria”), the origin of 3-methylglutaconic aciduria remains enigmatic but is hypothesized to be independent from leucine catabolism. Here we show the results of leucine loading test in 21 patients with different inborn errors of metabolism who present with 3-methylglutaconic aciduria. After leucine loading urinary 3-methylglutaconic acid levels increased only in the patients with an AUH defect. This strongly supports the hypothesis that 3-methylglutaconic aciduria is independent from leucine breakdown in other inborn errors of metabolism with 3-methylglutaconic aciduria and also provides a simple test to discriminate between primary and secondary 3-methylglutaconic aciduria in regular patient care.

Introduction

Currently, six inborn errors of metabolism with 3-methylglutaconic aciduria (3-MGA-uria) as a discriminative feature are known (3-MGA-uria-IEM; for classification and nomenclature, see (Wortmann et al.2013a). The “Primary 3-MGA-uria” 3-methylglutaconyl-CoA hydratase (3-MGH) deficiency or AUH defect is a disorder of leucine catabolism. For all other subtypes the origin of 3-MGA-uria has not been discovered but is hypothesized to be independent from leucine catabolism (for review (Wortmann et al.2012)). These three groups of “Secondary 3-methylglutaconic aciduria” encompass disorders of defective phospholipid remodeling (TAZ defect or Barth syndrome, SERAC1 defect, or MEGDEL syndrome) and mitochondrial membrane associated disorders (OPA3 defect or Costeff syndrome, DNAJC19 defect or DCMA syndrome, TMEM70 defect). The remaining patients with significant and consistent 3-MGA-uria in whom the abovementioned syndromes have been excluded are referred to as “not otherwise specified (NOS) 3-MGA-uria” until elucidation of the underlying pathomechanism enables proper classification.

One can discriminate AUH defect from the other 3-MGA-uria-IEMs based on (1) the level of 3-MGA increase which is higher than in all other subtypes, (2) the elevated urinary 3-hydroxyisovaleric acid (3-HIVA), and (3) the 2:1 ratio for cis:trans isoforms of 3-MGA (compared to 1:1 in all other types; (Engelke et al. 2006; Wortmann et al. 2009).

Here we test the hypothesis, that, as opposed to the observation in patients with the AUH defect, other types of 3-MGA-uria-IEM are independent of leucine catabolism by
presenting leucine loading test in 21 patients with different types of 3-MGA-uria-IEM.

**Material and Methods**

**Patients**

Twenty-one patients with different 3-MGA-uria-IEM (three AUH defect, nine TAZ-defect, one OPA3-defect, one SERAC1-defect, seven NOS-3-MGA-uria) were included in this study. In seven patients leucine loading tests were performed at our center, the other patients were reported in literature (Barth et al. 1999; Christodoulou et al. 1994; Duran et al. 1982; Ensenauer et al. 2000; Haan et al. 1987; Kelley et al. 1991; Ruesch et al. 1996).

**Leucine Loading Tests**

The tests of the patients P12–P18 were performed as part of the individual diagnostic work-up, not as a scientific study. Oral informed consent was obtained from all patients. In our center 100 mg/kg (max. 6 g) leucine powder dissolved in vanilla pudding or lemonade was given orally to all patients. Prior to the loading test, and 1 h after the leucine gift, a urine portion was collected for urinary organic acid analysis. Besides, a venous blood sample was drawn to determine ammonia, lactate and glucose, blood serum amino acids, and blood gas analysis 1 h after leucine loading. In children who were very scared no venous blood sample was taken, only bedside glucose measurements were performed (“finger prick”). After the loading, also a 24 h urine sample was also collected for another urinary organic acid analysis.

**Urinary Organic Acid Analysis**

Briefly, a urine sample, in volume equivalent to 4 μmol of creatinine, is acidified to pH 2, after which the organic acids are extracted by ethylacetate twice, derivatized with trimethylsilyl (TMS), and analyzed on an Agilent 7890A gas chromatograph (GC), coupled to a flame ionization detector (FID) and an Agilent 5975C inert XL MSD mass spectrometer. Quantification of organic acids was done by peak area. The concentration of 3-MGA was estimated by calculating the peak area of 3-MGA, which was compared to the calibration curve obtained with a 3-MGA standard. For this purpose the sum of both isoforms (cis–trans) was taken.

**Leucine Loading Tests in Patients Described in the Literature**

These were performed essentially as described above. Fasting hours before the challenge and time points for sampling after leucine loading slightly differed between studies. Loading was performed with 100 mg/kg leucine in nine patients, one patient received 580 mg, one 1,200 mg leucine; two patients received 4 g/kg protein (comparable with 200 mg/kg leucine); one patient received 100 mg/kg leucine followed by 4 g/kg protein. Leucine, isoleucine, or protein was administered orally in 12 and intravenously in three patients (for details see Table 1).

**Results**

The results are summarized in Table 1. Serum ammonia and lactate levels, blood gas analysis, and glucose levels 1 h after leucine loading in our patients were within normal limits (data not shown). Serum amino acid analysis showed elevated leucine levels proving the intake of leucine powder (see Table 1). The patients felt well during and after the test, no one showed signs or symptoms suggesting hypoglycemia, no one reported side effects. To improve comparability of results between different centers all 3-MGA-uria levels are provided in mmol/mol creatinine (in our laboratory reference <10) and the lowest and highest 3-MGA-uria levels before and after leucine loading per patient are presented, as well as the ratio between these levels.

In AUH defect 3-MGA-uria levels were generally higher than in all other 3-MGA-uria-IEM (range before loading 400–761 mmol/mol creatinine, range after loading 1,600–2,982 mmol/mol creatinine). The mean ratio after/before loading was 4.1 (N = 3, range 3.9–4.5).

In TAZ-defect 3-MGA-uria levels ranged between 36 and 202 mmol/mol creatinine. The mean ratio after/before leucine loading was 1.8 (N = 9, range 1.1–5.3). In OPA3-defect levels ranged between 26 and 29 mmol/mol creatinine. The ratio after/before leucine loading was 0.9 (N = 1). In SERAC1-defect levels ranged between 60 and 75 mmol/mol creatinine. The ratio after/before leucine loading was 1.25 (N = 1). In NOS-3-MGA-uria levels ranged between 21 and 410 mmol/mol creatinine. The mean ratio after/before leucine loading was 1.0 (N = 7, range 0.5–1.3).

**Discussion**

The pathomechanism leading to 3-MGA-uria in non-AUH-related defect (“Secondary 3-MGA-uria”) remains unclear. Based upon the finding of elevated serum 3-MGA in a subgroup of patients with Smith–Lemli–Opitz syndrome a link with cholesterol biosynthesis has been hypothesized (Kelley and Kratz 1995). Two possible shuntways are known, the Popjak shunt linking dimethylallyl-PPi in the cholesterol biosynthesis with 3-methylcrotonyl-CoA in the
<table>
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<th>Mutation Loading</th>
<th>Serum glucose level 1 h after loading (mmol/L, N 4–6 mmol/L)</th>
<th>Serum leucine after loading (mmol/L)</th>
<th>Elevated urinary 3-HIVA</th>
<th>Lowest urinary 3-MGA before loading</th>
<th>Peak urinary 3-MGA after loading</th>
<th>Ratio high/low</th>
<th>Ratio cis:trans isoforms</th>
<th>Mean ratio (range, N patients)</th>
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</thead>
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<tr>
<td><strong>P1</strong> (P1 Duran et al. (1982))</td>
<td><strong>AUH</strong></td>
<td>Protein 4 g/kg p.o.</td>
<td>NA</td>
<td>819&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>518</td>
<td>2,328</td>
<td>4,5</td>
</tr>
<tr>
<td><strong>P2</strong> (P2 Duran et al. (1982))</td>
<td><strong>AUH</strong></td>
<td>Protein 4 g/kg p.o.</td>
<td>NA</td>
<td>875&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>761</td>
<td>2,982</td>
<td>3,9</td>
</tr>
<tr>
<td><strong>P3</strong> Ensennauer et al. (2000)</td>
<td><strong>AUH</strong></td>
<td>100 mg/kg leucine followed by 4 g/kg protein</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
<td>400</td>
<td>1,600</td>
<td>4,0</td>
</tr>
<tr>
<td><strong>P4</strong> (P4 aged 6 years Kelley et al. (1991))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>NA</td>
<td>Three to fourfold elevated</td>
<td>–</td>
<td>61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,2</td>
</tr>
<tr>
<td><strong>P5</strong> (P5 aged 7 years Kelley et al. (1991))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,1</td>
</tr>
<tr>
<td><strong>P6</strong> (P6 Barth et al. (1999))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg i.v.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>159&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,3</td>
</tr>
<tr>
<td><strong>P7</strong> (P7 Barth et al. (1999))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg i.v.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>202&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5,3</td>
</tr>
<tr>
<td><strong>P8</strong> (P8 Barth et al. (1999))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg i.v.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>150&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,4</td>
</tr>
<tr>
<td><strong>P9</strong> (P9 Barth et al. (1999))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>123&lt;sup&gt;b&lt;/sup&gt;</td>
<td>192&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,6</td>
</tr>
<tr>
<td><strong>P10</strong> (JD Christodoulou et al. (1994))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>NA</td>
<td>211 (N 71–175)</td>
<td>–</td>
<td>100</td>
<td>110</td>
<td>1,1</td>
</tr>
<tr>
<td><strong>P11</strong> (ST Christodoulou et al. (1994))</td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>NA</td>
<td>NA</td>
<td>–</td>
<td>130</td>
<td>140</td>
<td>1,1</td>
</tr>
<tr>
<td><strong>P12</strong></td>
<td><strong>TAZ</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>4.3</td>
<td>NA</td>
<td>–</td>
<td>32</td>
<td>40</td>
<td>1,3</td>
</tr>
<tr>
<td><strong>P13</strong></td>
<td><strong>OPA3</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>5.2</td>
<td>NA</td>
<td>–</td>
<td>29</td>
<td>26</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>P14</strong></td>
<td><strong>SERAC1</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>4.5</td>
<td>536 (N 22–276)</td>
<td>–</td>
<td>60</td>
<td>75</td>
<td>1,3</td>
</tr>
<tr>
<td><strong>P15</strong></td>
<td><strong>NOS</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>5.8</td>
<td>425 (N 22–276)</td>
<td>–</td>
<td>21</td>
<td>23</td>
<td>1,1</td>
</tr>
<tr>
<td><strong>P16</strong></td>
<td><strong>NOS</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>5.5</td>
<td>310 (N 22–276)</td>
<td>–</td>
<td>52</td>
<td>53</td>
<td>1,0</td>
</tr>
<tr>
<td><strong>P17</strong></td>
<td><strong>NOS</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>4.9</td>
<td>NA</td>
<td>–</td>
<td>187</td>
<td>234</td>
<td>1,3</td>
</tr>
<tr>
<td><strong>P18</strong></td>
<td><strong>NOS</strong></td>
<td>Leucine 100 mg/kg p.o.</td>
<td>5.3</td>
<td>NA</td>
<td>–</td>
<td>187</td>
<td>234</td>
<td>1,3</td>
</tr>
</tbody>
</table>