It has been speculated that there are two different antibodies of the IgG class with different specificities. The spleen of M.K. requires corticosteroid therapy again decreased although corticosteroid therapy was continued. It may be associated with other therapeutic regimes or undetected primary infections. The duration of observation in hospital was too short to obtain definitive evidence.

In conclusion we describe two children with severe autoimmune neutropenia and thrombocytopenia. We were able to detect relevant autoantibodies with the unlabelled immunoperoxidase method. Corticosteroid therapy raised the platelet and neutrophil counts paralleled by diminution of antineutrophil and platelet antibodies and by clinical improvement.

References


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Activity of renal 25-hydroxyvitamin D3-1α-hydroxylase in a case of X-linked hypophosphataemic rickets


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Abstract. In 1974, a 2-year-old boy was diagnosed as having X-linked hypophosphataemic rickets (XLH) because of severe rickets and hypophosphataemia.

The vitamin D metabolite concentrations, blood and urine chemistry and renal 25-hydroxyvitamin D3 (25OHD3)-1α-hydroxylase were measured in 1982 (about 2 weeks after withdrawal of medication). 1α-hydroxylase was 392 pg/mg tissue/20 min in the patient, which was high compared with aged-matched controls. (69.7 ± 28.5 pg/mg tissue/20 min, mean ± SD, n = 7). Our present studies showed that the 1α-hydroxylase activity in the patient with XLH was elevated. Therefore, the normal or low 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) concentrations in XLH patients could be due to accelerated catabolism of 1,25-(OH)2D3 or abnormally regulated 25OHD3-1α-hydroxylase in response to hypophosphataemia, although significantly elevated above that in normal controls.

Key words: Hypophosphataemic rickets - 1,25(OH)2D – 1α-hydroxylase

Introduction

X-linked hypophosphataemic rickets (XLH) is characterized by reduced renal tubular reabsorption of phosphate (P), low plasma P and rickets in growing children. Recent studies have documented normal [2] or low [5, 7] plasma levels of 1,25-dihydroxyvitamin D (1,25-(OH)2D) in patients with hypophosphataemic rickets despite low levels of plasma phosphate that would be expected to increase the production of 1,25-(OH)2D. This finding suggests that an abnormality of vitamin D metabolism may contribute to the development of this skeletal disorder. Recently, Lyles and Drezner reported that the production of 1,25-(OH)2D after PTH stimulation is defective in patients with XLH [4]. Moreover, they demonstrated that the regulation of 25-hydroxyvitamin

Abbreviations:

XLH = X-linked hypophosphataemic rickets; CPBA = Competitive protein binding assay; AI-P = alkaline phosphatase; P = phosphate
The intra-assay coefficient of variation was 19.1 ± 9.0ng/ml (mean ± SD, range 8.0–36.1ng/ml). Serum 1,25-(OH)2D was measured by CPBA as described previously [9], using chicken embryonal duodenal cytosol (Yamasa 1,25-(OH)2D3 receptor; Yamasa Shoyu Company, Japan) as the binding protein [11]. The renal 1α-hydroxylase activity in the XLH mouse [3]. On the other hand, our previous study showed an accelerated catabolism of administered 1,25-(OH)2D3 in the XLH mouse [12]. Recently, we have developed a method for the measurement of renal 25OHD3-1α-hydroxylase (1α-hydroxylase) in small human biopsy specimens [15]. With this method, a study of the renal 1α-hydroxylase activity in XLH was possible for the first time.

Methods

All studies were performed on fasting blood and urine samples. Serum calcium (Ca), phosphate (P), alkaline phosphatase (ALP) and creatinine, as well as urine Ca, P and creatinine were measured by standard autoanalyzer techniques. Serum 25OHD was measured by a competitive protein binding assay (CPBA) which has been reported previously [13]. With this method the mean normal value of 25OHD in children (1–15 years, n = 62) was 19.1 ± 9.0ng/ml (mean ± SD, range 8.0–36.1ng/ml). Serum 1,25-(OH)2D3 was measured by CPBA as previously reported [9], using chicken embryonal duodenal cytosol (Yamasa 1,25-(OH)2D3 receptor; Yamasa Shoyu Company, Japan) as the binding protein [11]. The intra-assay coefficient of variation was 7.1%, the inter-assay coefficient of variation was 14.8%, and the sensitivity was 7.1%, the inter-assay coefficient of variation was 14.8%, and the sensitivity was 7.1%, the inter-assay coefficient of variation was 14.8%, and the sensitivity was 7.1%.

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The renal 1α-hydroxylase activity in the patient and in the controls was measured as follows. A 2.5% homogenate of kidney tissue was prepared in a Tris-acetate buffer (15mM Tris-acetate, pH 7.4, 0.19M sucrose, 2mM EGTA, 2mM DTT). Then 200μl homogenate and 100 μl sodium succinate solution (0.75mM sodium succinate in Tris-acetate buffer) were pipetted into each of two test tubes. The test tubes were placed in a water bath and shaken at 37°C. The reaction was terminated by the addition of 1.5ml ethanol to the other. The reaction was initiated by the addition of 5μg 25OHD3 per mg tissue to one test tube and 95% ethanol to the other. The reaction was terminated by the addition of 1.5ml methylene chloride and then labelled 1,25-(OH)2D3 (10,000 dpm) was added to the assay tubes to monitor the recovery of 1,25-(OH)2D3.

The methylene chloride phase was applied to high pressure liquid chromatography (HPLC; Waters Associates, Milford, MA) in a solvent system of hexane-2-propanol (9:1, vol/vol), and the fraction containing 1,25-(OH)2D3 was collected. Duplicate samples were assayed for 1,25-(OH)2D by CPBA as described previously [9]. The 1,25-(OH)2D detected in the tube incubated without substrate was subtracted from the value detected in the sample incubated with substrate.

Controls

Large-scale screening of school children by urinalysis has been carried out annually in Japan to detect renal disease. Seven children having persistent haematuria and proteinuria were subjected to renal biopsy for diagnostic purposes in August, 1982. Blood samples were obtained in the fasting state for serum analysis and renal function tests. Their statures and body weights were within normal ranges. As shown in Table 1, their normal creatinine and blood urea nitrogen values suggested no impairment of renal function. Histological study by light microscopy showed the tubules where 1α-hydroxylase should be localised to be normal in all cases. The numbers of glomeruli and distribution of proximal tubules in the specimens in the low power field were similar to each other, indicating that the specimens from the seven individuals were representative portions of the cortical tissue where 1α-hydroxylase is localised. The parents of all children studied had given their informed consent. The renal 1α-hydroxylase activities of the seven biopsied patients were between 25.1pg/mg tissue/20min and 117.0pg/mg tissue/20min (69.7±28.5pg/mg tissue/20min, mean ± SD), representing reference values for renal 1α-hydroxylase in children, aged 7–12 years.

Table 1. Clinical findings in patients with asymptomatic proteinuria and/or haematuria

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Serum analysis</th>
<th>Urinalysis</th>
<th>Histological findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Creatinine (mg/dl)</td>
<td>Urea-N (mg/dl)</td>
<td>RBC/HPF</td>
</tr>
<tr>
<td>1.</td>
<td>Y.I.</td>
<td>M</td>
<td>0.7</td>
<td>10</td>
<td>10–20</td>
</tr>
<tr>
<td>2.</td>
<td>C.S.</td>
<td>F</td>
<td>0.8</td>
<td>17</td>
<td>20–30</td>
</tr>
<tr>
<td>3.</td>
<td>A.K.</td>
<td>F</td>
<td>0.6</td>
<td>8</td>
<td>2–3</td>
</tr>
<tr>
<td>4.</td>
<td>K.M.</td>
<td>M</td>
<td>0.8</td>
<td>17</td>
<td>30–40</td>
</tr>
<tr>
<td>5.</td>
<td>M.N.</td>
<td>M</td>
<td>0.8</td>
<td>14</td>
<td>20–30</td>
</tr>
<tr>
<td>6.</td>
<td>J.Y.</td>
<td>F</td>
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<td>16</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7.</td>
<td>K.T.</td>
<td>F</td>
<td>0.8</td>
<td>13</td>
<td>7–10</td>
</tr>
</tbody>
</table>

DPGN: Diffuse Mesangio proliferative Glomerulonephritis
MGA: Minor Glomerular Abnormalities
MN: Membranous Nephropathy