Long-Term Follow-Up of a Girl with the Neonatal Form of Bartter’s Syndrome

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Abstract. We followed up a girl with the neonatal form of Bartter’s syndrome for sixteen years and determined the sensitivity to angiotensin II before and during the indomethacin treatment. A 4-month-old girl was admitted to our hospital, because of severe hypokalemia and growth retardation. Initially we treated her with spironolactone and potassium supplements. This treatment increased plasma potassium levels and her growth. At the age of one year she was diagnosed as having Bartter’s syndrome. Since then she has been treated with indomethacin at an initial dose of 3 mg/kg/day combined with spironolactone and potassium. After the start of the indomethacin treatment, her growth increased dramatically, and her final height was normal adult height. Her puberty developed normally and menarche occurred at the age of 12 years. Levels of serum sodium, chloride, plasma aldosterone and urinary prostaglandin E2 were also normalized. Levels of angiotensin I and II were improved but not within the normal range, but plasma potassium levels slightly decreased after plasma aldosterone levels were normalized and did not change during the treatment period. Plasma renin activity remained high until about the age of 8 years, after which it decreased to almost within the normal range. At 5 months after the start of indomethacin (3 mg/kg/day), her vascular sensitivity to angiotensin II had been improved, and after 2 years and 5 months, her vascular sensitivity was further improved. At this time renin activity had decreased after angiotensin II infusion, but plasma aldosterone did not change. At the age of 16 years (dose of indomethacin: 0.5 mg/kg/day), plasma aldosterone increased after angiotensin II infusion. These data suggest that indomethacin and spironolactone are effective treatments for the neonatal form of Bartter’s syndrome, especially during childhood.

Key words: Bartter’s syndrome, Indomethacin treatment, Angiotensin II, Long-term follow-up

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BARTTER’S syndrome is a rare renal tubular disorder characterized by normal blood pressure, decreased vascular sensitivity to angiotensin II, hyperaldosteronism, hypokalemia, hyperreninemia, hyperplasia of the juxtaglomerular apparatus of the kidney and increased renal synthesis of prostaglandins [1, 2]. Recently it has been clarified that Bartter’s syndrome is caused by mutations of Na-K-2Cl cotransporter gene (NKCC2) [3]. In Bartter’s syndrome the insensitivity to exogenous angiotensin II is thought to result from increased synthesis of vasodilator substance prostaglandin E by blood vessels, and the hypokalemia has been ascribed to excessive potassium wasting due to hyperaldosteronism. Prostaglandin synthethase inhibitors or antagonists of aldosterone have therefore been used in treating Bartter’s syndrome, and the treatment with prostaglandin synthetase inhibitor has been shown to correct most of the features of Bartter’s syndrome [4-6], but the long-term (over ten years) outcome of the treatment has not been fully evaluated.
In this paper we report the long-term clinical course of a girl with the neonatal form of Bartter's syndrome, treated with prostaglandin synthetase inhibitor (indomethacin) combined with spironolactone and potassium supplements and the influence of such a treatment on endocrinological status and growth.

**Subjects and Methods**

**Case report**

A 4-month-old girl was admitted to our hospital for growth retardation. She was born at 42 weeks of gestation from unrelated healthy parents. At admission, her weight was 4020 g (−3SD from the normal mean), and her length was 56.3 cm (−2.5SD). Her blood pressure was 90/62 mmHg. The levels of her plasma potassium, magnesium, bicarbonate, renin activity (PRA), aldosterone (PA), and angiotensin II were as follows (normal infant range in parenthesis): 2.7 mmol/L (3.4–4.8), 1.03 mmol/L (0.70–0.95), 35.5 mmol/L (24–28), 1.67 ng·L⁻¹·s⁻¹ (0.14–3.44), 2857 pmol/L (125–1110) and 622 pg/ml (<60). Urinary calcium was not determined.

Treatment with potassium supplement and spironolactone was started. At the age of one year we examined her vascular sensitivity to angiotensin II and she showed marked blood pressure resistance. She was diagnosed as having Bartter's syndrome, although renal biopsy could not be performed because of her parents' refusal. Since then indomethacin has been added to the previous treatment.

**Methods**

The vascular sensitivity to angiotensin II was determined according to the method of Kaplan and Silah [7] at the age of one year and 5 months, 3 years and 5 months, and 16 years. The responses of PA and PRA to exogenous angiotensin II were examined at the age of 3 years and 5 months and 16 years.

Chloride reabsorption was determined according to the method of Gill and Bartter [8] at the age of 16 years.

PRA was measured by RIA of angiotensin I generated in vitro (INCStar, Stillwater, MN). Aldosterone was measured with a commercial RIA kit (Diagnostic Products). Plasma electrolytes were measured with ion-specific electrodes (Corning 902, Corning, NY). Urinary prostaglandin E₂ was measured by enzyme immunoassay at the age of one year (before indomethacin treatment), and by prostaglandin E₂ assay (Amersham International plc, Bucks, UK) at the age of 16 years. Angiotensin I and II were measured after extraction by adsorption onto and elution from florisil in each RIA by using respective antibodies with sufficient specificities, as described elsewhere [9, 10].

**Results**

**Brief summary of treatment**

Treatment with potassium supplements (15 mEq/day) was started at 4 months of age. Spironolactone (20 mg/day) was added at 5 months. The dose of spironolactone was increased gradually. At 6 months, potassium supplements were stopped, because plasma potassium levels increased beyond 4.0 mmol/L. At one year, spironolactone was changed to indomethacin combined with potassium supplements. At 7 years, spironolactone was added again, because hypokalemia was not improved. The dose of spironolactone was gradually increased to 100 mg/day, but even with the high dose of spironolactone hypokalemia could not be improved. The dose of indomethacin was gradually increased to 25 mg/day at the age of 5 years and the dose has not been changed during the clinical course.

Her growth curve is shown in Fig. 1. With spironolactone and potassium treatment, there was catch-up growth. After indomethacin treatment, further catch-up growth was detected. Her weight increased from 5700 g to 6500 g in one week after the start of the indomethacin treatment. Her final height was reached at the age of 15 years (within the normal adult range). Menarche occurred at 12 years.

The levels of plasma sodium and chloride were increased promptly by spironolactone, and the levels of sodium and chloride were almost normal soon after the start of the indomethacin treatment. The levels of plasma potassium were slightly
decreased after the PA levels were normalized and the low levels of plasma potassium were not improved by the high dose of spironolactone (100 mg/day) and the increased dose of potassium, but they were not worsened by the low dose of indomethacin (0.5 mg/kg/day). Laboratory data and clinical symptoms were not changed by the dose of potassium. PA levels have been within the normal range throughout the treatment course. High PRA high continued until the patient was about 8 years old (Fig. 2). PRA decreased thereafter as her blood pressure slightly increased (data not shown).

Laboratory data before, 2 years and 5 months, and 15 years after the start of indomethacin treatment are shown in Table 1. The levels of plasma angiotensin I, II and urinary prostaglandin E₂ decreased after indomethacin treatment.

Chloride reabsorption was poor even after 15-years of treatment with indomethacin (Table 1).

During her clinical course, serum BUN and creatinine levels were slightly increased but within the normal range, and she showed no other adverse effects due to indomethacin or spironolactone.

**Angiotensin II infusion test**

Before indomethacin treatment, an angiotensin II infusion test revealed vascular insensitivity (more than 150 ng/kg/min for a 20 mmHg increase in diastolic blood pressure). At 5 months after the start of indomethacin treatment, her vascular sensitivity had been improved (60 ng/kg/min), and after 2 years and 5 months and after 15 years further improved (40 ng/kg/min and 25 ng/kg/min, respectively) (Fig. 3). At 2 years and 5 months after the start of indomethacin treatment, PRA to exogenous angiotensin II was from 10.72 to 3.22 ng.L⁻¹.s⁻¹, but PA had not responded. After 15 years, PA was
Fig. 2. Plasma potassium (K), sodium (Na), chloride (Cl), plasma renin activity (PRA) and plasma aldosterone (PA) levels during the clinical course. Shaded area shows normal range.

Table 1. Laboratory data during the treatment period

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>normal values</th>
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<tr>
<td><strong>Plasma</strong></td>
<td></td>
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</tr>
<tr>
<td>HCO₃⁻ (mEq/l)</td>
<td>35.5</td>
<td>30.7</td>
<td>26.5</td>
<td>24–28</td>
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<td>Angiotensin I</td>
<td>3875</td>
<td>541</td>
<td>536</td>
<td>&lt;200</td>
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<tr>
<td>Angiotensin II</td>
<td>860</td>
<td>85</td>
<td>151</td>
<td>&lt;60</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
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<td>0.82</td>
<td>0.7</td>
<td>0.7–0.95</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>140</td>
<td>137</td>
<td>137</td>
<td>135–147</td>
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<td>K (mmol/L)</td>
<td>3.8</td>
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<td>3.2</td>
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<tr>
<td>Cl (mmol/L)</td>
<td>98</td>
<td>99</td>
<td>96</td>
<td>95–105</td>
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<tr>
<td>PRA (ng·L⁻¹·s⁻¹)</td>
<td>10.2</td>
<td>10.7</td>
<td>5.7</td>
<td>0.14–3.44 in childhood</td>
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<td></td>
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<td></td>
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<td>0.3–1.1 in adult</td>
</tr>
<tr>
<td>pH</td>
<td>7.49</td>
<td>7.43</td>
<td>7.423</td>
<td>7.35–7.45</td>
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<td><strong>Urinary</strong></td>
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<tr>
<td>Prostaglandin E₂ (µg/day)</td>
<td>50¹⁺ 25²⁺</td>
<td>ND</td>
<td>453</td>
<td>&lt;700</td>
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<tr>
<td>Distal fractional chloride reabsorption (%)</td>
<td>ND</td>
<td>ND</td>
<td>66.4</td>
<td>&gt;80*</td>
</tr>
</tbody>
</table>

1: before indomethacin treatment, at the age of one year. The treatment of potassium supplement and spironolactone has already been started. 2: 2 years and 5 months after indomethacin treatment. 3: 15 years after indomethacin treatment. *from data suggested by Gill et al. [7]. §normal range in this age <20 ng/day in our laboratory. ²measured after 2 weeks of the start of indomethacin treatment. ND: not done.
LONG-TERM FOLLOW-UP OF BARTTER'S SYNDROME

Discussion

In patients with Bartter's syndrome, growth is usually impaired in infancy and childhood, and laboratory data at angiotensin II infusion tests are shown in Table 1.

Fig. 3. Vascular sensitivity to angiotensin II infusion before and during the treatment. •—•, before indomethacin treatment; ○—○, 5 months after indomethacin treatment; ○—○, 2 years and 5 months after indomethacin treatment; ▲—▲, 15 years after indomethacin treatment.

Fig. 4. The responses of plasma renin activity (PRA) and plasma aldosterone (PA) to angiotensin II during the indomethacin treatment. ○—○, after 2 years and 5 months of indomethacin treatment. ▲—▲, after 15 years of indomethacin treatment.

increased from 1100 to 3745 pmol/L (Fig. 4). Laboratory data at angiotensin II infusion tests are shown in Table 1.
failure to thrive is often the presenting symptom. It is well known that indomethacin or spironolactone therapy improves the growth of patients with Bartter's syndrome [11–13]. Our patient also showed signs of growth acceleration following the treatment with spironolactone and indomethacin. Indomethacin in particular had a powerful effect on growth during infancy. This growth acceleration was possibly induced by the sodium-retaining effect of indomethacin, because plasma sodium levels were also increased at this time.

The plasma potassium concentration increased after spironolactone treatment. The PA concentration decreased with age and was within the normal range after spironolactone was changed to indomethacin. Hypokalemia was not worsened by stopping spironolactone treatment.

Saruta et al. [14] reported a direct effect of prostaglandins on the biogenesis of aldosterone. Our patient showed normal urinary prostaglandin levels after indomethacin treatment. Indomethacin, prostaglandin synthetase inhibitor, may therefore have had an effect on hypokalemia by inhibiting the action of prostaglandins on the biogenesis of aldosterone.

Spironolactone and indomethacin were therefore effective on hypokalemia but the effects were not enough.

The hypokalemia in Bartter's syndrome has been ascribed to excessive potassium wasting due to hyperaldosteronism. In our patient, however, hypokalemia persisted even when the aldosterone excretion was suppressed to normal levels. This suggests that the cause of hypokalemia of Bartter's syndrome is heterogeneous.

The levels of urinary prostaglandins and PA were not increased and hypokalemia was not worsened, although the dose of indomethacin per unit of body weight decreased with age (at the age of 16 years, indomethacin 0.5 mg/kg/day; usual used dose, 2–3 mg/kg/day [15–16]. In our case, the low dose of indomethacin may be enough for the treatment of Bartter's syndrome, because she shows normal growth and electrolytes except for potassium.

In our patient, PRA was refractory to indomethacin during infancy and early childhood, but decreased to the almost normal range after 8 years of age. The cause is unknown but slightly increasing blood pressure after this age may be related.

Gill et al. [8] suggested that defective chloride reabsorption may be a primary cause of Bartter's syndrome and reported that indomethacin did not improve the defect in chloride reabsorption. On the other hand, Hornych et al. [17] reported that the defect in chloride reabsorption was improved by indomethacin. Our patient showed signs of defective chloride reabsorption even after 15 years' indomethacin treatment.

Recently Simon et al. have reported that the cause of Bartter's syndrome is the mutation of Na-K-2Cl cotransporter, which is primary mediator of sodium and chloride reabsorption in the thick ascending limb of the loop of Henle [3]. Therefore, it has been clear that the defective chloride reabsorption in Bartter's syndrome is caused by the mutation of NKCC2. But the effect of indomethacin on chloride reabsorption remains to be determined including the problems of dose or duration of indomethacin treatment.

At 5 months after the start of indomethacin treatment, our patient's vascular sensitivity to exogenous angiotensin II had been improved, and further improved after 2 years and 5 months and after 15 years. PRA had also responded to angiotensin II after 2 years and 5 months, but PA did not respond at this time. The cause of this different responsiveness to angiotensin II is unknown. Previous papers [19, 20] reported that the decreased vascular sensitivity was induced by the overproduction of prostaglandins and angiotensin II, and hypokalemia induced decreased production of aldosterone by the adrenal glands. We do not know why these phenomena occur in Bartter's syndrome, and the cause of the different responsiveness to angiotensin by blood vessels and adrenal glands remains to be determined.

At 15 years after the start of indomethacin treatment, PA in addition to vascular sensitivity also responded to angiotensin, although the levels of plasma potassium were not different from those at 2 years and 5 months. This suggests that unknown factors except for potassium influence the PA response to angiotensin, although previous studies reported that potassium regulated the PA production [19, 20].

These data suggest that spironolactone and indomethacin are effective in treating the neonatal form of Bartter's syndrome, because growth, sexual
development and most biochemical parameters except for hypokalemia have remained all normal from infancy through full maturity. Furthermore, this study indicated that the cause of hypokalemia in Bartter’s syndrome was not only aldosterone but heterogeneous, and the regulators of vascular response to All are different from those of adrenal response because the sensitivity to All was inconsistent in blood vessels and adrenal glands.

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References