Reciprocal Regulation of 11β-HSDs May Predict Steroid Sensitivity in Childhood Nephrotic Syndrome

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abstract

Synthetic glucocorticoids (GCs) are used as the first-line treatment of childhood idiopathic nephrotic syndrome (NS). The initial response to steroids is a major prognostic factor in childhood NS.1 More than 90% of children with minimal change nephrotic syndrome (MCNS) achieve initial remission after an 8-week course of prednisolone on the International Study of Kidney Disease in Children protocol.2 However, ~60% of steroid-responsive patients experience frequent relapses with some becoming dependent on steroids to prevent further relapse, but requiring higher doses to do so (exhibiting relative resistance to steroids).2 The mechanisms underlying the development of steroid resistance remain unclear, and there are currently no reliable biomarkers to identify patients who will go on to develop steroid dependence and resistance. A recent report suggests that low levels of glucocorticoid receptor (GR) in peripheral blood mononuclear cells (PBMCs) are associated with GC resistance in childhood NS.3 However, the density and binding affinity of GRs, as determined by dexamethasone binding assays in PBMCs, were not different in children with steroid sensitive and resistant NS.4 Thus, whether GR in PBMCs are a useful biomarker in NS is unclear. We have previously shown that both the type 1 and type 2 isozymes of the prereceptor GC metabolizing enzyme, 11β-hydroxysteroid dehydrogenase (11β-HSD1 and 11β-HSD2, respectively), are associated with steroid sensitivity in childhood acute lymphoblastic leukemia and indeed that 11β-HSD2 may contribute to steroid resistance.5, 6 Therefore, we hypothesized that 11β-HSDs in PBMCs could be associated with steroid responsiveness in childhood NS.

CASES AND METHODS

We studied all the Japanese children with NS who were treated at Seirei Hamamatsu General Hospital between April 2013 and October 2015 (a total of 8). Peripheral blood samples were obtained with informed consent. Study protocols were approved by the ethics committee of our hospital. Diagnosis of idiopathic NS was made by pediatric nephrologists on the basis of clinical and laboratory findings. Patient...
profiles are shown in Table 1. We had 4 newly diagnosed and 4 relapsed NS. Prednisolone treatment was initiated in all patients. For newly diagnosed patients (patients 1, 3, 4, and 8; Table 1), induction therapy used 2 mg/kg/day prednisolone for 4 weeks (60 mg/m² per day) according to the International Study of Kidney Disease in Children protocol. Because we hypothesized that there would be a qualitative difference between early and late responders, we determined clinical GC-sensitivity/resistance from the initial response to prednisolone treatment at 2 weeks. This is earlier than the common definition (at 4 weeks), but in concordance with a recent report suggesting that the initial response to steroids (at 7 days) is a major prognostic factor in idiopathic NS. Therefore, patients 1, 3, and 4, who showed complete resolution of proteinuria (urine protein/creatinine ratio less than 0.2) at 2 weeks and remained negative until 4 weeks, were classified as GC-sensitive. Patient 8 was classified as GC-resistant as she failed to respond to the initial prednisolone treatment. Her histologic finding revealed focal segmental glomerulosclerosis on renal biopsy (Table 1).

Of the 4 patients with relapsing NS, 1 was classified as infrequent relapse (patient 7). The remaining 3 (patients 2, 5, and 6) were frequent relapsing nephrotic syndrome (FRNS) patients. Modified induction therapy was initiated according to our regional protocol (Nagoya City Kidney Disease Study Group). According to this protocol, if patients with FRNS are not prescribed prednisolone before relapse, to reduce steroid toxicity, 1 mg/kg dose of prednisolone (maximum dose of 30 mg/m² per day) is administered initially, which is lower than the common protocol. Patients already undergoing treatment with ≥1 mg/kg prednisolone are started on 2 mg/kg prednisolone daily. For infrequent relapse patient 7, 2 mg/kg prednisolone was administered daily (60 mg/m² per day) until the proteinuria disappeared. We classified patient 7 as GC-resistant due to the presence of proteinuria at 2 weeks, although this had disappeared at 4 weeks. FRNS patients 2, 5, and 6 were all treated with 1 mg/kg prednisolone therapy at relapse.

Blood sampling was carried out before prednisolone treatment and at 2 weeks and 4 weeks after initiating prednisolone treatment. PBMC were isolated by density gradient centrifugation (Ficoll-Paque; Pharmacia, Stockholm, Sweden). Cells were cultured for 24 hours in the presence or absence of 10–6 M dexamethasone in RPMI-1640 with 10% fetal calf serum, penicillin (100 U/mL) and streptomycin (100 μg/mL; all Invitrogen, Carlsbad, CA), at 37°C, 5% CO₂. RNA was extracted after homogenization in Trizol (Invitrogen) and resuspended in RNase-free water. RNA (1 μg) was reverse transcribed using SuperScript III (Invitrogen) and quantified by real-time polymerase chain reaction on a LightCycler (Roche) as previously described.

All experimental procedures were carried out at Hamamatsu University School of Medicine. Mastermix and primer-probe sets for HSD11B1, HSD11B2, NR3C1 (encoding GR), and 18S were purchased from Applied Biosystems (Foster City, CA). 18S RNA served as the internal control. The fold induction of HSD11B1, HSD11B2, and NR3C1 mRNA levels were calculated, relative to levels in vehicle-treated cells. Values are mean ± SEM. Data were analyzed in each group using the Wilcoxon matched pairs test. Significance was set at P < .05.

### RESULTS AND DISCUSSION

HSD11B1, HSD11B2, and NR3C1 mRNA were expressed in PBMC in all cases. Absolute levels varied between patients, so findings are expressed as fold induction after dexamethasone. HSD11B1 mRNA levels were decreased after in vitro dexamethasone treatment of PBMC collected from all patients, before or after prednisolone treatment (Fig 1A). The decrease in HSD11B1 mRNA levels after dexamethasone tended to be lower in GC-resistant than in GC-sensitive NS, although this did not achieve significance (Fig 1A). In contrast, HSD11B2 mRNA levels were unchanged by dexamethasone treatment of PBMC collected from patients with GC-sensitive NS either before or after their prednisolone

### TABLE 1 Patient Profiles

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis, y/sex</th>
<th>Disease Status at Sampling</th>
<th>Pathophysiology</th>
<th>Urine Protein/Creatinine Ratio, g/gCr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
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<tr>
<td>GC sensitive</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4/M</td>
<td>diagnosis</td>
<td>ND</td>
<td>10.73</td>
</tr>
<tr>
<td>2</td>
<td>11/M</td>
<td>relapse</td>
<td>FSGS</td>
<td>6.72</td>
</tr>
<tr>
<td>3</td>
<td>2/F</td>
<td>diagnosis</td>
<td>ND</td>
<td>13.35</td>
</tr>
<tr>
<td>4</td>
<td>6/M</td>
<td>diagnosis</td>
<td>ND</td>
<td>3.65</td>
</tr>
<tr>
<td>GC resistant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4/M</td>
<td>relapse</td>
<td>FSGS</td>
<td>2.39</td>
</tr>
<tr>
<td>6</td>
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<td>FSGS</td>
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<tr>
<td>7</td>
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<td>relapse</td>
<td>FSGS</td>
<td>17.68</td>
</tr>
<tr>
<td>8</td>
<td>3/F</td>
<td>diagnosis</td>
<td>FSGS</td>
<td>6.18</td>
</tr>
</tbody>
</table>

FSGS, focal segmental glomerulosclerosis; ND, not determined.
treatment (the fold induction was not significantly different to 1; Fig 1B). However, HSD11B2 mRNA levels were significantly increased by dexamethasone treatment of PBMC collected from patients with GC-resistant NS both before, and after, their prednisolone therapy at 2 weeks (Fig 1B). The different responses of HSD11B1 and HSD11B2 mRNAs after in vitro dexamethasone revealed a qualitative difference between GC-sensitive and resistant NS, as seen in GC-sensitive and resistant childhood lymphoblastic leukemia. The patterns of HSD11B1 and 2 remained similar during the course of prednisolone therapy, suggesting that GC-sensitivity/resistance is intrinsic in childhood NS. Levels of NR3C1 mRNA, encoding GR, were unaffected by in vitro treatment of PBMC with dexamethasone (Fig 1C).

Expression of 11β-HSD1 and 11β-HSD2 is normally reciprocally regulated. In vivo, 11β-HSD1 predominantly activates endogenous GCs (converting cortisone to cortisol), whereas 11β-HSD2 catalyses the reverse reaction, inactivating GCs. However, it should be noted that 11β-HSD2 is a high affinity though low capacity enzyme and thus high levels of GCs can escape inactivation. The 11β-HSD enzymes potentially modulate exogenous GC action as well. They interconvert prednisolone (active) and prednisone (inert), which itself does not bind to GR. In many tissues, there is a switch from 11β-HSD2 to 11β-HSD1 expression as cells differentiate and mature. The precise mechanism of this reciprocal regulation remains largely unknown, but transcriptional regulator CAAT/enhancer-binding proteins (C/EBPs) are likely involved, as we have previously shown that they regulate 11β-HSDs in a variety of cell types.

In normal PBMCs, 11β-HSD1 expression is low and 11β-HSD2, negligible. However, in certain disease conditions, such as early rheumatoid arthritis, levels of mRNA encoding 11β-HSD2 in PBMC are increased; indeed, 11β-HSD2 has been reported as a PBMC marker of early rheumatoid arthritis. Similarly, 11β-HSD2 is expressed in glucocorticoid-resistant leukemic cell lines where it contributes to prednisolone resistance. Up-regulation of HSD11B2 coupled with down-regulation of HSD11B1 may reflect a return to an earlier developmental state in GC resistant NS. Alternatively, the qualitative difference in 11β-HSD expression between steroid sensitive and resistant NS may reflect a stable state. This may arise, for example from epigenetic mechanisms which, once established, become self-perpetuating. In steroid-resistant NS, induction of 11β-HSD2 is predicted to inactivate and thus attenuate prednisolone action, whereas the opposite is predicted for 11β-HSD1. Thus, in steroid-resistant NS, higher doses of prednisolone are needed to overcome the 11β-HSD2 inactivation. Therefore, we suggest that dexamethasone should be used to treat this condition, rather than prednisolone. The current study has some limitations. We evaluated only Japanese patients with NS at our hospital, who may not be representative of childhood idiopathic NS patients in other ethnic groups. Frequent relapse patients were treated with a lower dose of prednisolone (1 mg/kg per day), according to our regional protocol, which is intended to reduce steroid toxicity. Moreover, the sample size is small, and statistical significance was not achieved for the HSD11B1 mRNA findings. Therefore, our findings are preliminary, qualitative rather than quantitative, and require confirmation in a larger number of patients. Nevertheless, they suggest that analysis of expression of these isozymes, and their regulation by GCs in NS, may aid in tailoring steroid treatment to the patient, by serving as prognostic biomarkers of disease activity.
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ABBREVIATIONS
11β-HSD: 11β-hydroxysteroid dehydrogenase
FRNS: frequent relapsing nephrotic syndrome
GC: glucocorticoid
GR: glucocorticoid receptor
NS: nephrotic syndrome
PBMC: peripheral blood mononuclear cell

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