Original Article

Steroid therapy reduces mesangial matrix accumulation in advanced IgA nephropathy

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Abstract

Background. Steroid therapy for IgA nephropathy (IgAN) has been reported to ameliorate the long-term prognosis of IgAN, but its mode of action has not been fully elucidated. In this study, we examined the effect of steroids on glomerular morphological changes in IgAN.

Methods. We examined 16 patients with biopsy-proven IgAN (male:female = 11:5, mean age 32.1 years) who were divided into prognosis groups according to criteria set by the Japanese Society of Nephrology. Initially, they received a loading dose of steroids, followed by a daily dose of 10–15 mg prednisolone. After 12 months, they underwent a second biopsy, and their histological and clinical features were examined.

Results. Before and after therapy, systolic blood pressure, diastolic blood pressure, serum creatinine and creatinine clearance all remained unchanged. However, urinary protein excretion decreased dramatically, from 1.6±1.7 to 0.4±0.2 g/day (P < 0.005). Furthermore, computerized imaging revealed a significant reduction of the mesangial matrix index (MMI) from 14.5±5.2 to 9.5±3.6% (P < 0.001). The numbers of sclerosing glomeruli did not change.

Conclusions. Steroid therapy reduces mesangial matrix accumulation and reduces urinary protein excretion in advanced IgAN.

Keywords: glomerular sclerosis; image analyser; renal biopsy

Introduction

IgA nephropathy (IgAN) was first reported by Berger and Hinglais in 1968 [1]. Its histology is characterized by diffuse and global mesangial proliferation at the glomerular level with increased cellular density. Its immunohistology is characterized by intensely fluorescent IgA deposits in all glomerular structures, mainly at the mesangial and sometimes the segmental wall level. IgAN was initially regarded as having a benign prognosis, but it has become apparent that 30–40% of patients develop end-stage renal failure after ~20 years [2,3]. In Japan, IgAN is the most common of primary glomerulonephritis, accounting for ~40–50% of the total [2,4]. Steroid therapy is reported to be a potential approach to managing IgAN, and recent reports suggest that steroids improve long-term renal prognosis [5,6]. However, the mechanism of steroid action on IgAN has not been fully elucidated. In this study, therefore, we evaluated the effect of steroids on glomerular mesangial matrix accumulation, which is considered to be a specific feature of progressive glomerular damage in IgAN [7], using a computerized imaging system to examine the glomerular histology before and 12 months after the initiation of steroid therapy.

Subjects and methods

Subjects

From April 1998 to October 1999, 72 patients underwent renal biopsies in the Third Department of Medicine, Fukushima Medical University Hospital, Fukushima, Japan. Renal biopsies were performed on patients with proteinuria (>2+ by dipstick urine test) or with both proteinuria (>1+) and haematuria or with haematuria (>1+) for >1 year without urological abnormalities. Using immuno-fluorescent and electron microscopy (EM), 31 patients were diagnosed as having IgAN. Purpural nephritis, lupus nephritis and hepatic disease were excluded based on clinical symptoms, blood chemistry and serological examinations. Of the 31 patients, 16 (male:female = 11:5; mean age 32.1 years old, range 21–51 years) were categorized as described below into poor (n = 8) or relatively poor (n = 8) prognosis groups according to light microscopic glomerular findings and criteria defined in the ‘Clinical guidelines of IgAN issued...
by the Ministry of Health and Welfare of Japan and the Japanese Society of Nephrology [8].

Poor prognosis group. Severe diffuse mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation or adhesion to Bowman’s capsule were seen in >30% of all biopsied glomeruli. When sites of sclerosis are totalled and converted to global sclerosis, the sclerosis rate is >50% of all glomeruli. Some glomeruli also show compensatory hypertrophy.

Relatively poor prognosis group. Moderate, diffuse mesangial cell proliferation and increased matrix. Glomerulosclerosis, crescent formation or adhesion to Bowman’s capsule seen in 10–30% of all biopsied glomeruli.

Methods

All subjects then received corticosteroid therapy. Initially they received a loading dose of steroids (methylprednisolone 500 mg/day for 3 days) followed by prednisolone treatment at an initial dosage of 0.6 mg/kg/day for 4 weeks, tapered by 5 mg every 4 weeks, and maintained at 10–15 mg/day. Twelve months later, they underwent a second renal biopsy. Blood pressure was defined as the mean of three consecutive measurements on different days. Systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg was regarded as hypertension according to the WHOISH criteria, and calcium antagonists were administered (n = 2). Serum creatinine concentrations were <1.5 mg/dl in all patients. The initial clinical parameters and characteristics of each patient are shown in Table 1.

Angiotensin converting enzyme inhibitors or angiotensin II receptor blockers were not administered. All patients were given dilazep dihydrochloride, an anti-platelet agent, its effect on the progression of IgAN has not been established. Medication was not changed during the study except for the tapering of prednisolone. The control parameters were derived from five biopsy samples from patients (male/female = 3/2, age 21–66 years old) with minimal change nephrotic syndrome. Informed consent was obtained from all patients. This study was approved by the Ethical Committee of Fukushima Medical University.

Analytical

Renal specimens were taken by standard needle biopsy methods, then fixed in 10% buffered formalin, embedded in paraffin and serially sectioned at a 2 μm thickness. Only those glomeruli that were visible in their entirety were studied (mean number 12 ± 5, range 8–18). The analysis was conducted on specimens stained by periodic acid-Schiff (PAS), which allowed the image analyser to discriminate between individual glomeruli and the surrounding renal parenchyma. The morphometric analysis was performed with a system composed of a microscope (BX50, Olympus, Tokyo, Japan) attached to a colour CCD camera (HC-2500, Fujix, Tokyo, Japan) and a computer (FMV 6400 TX2, Fujitsu, Tokyo, Japan), using image analysis software Winroof Ver. 3.31 (Mitani, Tokyo, Japan). Imaging analysis consisted of the following steps [9,10]: (i) capturing glomeruli on the PAS preparation at a magnification of 200×; (ii) tracing the outline of the glomeruli to obtain the total glomerular area; (iii) selecting the mesangial area manually with the mouse pointer; however, as that still included the basement membrane; and (iv) erasing the basement membrane and any ‘garbage’ on the screen. Finally, the mesangial matrix index (MMI) was calculated from the ratio of the mesangial area to the glomerular area measured as above. The mean of glomerular MMIs in each specimen was regarded as representing the magnitude of mesangial matrix accumulation in each subject. Globally sclerosed glomeruli were not included in the MMI analysis. The globally sclerosed glomeruli were counted and the sclerosing index was calculated as the number of globally sclerosed glomeruli per number of all glomeruli, which was regarded as representing the progression of glomerular sclerosis in each case.

The morphometric results before and after steroid therapy were expressed as a mean value and standard deviation, and were compared using the paired t-test. Blood and urine samples were analysed by standard laboratory methods.

Table 1. Clinical and histological characteristics of 16 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Gender (male/female)</th>
<th>Prognostic category</th>
<th>Blood pressure (mmHg)</th>
<th>Serum Cr (mg/dl)</th>
<th>Urinary protein excretion (g/day)</th>
<th>MMI (%)</th>
<th>Sclerosing index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>M</td>
<td>RP</td>
<td>132/70</td>
<td>0.9</td>
<td>0.2</td>
<td>10.0</td>
<td>30.0</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>M</td>
<td>P</td>
<td>142/80</td>
<td>1.1</td>
<td>0.1</td>
<td>13.5</td>
<td>18.2</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>M</td>
<td>RP</td>
<td>120/60</td>
<td>1.2</td>
<td>2.0</td>
<td>12.6</td>
<td>16.7</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>M</td>
<td>P</td>
<td>150/92</td>
<td>1.4</td>
<td>1.0</td>
<td>10.1</td>
<td>26.0</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>M</td>
<td>RP</td>
<td>118/68</td>
<td>1.0</td>
<td>0.6</td>
<td>14.1</td>
<td>10.0</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>M</td>
<td>RP</td>
<td>126/68</td>
<td>1.1</td>
<td>1.3</td>
<td>9.0</td>
<td>10.0</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>M</td>
<td>P</td>
<td>120/70</td>
<td>1.0</td>
<td>4.3</td>
<td>25.9</td>
<td>16.7</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>M</td>
<td>P</td>
<td>118/70</td>
<td>1.0</td>
<td>0.9</td>
<td>9.3</td>
<td>28.6</td>
</tr>
<tr>
<td>9</td>
<td>39</td>
<td>M</td>
<td>P</td>
<td>122/78</td>
<td>1.1</td>
<td>0.5</td>
<td>18.8</td>
<td>52.9</td>
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<tr>
<td>10</td>
<td>31</td>
<td>F</td>
<td>P</td>
<td>124/74</td>
<td>1.1</td>
<td>6.1</td>
<td>19.3</td>
<td>30.0</td>
</tr>
<tr>
<td>11</td>
<td>35</td>
<td>F</td>
<td>RP</td>
<td>116/68</td>
<td>0.7</td>
<td>0.8</td>
<td>9.6</td>
<td>13.3</td>
</tr>
<tr>
<td>12</td>
<td>36</td>
<td>M</td>
<td>RP</td>
<td>130/68</td>
<td>1.3</td>
<td>4.0</td>
<td>10.3</td>
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<tr>
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<td>F</td>
<td>P</td>
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<td>1.1</td>
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<td>22.6</td>
<td>30.4</td>
</tr>
<tr>
<td>14</td>
<td>34</td>
<td>M</td>
<td>P</td>
<td>124/84</td>
<td>0.9</td>
<td>0.4</td>
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<td>23.8</td>
</tr>
<tr>
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<td>23</td>
<td>F</td>
<td>RP</td>
<td>118/58</td>
<td>0.8</td>
<td>1.1</td>
<td>15.8</td>
<td>17.4</td>
</tr>
<tr>
<td>16</td>
<td>31</td>
<td>F</td>
<td>RP</td>
<td>126/74</td>
<td>1.1</td>
<td>0.7</td>
<td>11.8</td>
<td>34.3</td>
</tr>
</tbody>
</table>

RP, relatively poor prognosis group; P, poor prognosis group.
Urinary protein excretions were measured for 2 days before the renal biopsies. Data was expressed as average of each measurement. Creatinine clearance was estimated using 24-h collections of urine. The accepted level of significance was $P < 0.05$.

**Results**

**Steroid therapy significantly reduced urinary protein excretion and attenuated the progression of renal dysfunction**

Before and after steroid therapy, the systolic blood pressure (126 ± 9 vs 122 ± 12 mmHg, respectively), diastolic blood pressure (72 ± 8 vs 74 ± 7 mmHg), serum creatinine (Cr) (1.1 ± 0.2 vs 1.0 ± 0.2 mg/dl) and Cr clearance (59.3 ± 19.0 vs 70.7 ± 16.0 ml/min) remained unchanged. However, the amount of proteinuria significantly decreased from 1.6 ± 1.7 to 0.4 ± 0.2 g/day (Figure 1; $P < 0.005$).

**Steroid therapy significantly reduced mesangial matrix accumulation**

Before steroid therapy, the MMI was 14.5 ± 5.2% (range 9.0–25.9%), which significantly decreased to 9.5 ± 3.6% (range 5.0–17.5%) after the therapy (Figure 2; $P < 0.001$). In one case (patient 7), the MMI was remarkably decreased from 25.9 to 7.2% after 1 year of treatment (Figure 3). MMI in those with minimal change nephrotic syndrome ($n = 5$) was 4.5 ± 2.8% which was significantly different from those with IgAN before and after the therapy (Figure 2; $P < 0.001$). However, the sclerosing index did not change significantly, from 23.4 ± 11.2 to 19.9 ± 12.4% (Figure 4).

**Adverse effects of steroid therapy**

During steroid therapy, no major adverse effects, such as life-threatening infectious disease, gastric ulcer,
Discussion

In this study, we demonstrated that steroid therapy for IgAN in advanced stage reduced urinary protein excretion and mesangial matrix accumulation. The mechanism of this therapeutic effect of a steroid was elucidated—for the first time, using image analysis—to be its suppressive effect on mesangial matrix accumulation. Steroid therapy for IgAN has been controversial. Although steroid therapy was initially considered to be ineffective for IgAN [11], there have been studies suggesting that it may have a beneficial effect [12, 13]. Pozzi et al. [5] demonstrated that a 6-month course of steroid therapy effectively inhibited the deterioration of renal function over a follow-up period of 5 years. Tamura et al. [6] reported that steroid therapy attenuated the progression of renal dysfunction, determined by serum Cr slope in a 6-month observation period, and that the therapy also decreased urinary protein excretion significantly. These findings are compatible with our results that the serum Cr, as well as Cr clearance, was preserved during the 1-year follow-up period and that steroids reduced urinary protein excretion.

Mesangial cells synthesize various extracellular matrix proteins including collagen type I, collagen type IV, fibronectin and laminin in response to pathological signals. Matrix proteins accumulate in the mesangial area leading to the development of mesangio proliferative glomerulonephritis with an increased number of mesangial cells. The extracellular matrix is closely related to the functions of glomerular cells, and its proliferation has been recognized as one of the most prominent causative and histological features of IgAN. In this study, we demonstrated that steroid therapy significantly reduced mesangial matrix accumulation, which suggests that steroids not only preserve glomerular structure but also regress the glomerular lesion involved in the pathological process of IgAN. Although the pathophysiological mechanism of the action of corticosteroids on matrix metabolism in glomeruli is still not fully known, one possible explanation may involve the suppression of matrix production by their negative regulatory effects on the pro-inflammatory genes, including NF-κB, leading to a negative matrix balance as a result of the action of a matrix degradation system [14]. Further, the use of angiotensin converting enzyme inhibitors or angiotensin II receptor blockers, which act on progressive renal diseases independently of corticosteroids [15], may have additive effects in IgAN. As for tubulointerstitial lesions, we preliminarily observed the regressive effects of steroid on inflammatory cell infiltration and fibrosis. However, further quantitative studies are needed to prove these effects.

The number of patients with hypertension (n = 2) seems to be too small for advanced IgAN, possibly related to the limited number of subjects. Noteworthy also is that all patients in this study took dilazep dichloride. Although an uncontrolled observational study was published [16], and the clinical guideline mentioned above recommends the use of an anti-platelet agent [8], there has been little evidence for the beneficial effect of dilazep dichloride on the clinical course of IgAN. Thus, the changes observed in our study we considered to be due mainly to corticosteroids.

It has been suggested that steroid therapy for advanced IgAN, where Cr clearance is < 70 ml/min, might accelerate glomerular sclerosis and that steroid therapy is contraindicated [8]. However, this study demonstrated that steroid therapy in patients with an initial mean Cr clearance of 59 ml/min did not increase the number of globally selerosed glomeruli. This finding suggests that steroid therapy is indicated for IgAN in any stage.

In conclusion, these findings indicate that steroid therapy for IgAN in the advanced stage reduces mesangial matrix accumulation and reduces urinary protein excretion. Although these effects are clearly favourable for the kidney, long-term observation by controlled study is required to prove that steroid therapy benefits the prognosis of advanced IgAN.

Conflict of interest statement. None declared.

References

Steroid therapy on IgA nephropathy


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