Comparison of Glucocorticoids Alone and Combined with Cyclosporine A in Patients with IgA Nephropathy: A Prospective Randomized Controlled Trial

Hong Liu, Xialian Xu, Yi Fang, Jun Ji, Xiaoyan Zhang, Ming Yuan, Chunfeng Liu and Xiaoqiang Ding

Abstract

Objective The aim of this study was to investigate the effects of two different treatment regimes in patients with IgA nephropathy (IgAN): steroids alone and in combination with a medium dose of cyclosporine A (CsA).

Methods Forty-eight IgAN patients 18-69 years of age with proteinuria >1.0 g/24 hours and an estimated glomerular filtration rate (eGFR) of >30 mL/min/1.73 m$^2$ were randomly given either steroids alone (methylprednisolone (MP) group; n=25) or steroids plus CsA treatment (combination group; n=23). The primary endpoint was the reduction of proteinuria by 50% or more of the baseline value. The secondary endpoint was an increase in the baseline serum creatinine level of 50% or a decrease in the baseline eGFR of 25%.

Results After 12 months of treatment, all patients in the combination group and 87.50% of the patients in the MP group reached the primary endpoint. The complete remission rates in the combination group and MP group were 50.0% and 45.83%, respectively. The level of urinary protein excretion declined from 3.17 ± 3.25 g/24 hours to 0.36 ± 0.23 g/24 hours (p<0.001) in the combination group and from 2.60 ± 2.03 g/24 hours to 0.53 ± 0.71 g/24 hours (p<0.001) in the MP group. Two patients in the combination group reached the secondary endpoint, with a decrease in the eGFR of 25% from the baseline value, while no patients in the MP group achieved this goal. The patients in the combination group exhibited significant improvements in the eGFR after nine months (90.16 ± 28.78 vs. 80.46 ± 22.73 mL/min/1.73 m$^2$, p=0.011), while the patients in the MP group showed significant increases in the eGFR after six months of treatment (92.18 ± 22.71 to 81.63 ± 18.36 mL/min/1.73 m$^2$, p=0.019). Four patients (8.33%) developed severe pneumonia during treatment.

Conclusion Both the full dose of steroids alone and combined treatment with steroids and a medium dose of CsA remarkably reduced the levels of proteinuria and ameliorated the renal function in the IgAN patients. Infection was the most serious complication during the treatment.

Key words: IgA nephropathy, cyclosporine, methylprednisolone, proteinuria, glomerular filtration rate

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effects, such as infection, diabetic mellitus and abnormal bone metabolism. Low-dose glucocorticoid treatment can reduce proteinuria, although without protection of the renal function (6). On the other hand, whether glucocorticoid monotherapy is adequate to treat severe IgAN cases remains in dispute. Cyclosporine A (CsA) has been confirmed to effectively attenuate proteinuria caused by many types of primary glomerulonephritis (7-10). In addition, proteinuria is associated with long-term renal survival (11-14). However, currently, there are few randomized controlled trials (RCTs) of CsA treatment in patients with IgAN. In 1987, Lai et al. reported that treatment with CsA (5 mg/kg) effectively reduces proteinuria, increases serum albumin and partially lowers plasma IgA, although, in that study, the serum creatinine (Scr) level increased in association with a decline in glomerular filtration rate (GFR) in the patients with IgAN (15). Since then, a few non-controlled small sample studies have reported that CsA is effective in treating IgAN without causing significant effects on the renal function (16-18). In the present study, we prospectively compared the efficacy and safety of glucocorticoids alone with that of combined treatment with steroids and a medium dose of CsA in IgAN patients with proteinuria of >1.0 g/24 hours and an eGFR of >30 mL/min/1.73 m².

Materials and Methods

This study was a single-center, prospective, randomized, controlled trial approved by the Ethics Committee of Zhongshan Hospital, Fudan University. All participating patients provided their written, informed consent.

Patients

The inclusion criteria included: (1) biopsy-proven IgAN; (2) an age of 18 to 69 years; (3) a level of urinary protein excretion of >1.0 g/24 hours; (4) an estimated glomerular filtration rate (eGFR) of >30 mL/min/1.73 m² (Modification of Diet in Renal Disease (MDRD) equation (19)) on at least two consecutive measurements. The exclusion criteria included: IgAN with severe chronic tubulointerstitial damage or crescentic formation of ≥50% of the glomeruli; IgAN with minimal change syndrome; secondary IgAN, such as that due to lupus nephritis, Henoch-Schonlein purpura or hepatitis B virus (HBV)-associated glomerulonephritis; consecutive treatment for more than three months with corticosteroids or immunosuppressive drugs within the previous one year; diabetes mellitus; severe uncontrolled hypertension (a diastolic blood pressure of ≥120 mmHg); severe liver disease; pregnancy (a pregnancy test must be performed in fertile female patients before the administration of therapy) or lactation; and an known allergy or intolerance to the study medication.

Pathological assessment

All eligible patients underwent a renal biopsy with light microscopy, immunofluorescence and electron microscopy at the beginning or within three months before the trial. The histological grading of all renal biopsy samples was performed in accordance with the Oxford classification (20). Areas of global sclerosis, cellular crescents and vascular damage, such as vessel hyalinization or vascular wall thickening (all or none), were also counted.

Treatment regimen and evaluation

All eligible patients were assigned to the methylprednisolone alone group (MP group) or methylprednisolone plus CsA group (combination group) successively and randomly. In the MP group, methylprednisolone was administered at a dose of 0.8 mg/kg/d (the highest dose was 48 mg/d) orally for eight weeks. The dose was then reduced by 4-8 mg every two weeks until reaching a maintenance dose of 4 mg/d or 4 mg every other day. In the combination group, the 12-month course of CsA began with a dose of 3 mg/kg/day (before meals, the highest dose was 200 mg/d). The dose was reduced by 25% when the serum creatinine level increased by more than 25% of the baseline value. Twelve weeks later, the dose was gradually reduced by 50 mg every month then maintained at a maintenance dose of 25 mg/d. At the same time, the patients were given a medium dose of methylprednisolone of 0.4 mg/kg/d (the highest dose was 36 mg/d) orally for eight weeks, after which the dose was tapered by 4-8 mg every two weeks to a maintenance dose of 4 mg/d or 4 mg every other day. In both groups, losartan (50 mg/d) and dipyridamole (50 mg t.i.d.) were administered. Other immunosuppressants (except CsA), angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor antagonists (ARBs) were not allowed in any phase of the study. During the study, blood pressure was maintained under 140/90 mmHg (other antihypertensives, such as calcium antagonists, were given for poorly controlled hypertension).

After initiating the treatment protocol, the patients were followed up regularly every month for three months, then every three months thereafter. The doses of methylprednisolone and CsA were reduced or stopped if adverse events, such as severe infection or diabetes mellitus, occurred. Patients were withdrawn from the study if their symptoms did not disappear after one month.

Study endpoints

We used the rate of remission of urinary protein as the primary endpoint. The rate of remission of urinary protein included complete remission (a 24-hour urinary protein level less than 0.3 g/24 hours) and partial remission (a reduction of 24-hour proteinuria by 50% or more of the initial level and an absolute value of >0.3 g/d). The secondary endpoints included the following: an increase in the Scr level of 50% or a decrease in eGFR of 25% from the baseline values; the onset of end-stage renal disease with the need for renal replacement therapy; a relapse in proteinuria (defined as an increase in proteinuria of 50% and a value of ≥1.0 g/24 hours in patients with a complete or partial remission); and severe
Table 1. Baseline Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>MP group (n=25)</th>
<th>Combination group (n=23)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: male: female</td>
<td>12: 13</td>
<td>10: 13</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.84±8.06</td>
<td>42.39±13.10</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>24.5±3.53</td>
<td>23.07±2.97</td>
<td>NS</td>
</tr>
<tr>
<td>Course (months)</td>
<td>16.14±22.43</td>
<td>19.86±47.23</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14(56.0%)</td>
<td>11(47.83%)</td>
<td>NS</td>
</tr>
<tr>
<td>Macrohematuria</td>
<td>5(20.0%)</td>
<td>6(26.09%)</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR (mL/min.1.73m²)</td>
<td>81.63±18.36</td>
<td>78.93±23.11</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.01±0.26</td>
<td>1.02±0.28</td>
<td>NS</td>
</tr>
<tr>
<td>Serum uric acid (mg/dL)</td>
<td>4.58±1.72</td>
<td>6.08±1.45</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>36.96±6.69</td>
<td>33.57±7.86</td>
<td>NS</td>
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<tr>
<td>Serum IgA level (g/L)</td>
<td>2.61±0.91</td>
<td>2.75±0.80</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>204.5±130.16</td>
<td>161.15±60.21</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>2.60±2.03</td>
<td>3.17±3.25</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2. Baseline Histological Characteristics

<table>
<thead>
<tr>
<th></th>
<th>MP group (n=25)</th>
<th>Combination group (n=23)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glomeruli samples</td>
<td>16.1±10.40</td>
<td>19.65±10.29</td>
<td>NS</td>
</tr>
<tr>
<td>Glomerular sclerosis (%)</td>
<td>11.90±12.44</td>
<td>17.31±20.50</td>
<td>NS</td>
</tr>
<tr>
<td>No cellular crescents: n(%)</td>
<td>17(68.0)</td>
<td>11(47.82)</td>
<td>NS</td>
</tr>
<tr>
<td>Crescents : n(%)</td>
<td>8(32.0)</td>
<td>12(52.17)</td>
<td>NS</td>
</tr>
<tr>
<td>1-25% cellular crescents: n(%)</td>
<td>7(28.0)</td>
<td>11(47.83)</td>
<td>NS</td>
</tr>
<tr>
<td>25-50% cellular crescents: n(%)</td>
<td>1(4.0)</td>
<td>1(4.35)</td>
<td>NS</td>
</tr>
<tr>
<td>Oxford classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1: n (%)</td>
<td>22(88.0)</td>
<td>20(86.97)</td>
<td>NS</td>
</tr>
<tr>
<td>S1: n (%)</td>
<td>17(68.0)</td>
<td>18(78.26)</td>
<td>NS</td>
</tr>
<tr>
<td>E1: n (%)</td>
<td>7(28.0)</td>
<td>7(30.43)</td>
<td>NS</td>
</tr>
<tr>
<td>T0: n (%)</td>
<td>17(68.0)</td>
<td>13(56.52)</td>
<td>NS</td>
</tr>
<tr>
<td>T1: n (%)</td>
<td>8(32.0)</td>
<td>8(34.78)</td>
<td>NS</td>
</tr>
<tr>
<td>T2: n (%)</td>
<td>0</td>
<td>2(8.70)</td>
<td>NS</td>
</tr>
<tr>
<td>Present vascular change: n (%)</td>
<td>7(28.0)</td>
<td>8(34.78)</td>
<td>NS</td>
</tr>
</tbody>
</table>

M1: half the glomeruli have more than three cells in a mesangial area, S1: present segmental glomerulosclerosis or tuft adhesion, E1: present endocapillary hypercellularity, T: percentage of cortical area involved by the tubular atrophy or interstitial fibrosis, T0:0-25%, T1:26-50%, T2: > 50%

adverse effects.

Statistical analysis

The data are expressed as the mean ±SD. Univariate baseline and 12-month comparisons were made between the two groups using the Wilcoxon rank-sum test or chi-square test. Changes in the 24-hour urinary protein levels, the remission status of proteinuria, the renal function and the serum albumin levels over time between the two groups were compared using repeated measures and multivariate analyses of variance of the general linear model (ANOVA), with the baseline values as references. Two-tailed p values of <0.05 were considered to be significant. In all analyses, the SPSS statistical software program (17.0) was used.

Results

Patients

A total of 51 biopsy-proven IgAN patients were assessed for eligibility between January 2008 and November 2010. Of the patients, 26 were treated with methylprednisolone alone and 25 were treated with the combined therapy. Three patients were lost to follow-up (one from the MP group, two from the combination group), and two patients in the combination group discontinued CsA after three months of treatment due to severe pulmonary infections. After six months of treatment, one patient in the MP group without a response to treatment was converted to CsA (3 mg/kg/day) in combination with low-dose MP (8 mg/day). The other pa-
patients were followed for at least 12 months, with an average follow-up period of 35.64 ± 15.74 months (12-60 months) in the MP group and 36.45 ± 17.08 months in the combination group (12-58 months). The clinical and renal pathology characteristics of the patients are summarized in Tables 1 and 2.

**Serum concentrations of CsA**

The blood trough concentration (C0) and concentration at two hours post-dose (C2) were monitored during the CsA treatment. The dose of CsA was adjusted based on a target C0 level of less than 200 ng/mL and a C2 level of less than 800 ng/mL during the first 12 weeks. After two weeks of treatment, the mean C0 level among 19 patients was 84.65 ± 20.33 ng/mL (60-180 ng/mL), and the mean C2 level was 408.36 ± 146.40 ng/mL (300-660 ng/mL). After 24 weeks of treatment, the CsA concentration was measured in only five patients. The maintenance concentrations of C0 and C2 were 31.68±26.57 ng/mL and 192.79±116.51 ng/mL, respectively.

**Primary endpoints (rate of remission of urinary protein)**

By the end of the first month of treatment, 71.43% of the patients in the combination group and 68.0% of the patients in the MP group reached the primary endpoint. The complete remission rate in each group was 19.05% and 8.0%, respectively. After 12 months of treatment, all patients in the combination group and 87.50% of the patients in the MP group reached the primary endpoint. The complete remission rate in each group was 50.0% and 45.83%, respectively.

After 12 months of treatment, the proteinuria levels decreased from 2.60 ± 2.03 g/24 hours to 0.53 ± 0.71 g/24 hours in the MP group (p<0.001) and from 3.17 ± 3.25 g/24 hours to 0.36 ± 0.23 g/24 hours in the combination group (p<0.001). The 24-hour proteinuria levels decreased markedly in both groups after the first, third, sixth and ninth months of treatment and at the last follow-up (Fig. 1). There were no significant differences in the partial or complete remission rates or levels of urinary protein excretion (p>0.05) between the two groups over time.

During the therapy, parallel to the reduction in the levels of urinary protein, the levels of serum albumin were significantly higher than the baseline values in both groups. After 12 months of treatment, the mean serum albumin level was 43.32 ± 2.54 g/L in the combination group and 42.47 ± 4.28 g/L in the MP group (vs. pretreatment, p<0.001, respectively); however, there were no significant differences between the two groups (p=0.05) (Fig. 2).

**No response or relapse in proteinuria**

One patient in the MP group with sustained nephrotic syndrome after six months of treatment was transferred to the CsA group, achieved a complete remission after two months and maintained a partial remission after 24 months of follow-up. Two patients with a complete remission in the MP group experienced a relapse of proteinuria after 11 and 18 months of follow-up, respectively. Both patients restarted MP therapy (0.4 mg/kg per day) for more than two months and did not achieve a partial remission until CsA (2.5 mg/kg per day) was added.

**Secondary endpoints (kidney survival)**

During the 12 months of follow-up, there were no significant differences in the changes in the serum creatinine levels before and after treatment between the two groups; no patients exhibited an increase in the baseline serum creatinine level of 50%. Only one patient with a long history of nephritis (more than 20 years) and a baseline eGFR of 34.67 mL/min. 1.73 m² in the combination group demonstrated an increase in the serum creatinine level of 50% in the 30th month. That patient began dialysis in the 48th month after undergoing surgery for a benign breast tumor.

The patients in the combination group achieved significant improvements in eGFR after nine and 12 months of treatment (9 months, 90.16 ± 28.78 vs. 80.46 ± 22.73 mL/min. 1.73 m², p=0.011; 12 months, 96.44 ± 32.90 vs. 80.46 mL/min. 1.73 m², p<0.001). The maintenance concentration of C0 and C2 was 31.68±26.57 ng/mL and 192.79±116.51 ng/mL, respectively.
The pathogenesis of IgAN is complex and diverse, and the severity of the condition varies. Therefore, there are many kinds of therapies. Research has found that proteinuria is the strongest prognostic factor in patients with IgAN and has a “dose-dependent” effect that is independent of other risk factors for the disease. Reducing the level of proteinuria can effectively protect the renal function in patients with IgAN (11-13). It is important for IgAN patients who continue to exhibit proteinuria of >1.0 g/24 hours after receiving optimized supportive care to be treated with combined...
immunosuppressive and non-immunosuppressive therapy. However, there is controversy over the efficacy of immunosuppressants. Some low-quality evidence has shown that treatment with glucocorticoids and cyclophosphamide (CTX) or azathioprine in progressive IgAN patients is significantly better than other treatment regimens (21-25). However, issues of case enrollment, observation bias and lack of better supportive care measures in these patients make these studies difficult to interpret (26). Compared to that observed in single-steroid therapy, the risk-benefit assessment is strongly impacted by the potential for severe adverse effects in cases of combination steroid treatment. The Kidney Disease: Improving Global Outcomes (KDIGO) organization guidelines suggest not treating IgAN patients with corticosteroids combined with cyclophosphamide or azathioprine, unless the patient exhibits crescentic IgAN with a rapidly deteriorating kidney function (27). The KDIGO organization guidelines also suggest not using mycophenolate mofetil (MMF) in IgAN patients because the findings of RCTs evaluating the efficacy of MMF in treating IgAN are variable (12, 13, 28, 29). CsA has multifactorial mechanisms of antiproteinuric actions. It can inhibit the activation of T-lymphocytes and the production of IL-2, improving the permeability of the glomerular basement membrane (30, 31). On the other hand, CsA has an antiproteinuric effect independent of its immunosuppressive function in T cells, directly resulting from the stabilization of the podocyte actin cytoskeleton (32). Research has shown that the administration of CsA can reduce proteinuria in patients with many types of chronic glomerulonephritis and nephrotic syndrome, even those who are resistant to steroids (33, 34). In our prospective, randomized, controlled trial, we showed that treatment with both full-dose corticosteroids alone and medium-dose glucocorticoids combined with CsA rapidly reduces proteinuria, even by the end of the first month of treatment. CsA therapy also reduces proteinuria in patients who exhibit no response to or relapse after receiving a full dose of MP treatment. In the present study, the levels of proteinuria decreased dramatically in the patients treated with the combined therapy, while the eGFR values increased, indicating that the role of CsA in reducing proteinuria is not limited to its effects in improving glomerular hemodynamics and reducing glomerular filtration. The biggest problem associated with CsA treatment is renal toxicity, which can result in damage to the renal function by inducing renal vasoconstriction and interstitial fibrosis (34-36). Our study showed that even though we used a medium dose of 3 mg/kg/d as the initial dose of CsA, more than 25% of the patients treated with the combined therapy showed a transient drop in eGFR, although most of them quickly returned to their previous values. After 12 months of treatment, the average eGFR of the patients in the combination group was significantly increased compared to the baseline levels. Only one patient in the combination group who had a long history of glomerulonephritis and a low baseline eGFR progressed to end-stage renal disease after 48 months of follow-up. In general, it is safe and effective to treat IgAN patients with moderate to severe proteinuria using a medium dose of CsA, which not only reduces proteinuria quickly and effectively, but also improves the renal function. We believe that medium-dose CsA therapy combined with steroid treatment can be used as an effective alternative treatment strategy in IgAN patients who are unwilling to accept or not suitable for high-dose glucocorticoid therapy. In our study, one patient exhibited no response to the MP treatment, and two patients relapsed. These patients achieved urinary protein remission following CsA treatment, suggesting that the administration of combination treatment may be useful in patients who relapse or exhibit no response to MP treatment. Of course, the renal function must be monitored during treatment by measuring the Scr level and GFR. If the GFR starts to decline consistently, the dose of CsA must be reduced or discontinued. Recently, studies have reported that treating children with IgAN with 5 mg/kg/d of CsA reduces the urine protein level and increases the serum albumin level without changing the Scr level or GFR. Repeated renal biopsies in these patients showed decreased, and even disappeared, deposition of IgA in the glomeruli with a reduction of diffuse mesangial proliferation; however, renal interstitial fibrosis appeared or became aggravated in 50% of the patients associated with an increase in the level of interstitial transforming growth factor β (37). Therefore, further large-sample studies of the efficacy and safety of long-term CsA treatment are needed.

There were no significant differences in the side effects of treatment, compliance or tolerability between the two groups. Severe pneumonia was the most serious complication, occurring in both groups within two to three months of treatment. This is the time when CsA and steroids have the greatest effects in suppressing the immune function of the body and when the plasma albumin level has not yet returned to normal; therefore, patients are more vulnerable to developing complications with severe infections.

There are some limitations to our study. This was a single-center study that included a relatively small sample size. Some patients have just recently completed 12 months of follow-up; therefore, a longer follow-up period is required.

### Conclusion

Treatment with either steroids alone or in combination with medium-dose CsA remarkably reduced proteinuria, increased the serum albumin level and ameliorated the renal function in IgAN patients with a proteinuria level of >1.0 g/24 hours and an eGFR of >30 mL/min/1.73 m². Combination therapy can also be used as an effective alternative treatment in IgAN patients who are unwilling to accept or are not suitable for high-dose glucocorticoid treatment. This therapy is also useful in patients who relapse or exhibit no response to MP treatment. It is necessary to monitor the renal function carefully during treatment. Future large-sample,
multicenter, long-term follow-up studies are needed to confirm our findings.

The authors state that they have no Conflict of Interest (COI).

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References

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