Original Article

Efficacy of methylprednisolone and urokinase pulse therapy combined with or without cyclophosphamide in severe Henoch-Schoenlein nephritis: a clinical and histopathological study

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Abstract

Background. There have been few controlled studies of combined therapy with multiple drugs, including immunosuppressives, for severe Henoch-Schoenlein nephritis (HSPN). We evaluated the efficacy of methylprednisolone and urokinase pulse therapy combined with cyclophosphamide for patients with HSPN of at least grade IVb.

Methods. We studied 37 patients who had been diagnosed with HSPN of at least grade IVb. Of them, 20 (Group A) were treated with methylprednisolone and urokinase pulse therapy, and 17 (Group B) were treated with methylprednisolone and urokinase pulse therapy combined with cyclophosphamide. We analysed the clinical features, laboratory and pathological findings of the two groups retrospectively.

Results. After 6 months of treatment, mean urinary protein excretion in Group B had significantly decreased compared with Group A, and the activity index of both groups at the second biopsy was lower than that at the first. Furthermore, at the second biopsy, the chronicity index of Group B was lower than that of Group A. Four patients of Group A but none of Group B had persistent nephropathy ($P$ < 0.05).

Conclusions. Our study suggests that methylprednisolone and urokinase pulse therapy combined with cyclophosphamide is useful for patients with severe HSPN.

Keywords: cyclophosphamide; HSPN; methylprednisolone pulse therapy; urokinase pulse therapy

Introduction

Henoch-Schoenlein purpura (HSP) is an immunoglobulin (Ig) A-mediated immune-complex vasculitis that affects predominantly the skin, joints, gastrointestinal tract and kidneys. It occurs most frequently in childhood, and its prognosis is mainly predicted by the severity of renal involvement.

The proportion of patients reported to have renal involvement varies between 20 and 80%. The majority of children with Henoch-Schoenlein nephritis (HSPN) present only with haematuria and/or low-grade proteinuria, or both, and have a good chance to recover. However, patients with massive proteinuria at onset frequently have a progressive course [1,2]. In specialized centres, the proportion of children with HSPN who progress to renal failure or end-stage renal disease varies from 12 to 19% [3–6].

As for the treatment of severe HSPN, there are a few reports dealing with the use of multiple combined agents, including immunosuppressive drugs [7–10]. However, those studies were not controlled studies. We have recently reported that methylprednisolone and urokinase pulse therapy (MUT) was effective in HSPN patients of least type IIIb, though some of them nevertheless progressed and had increased sclerotic lesions [11].

In this study, we evaluate the efficacy of cyclophosphamide in severe HSPN, comparing clinical and histological outcomes in patients treated with methylprednisolone, warfarin, dipyridamole and urokinase pulse therapy (MUT) between 1988 and 1994 and with
Efficacy of methylprednisolone and urokinase pulse therapy

MUT plus cyclophosphamide (MUTC) between 1994 and 1998.

Subjects and methods

Patients

We collected data on 37 patients, who had been diagnosed to have HSPN of at least grade IVb, in the Department of Pediatrics of Fukushima Medical University School of Medicine between January 1988 and December 1998. Entry criteria included: (i) a diagnosis of HSP, made if the major manifestations of the illness consisted of a purpuric rash and abdominal pain without thrombocytopenia, with additional features, arthritis and nephritis, accepted as being consistent with the diagnosis; (ii) age at the initiation of therapy and follow-up under 15 years; (iii) no previous treatment with corticosteroids or immunosuppressive drugs. These patients were retrospectively divided into two groups without randomization. Group A consisted of 20 patients treated with MUT, and Group B consisted of 17 patients treated with MUTC. The clinical features, laboratory data and pathological findings of both groups were retrospectively studied and comparisons were made between data obtained before and after therapy.

Therapeutic intervention

A timetable of treatment for both groups is shown in Figure 1. Following diagnostic renal biopsy, the patients were treated with MUT before 1994 and were designated Group A. MUT was a combination of ‘pulse’ methylprednisolone, at 30 mg/kg/day i.v. bolus (maximum 1 g) for 3 consecutive days, and pulsed UK, at 5000 units/kg/day i.v. bolus (maximum 180,000 units) for 7 consecutive days, followed by daily oral prednisolone (1 mg/kg/day) for 6 months, along with anti-platelet agents (dipyridamole 5 mg/kg/day), and anti-coagulant (warfarin) for 24 months. Warfarin was given orally at a single morning dose of 1 mg (for <7-year-old patients) or 2 mg (for >7-year-old patients) to maintain the thrombotest at 30–50%. The corticosteroid was reduced subsequently over 3 months and dipyridamole and warfarin reduction was individualized according to individual improvement in 24 h creatinine clearance (24h Ccr), urinary sediment and urinary protein.

After 1994, the patients were treated with MUTC, and were designated Group B. MUTC involved the addition to the above described regimen of cyclophosphamide 2.5 mg/kg/day for 12 weeks. Cyclophosphamide was given orally at a single morning dose to maintain the pseudocholinesterase (ChE) level at more than half of the pre-therapy ChE or lymphocyte counts at >1500/μl, or both.

Definitions

‘Haematuria’ was considered a positive finding if urinary microscopic examination showed five or more red blood cells per high power field, and macrohaematuria if blood was visible to the naked eye [12]. Proteinuria was evaluated by 24 h quantitative measurements. Nephrotic syndrome was
defined as the presence of proteinuria (≥40 mg/m²/h) and a serum albumin level <25 g/l, with or without oedema [12]. Hypertension was defined as a systolic or diastolic blood pressure greater than the 95th percentile for the patient’s age, based on the recommendations of Pediatric Task Force [13].

The clinical status of each patient was classified as follows: Stage 0, normal: the patient was normal on physical examination, with normal urine and renal function; Stage 1, minor urinary abnormalities: the patient was normal on physical examination, and had microscopic haematuria or proteinuria <20 mg/m²/h; Stage 2, persistent nephropathy: the patient had proteinuria of 20 mg/m²/h or greater, and 24h Ccr of 40 ml/min/1.73 m² or greater; Stage 3, renal insufficiency: the patient had 24h Ccr <40 ml/min/1.73 m² (this category included patients who were on dialysis, were transplanted or who had died).

Pathology

First renal biopsy was performed initially in all patients; second biopsies were performed in the recovery phase (5.5±2.5 months after onset) in 15 of 20 patients (75%) in Group A and 13 of 17 patients (76%) in group B in order to assess the efficacy of the therapy. Material for histological studies was fixed in 20% neutral formalin, embedded in paraffin, stained with haematoxylin and eosin or periodic acid-Schiff reagent. The specimens were assessed by light microscopy (LM) and immunofluorescence (IF). The mean (±SD) number of glomeruli found in the biopsy specimens was 17.3±8.7 (range 10–46).

The glomerular changes were graded according to the classification devised by the pathologists of the International Society of Kidney Disease in Children [12], as follows: I, minor glomerular abnormalities; II, pure mesangial proliferation [(a) focal, (b) diffuse]; III, minor glomerular abnormalities or mesangial proliferation, with crescents in <50% of glomeruli [(a) focal, (b) diffuse mesangial proliferation]; IV, same as III but with crescents in 50–75% of glomeruli [(a) focal, (b) diffuse mesangial proliferation]; V, same as III but with crescents in >75% glomeruli [(a) focal, (b) diffuse mesangial proliferation]; VI, membrano-proliferative-like lesions.

To compare the biopsies, a histological scoring system was modified to evaluate acute and chronic changes. Acute changes included mesangial proliferation (grades 0–3, 0 = normal, 1 = slight, 2 = moderate, 3 = severe), segmental necrosis with cellular crescent formation (scored according to the percentage of glomeruli involved, 0 = 0%, 1 = 1–20%, 2 = 20–50%, 3 = >50%), and interstitial oedema with mononuclear cell infiltration (0–3). The acuity index (AI) is the sum of the scores of those changes. Chronic renal injury was estimated by determining the number of glomeruli with fibrous crescents and segmental or global sclerosis. Each abnormality was scored 0–3 according to the number of glomeruli involved, as for the scoring of acute crescent formation. In addition, the combination of tubular atrophy and interstitial fibrosis was graded 0–3. The chronicity index (CI) is the sum of the scores of those chronic renal changes. Scoring was performed in a blinded fashion on specimens identified only by codes.

Tissue for IF was immediately fixed in OCT compound and frozen at −80°C until use for IF. IF searched for IgG, IgA, IgM, C1q, C3, C4 and fibrinogen. The intensity of immunofluorescence was graded on a scale where negative = 0, mild = 1, moderate = 2 and severe = 3.

Statistics

Data are expressed as mean values±SEM. Statistical analysis was performed on a Macintosh computer with a software package for statistical analysis (Stat View, Abacus Concepts, Berkeley, CA, USA). Several variables, that are clearly not normal in their distribution, were compared using non-parametric statistics such as the Mann–Whitney test or Wilcoxon signed-rank test. The renal survival rates were calculated using the life-table method (Kaplan-Meier). P<0.05 was considered significant.

Results

Comparison of baseline characteristics and pathological findings between two groups at the time of the first renal biopsy (Table 1)

Age at onset and duration since onset were 8.0±2.8 and 7.4±3.1 years in Group A, and 7.9±3.1 and 6.2±1.7 years in Group B; male:female ratios were 9:11 and 8:9, respectively, in the two groups.

The glomerular abnormalities in the first biopsies of both groups were as follows: in Group A, grade IV in 18 patients, grade V in two patients; in Group B, grade IV in 15 patients, grade V in two patients. The percentage of crescentic formations was 55.5±6.5% in Group A and 60.7±12.3% in Group B.

Table 1. Comparison of laboratory findings between both groups at first renal biopsy and most recent follow-up

<table>
<thead>
<tr>
<th></th>
<th>At first renal biopsy</th>
<th>At most recent follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Urinary protein excretion (mg/m²/h)</td>
<td>154±13</td>
<td>181±85</td>
</tr>
<tr>
<td>Haematuria (%)</td>
<td>20 (12)</td>
<td>17 (10)</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>29±4</td>
<td>27±6</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>63±32</td>
<td>71±48</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>83±14</td>
<td>87±10</td>
</tr>
</tbody>
</table>

NS, not significant.
Comparison of clinical and laboratory findings between two groups at the time of the first renal biopsy (Table 1)

In the pre-biopsy period, haematuria and proteinuria were present in all patients in either group. Proteinuria varied from 78 to 375 mg/m^2/h (mean 154 ± 73 mg/m^2/h) in Group A and from 105 to 458 mg/m^2/h (mean 181 ± 85 mg/m^2/h) in Group B. Nephrotic syndrome was present in 12 patients of Group A and in 14 patients of Group B ($P = 0.15$). 24h Ccr was decreased in eight patients of Group A and in eight patients of Group B, ranging from 16 to 67 ml/min/1.73 m^2. Mean urinary protein excretions, incidence of haematuria, serum creatinine and creatinine clearances did not differ between the two groups.

Comparison of renal symptoms and laboratory data between two groups and the clinical stage at most recent follow-up (Figure 2 and Table 2)

We compared the renal symptoms and laboratory data before and after therapy. The mean urinary protein excretions of Group A and Group B were reduced from 154 ± 73 and 181 ± 85 mg/m^2/h to 29 ± 19 ($P < 0.001$) and 10 ± 5 mg/m^2/h ($P < 0.001$), respectively, at 6 months of follow-up. From 2 months after the initiation of treatment to the most recent follow-up, the mean urinary protein excretion in Group B was lower than that in Group A. At the most recent follow-up, the incidence of haematuria in Group A was higher than in Group B ($P < 0.05$); however, serum albumin, serum creatinine and the mean blood pressure did not differ between the groups.

![Fig. 2. Comparison of proteinuria between Group A and Group B up to the most recent follow-up.](image)

**Table 2.** Comparison of clinical stage between both groups at first renal biopsy and most recent follow-up

<table>
<thead>
<tr>
<th>At first renal biopsy</th>
<th>At most recent follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td><strong>Group B</strong></td>
</tr>
<tr>
<td>Stage 0</td>
<td>0/20 (0%)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>16/20 (80%)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>4/20 (20%)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>0/20 (0%)</td>
</tr>
</tbody>
</table>

NS, not significant.

a,b $P < 0.05$. 

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At the most recent follow-up, 24hCcr had returned to within the normal range in eight patients of Group A and in eight patients of Group B who had 24hCcr \(<70\) ml/min/1.73 m\(^2\) at the initiation of therapy. Eight patients of Group A and 13 patients of Group B had recovered clinically, and eight patients of Group A and four patients of Group B had minimal urinary abnormalities. Four patients of Group A and none of Group B had persistent nephropathy \((P<0.05)\) and no patient in either group had renal insufficiency.

**Results of repeat renal biopsies**

**Comparison of ISKDC classification between both groups at first and second biopsies** (Table 3). The number of patients with grade IV and grade V did not differ between the two groups. At the second biopsy, there were more patients with grade IV in Group A than in Group B.

**Comparison of AI and CI between both groups at first and second biopsies** (Table 4). The AI of both groups at second biopsy was lower than that at first biopsy. At second biopsy, the AI of Group B was lower than that of Group A \((P<0.05)\). The CI of Group A did not differ between the first and second biopsies, but the CI of Group B at second biopsy decreased when compared with the first biopsy. At second biopsy, the CI of Group B was lower than that of Group A \((P<0.05)\).

**Side effect of treatment**

Five patients of Group A and five patients of Group B showed Cushingoid changes, three patients in Group A and two patients in Group B developed mild glaucoma, and one patient in Group A and one patient in Group B presented with mild growth retardation. One patient in Group A (but none in Group B) developed mild hypertension, which was well controlled by treatment with nifedipine. The doses of urokinase and anticoagulants used in the treatment regimen did not result in bleeding tendencies such as epistaxis or gastrointestinal haemorrhage. One patient in Group B developed leucopaenia and discontinued the cyclophosphamide for 8 weeks. One patient in Group A and one patient in Group B developed infections—the former acute bronchitis, the latter acute pneumonia. Both recovered with antibiotic treatment. Most of the side effects were mild and well controlled; all were reversible.

**Discussion**

Our study suggests that MUTC significantly reduced urinary protein excretion and prevents any increase of crescentic and sclerosed glomeruli in HSPN patients with at least type IV, when compared with MUT. At their most recent follow-ups, there were no patients with persistent nephropathy and renal insufficiency among the patients treated with MUTC.

As for reports concerning the prognosis of HSPN, Counahan *et al.* [6] reviewed 88 patients with HSPN and found that 15 of 26 patients (58%) with at least grade IV and five of 38 (13%) without treatment had active renal disease, renal insufficiency or both. Yoshikawa *et al.* [14] reported that HSPN was a significant cause of childhood chronic renal failure, accounting for 16% of all children entering dialysis in Japan. Specifically, 52% (13 of 25) of patients studied by them, with at least grade IV, had renal insufficiency.

The pathological features that are valuable portents of prognosis are histological grade, especially the

<table>
<thead>
<tr>
<th>ISKDC classification</th>
<th>Group A ((n=20))</th>
<th>Group B ((n=17))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First biopsy ((n=20))</td>
<td>Second biopsy ((n=15))</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>IV</td>
<td>18</td>
<td>4(^a)</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^aP<0.05.\)

<table>
<thead>
<tr>
<th>Group</th>
<th>AI First biopsy</th>
<th>Second biopsy</th>
<th>(P)</th>
<th>CI First biopsy</th>
<th>Second biopsy</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.3±1.6</td>
<td>3.8±1.8(^a)</td>
<td>(&lt;0.05)</td>
<td>2.5±1.2</td>
<td>2.9±1.1(^b)</td>
<td>NS</td>
</tr>
<tr>
<td>B</td>
<td>6.1±1.6</td>
<td>2.1±0.8(^a)</td>
<td>(&lt;0.05)</td>
<td>3.5±2.4</td>
<td>1.8±1.2(^b)</td>
<td>(&lt;0.05)</td>
</tr>
</tbody>
</table>

NS, not significant.

\(^a,bP<0.05.\)
Efficacy of methylprednisolone and urokinase pulse therapy

percentage of glomeruli with crescents, and higher
cr
chronicity indices [1,2,5,6]. Therefore, it is necessary to
cr
assure that therapy for severe HSPN is adequate.

Niaudet and Habib [3] reported that methylpredniso-
cr
lone pulse therapy was effective in patients at risk of
progression of their nephropathy, especially if it was
started early during the course of the disease, before
the crescents become fibrosed. Iijima et al. [7] mentioned
that a multiple combined therapy with prednisolone,
cyclophosphamide, heparin or warfarin, and dipyrida-
cr
mole could be effective in histologically severe HSPN.

In addition, Flynn et al. [9] report that treating children
with HSPN with high-dose corticosteroids plus oral
cyclophosphamide is safe and, as in nephrotic syn-
drome, appears to significantly reduce proteinuria.
This study, however, was not a controlled study.
Therefore, we resorted to a controlled study to
investigate the efficacy of that therapy combined with
cyclophosphamide in patients with severe HSPN.

Cyclophosphamide is a potent alkylating agent
which inhibits lymphocyte proliferation, leading to
repression of the function of B and T lymphocytes as
well as to a reduction of their numbers [15]. The
dose of cyclophosphamide we used was based on the classic
approach to minimal-change nephrotic syndrome,
where cyclophosphamide has been shown to have a
talent immunomodulating effect, especially in steroid-
sensitive patients [16–18]. Cyclophosphamide has also
been used with success in the treatment of several
other forms of crescentic glomerulonephritis, particu-
larly those characterized by altered autoimmunity,
including Wegener’s granulomatosis and systemic
lupus erythematosus [15,19,20].

Some side effects of cyclophosphamide have been
observed, such as myelosuppression, haemorrhagic
cystitis and interstitial pneumonia [15]. In our hospital,
we have used serum cholinesterase activity in addition
to WBC and lymphocyte counts to monitor for the side
effects of cyclophosphamide. Serum cholinesterase
activity might be an index of cyclophosphamide
therapy. Imai et al. [21] showed the importance of
maintaining serum cholinesterase (ChE) levels at more
than half of the pre-therapy ChE level for preventing
the side effects. In our study, it is impossible that the side
effects of cyclophosphamide were minimized by careful
regulation of serum cholinesterase activity. Therefore,
we suggest that it would be useful to monitor serum
cholinesterase activity during cyclophosphamide
therapy.

Our cocktail therapy with cyclophosphamide
included reduced urinary protein excretion and mesan-
gial IgA deposition, and prevented the increase of
sclerosed glomeruli in children with severe HSPN of
at least grade IVb. The rationale for using prednisolone
and cyclophosphamide in severe HSPN is that corti-
costeroids and immunosuppressive agents reduce IgA
production and minimize the abnormal immune
response and inflammatory events that follow IgA
deposition in glomeruli. Urokinase, warfarin and
dipyridamole are used to inhibit the mediators of
glomerular damage [4,8,22].

UK, a plasminogen activator derived from fresh
human urine, first attracted attention as a therapeutic
agent for thrombotic diseases such as cardiovascular
diseases or cerebral thrombosis. The molecular weight
of UK is 54 000. UK has been used in patients with
chronic glomerulonephritis, including IgA nephrone-
pathy, with fibrinogen or fibrin deposits detected by
immunofluorescence. The rationale for such treatment
was as follows: (i) the defibrinating activity of UK was
stronger than of anti-coagulants; (ii) specific accumula-
tions of UK were seen in the kidney and liver despite a
very short turnover rate; (iii) adverse effects were very
rare even if UK was administered for a long period
[23,24].

Recently, Glass et al. [25] reported that UK may
mediate proteolysis in the mesangial extracellular
matrix, and Yasunaga et al. [26] showed in vitro that
plasminogen enhanced the angiogenesis of bovine
capillary endothelial cells and an anti-UK antibody
inhibited this effect. We have reported that the
improvement of hypercoagulable states and acute
inflammatory responses, and the suppression of sclero-
tic changes in glomeruli, were found in patients on
MUT. The studies mentioned suggest that UK and
plasminogen may regulate the repair of affected
capillaries and may digest the proliferated matrix in
diseased glomeruli.

Dipyridamole is an anti-platelet agent, and it
abrogates the mitogenic effects of platelet-derived
growth factor [27]. Hillis et al. [27] reported that
dipyridamole significantly inhibited the growth of
human mesangial cells in vitro in a dose-dependent
fashion. Nagai et al. [28] showed that warfarin
ameliorated hyperfiltration and urinary albumin excre-
tion in streptozotocin rats. Futhermore, Lee et al. [29]
found that dipyridamole and low-dose warfarin were
effective in patients with IgA nephropathy and renal
impairment.

The beneficial effects of combined prednisolone,
cyclophosphamide, urokinase, warfarin and dipyrida-
mole treatment were accompanied by few serious side
effects specifically attributable to those drugs. Most of
the side effects were mild and well controlled, and all
were reversible. The cocktail therapy was well tolerated
in all patients, and its safety was monitored by using
serum cholinesterase as the index of the side effects
of cyclophosphamide.

In conclusion, our study suggests that cocktail
therapy with MUTc is effective in patients with the
risk of the progression of their severe HSPN. Long-
term and additional observations are necessary to
evaluate the long-term efficacy of this cocktail therapy.

Conflict of interest statement. None declared.

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eds. Renal Pathology with Clinical and Functional Correlations,

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