Antiproteinuric effect of angiotensin-converting enzyme inhibition and C5b-9 urinary excretion in membranous glomerulonephritis

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Abstract

Background. Angiotensin-converting enzyme (ACE) inhibitors have an antiproteinuric effect in membranous glomerulonephritis (MGN). However, no studies have investigated whether this antiproteinuric effect is influenced by urinary C5b-9 excretion, a marker of immunological activity in this disease.

Methods. Eleven patients with biopsy-proven MGN were treated with captopril for 8 weeks. The evolution of several clinical and biochemical parameters, including 24-h urinary protein excretion was evaluated every 4 weeks. Urinary C5b-9 excretion was measured at the onset and at the end of captopril treatment.

Results. Patients with MGN had significantly higher C5b-9 excretions than a group of 14 healthy controls (89 ± 23 vs 3.7 ± 1.4 ng/mg UCr; \( P < 0.001 \)). A significant correlation was found between urinary C5b-9 and the magnitude of proteinuria, both at the onset and at the end of treatment. After 8 weeks of captopril treatment, proteinuria had decreased from 8 ± 1.8 to 5.2 ± 1.3 g/day (\( P < 0.05 \)). Four weeks after captopril discontinuation, proteinuria rose to 7.3 ± 1.7 g/day (\( P < 0.05 \)). A marked variability in the antiproteinuric response was observed, ranging from 0 to 85% with respect to baseline values. No correlation between decrease in proteinuria and baseline urinary C5b-9 levels was observed. Several patients with elevated urinary C5b-9 levels had captopril-induced decrease in proteinuria.

Conclusions. ACE inhibition induces an antiproteinuric effect in patients with MGN. The urinary C5b-9 excretion does not predict the magnitude of this response.

Key words: ACE inhibitors; C5b-9 urinary excretion; complement terminal complexes; membranous glomerulonephritis; proteinuria decrease

Introduction

Several studies have reported that angiotensin-converting enzyme (ACE) inhibitors induce a sustained proteinuria decrease in MGN and other primary glomerulonephritis [1–5]. This antiproteinuric response is markedly variable in patients with MGN [3,4].

The mechanisms involved in the antiproteinuric effect of ACE inhibitors are currently unknown; theoretically, ongoing immune-complex formation in MGN could influence the antiproteinuric response. Urinary levels of complement terminal complexes (C5b-9) have been proposed as a useful marker of immunological activity in MGN, both in experimental models of the disease and in clinical studies [6–14]. We investigated whether the antiproteinuric effect of ACE inhibitors is influenced by urinary C5b-9 levels in this disease.

Subjects and methods

Patients

Eleven patients with biopsy-proven MGN were included in the study. The main characteristics of the patients are expressed in Table 1. Date of renal biopsy was considered as the onset of the disease. All the patients had normal blood pressure; two of them were taking calcium-channel blockers because of previous moderate arterial hypertension. None of them had previously been treated with ACE inhibitors, steroids or immunosuppressive drugs. The patients were following low-salt, normal-protein diets.

Study protocol

Patients were evaluated 4 weeks prior to the study to assess whether renal function and blood pressure were stable (period from −4 week to baseline; see Table 2). Diets remained the same and previous medications (diuretics and calcium-channel blockers) were maintained at the same doses throughout the entire study. At baseline, patients received 25 mg captopril b.i.d. for 4 weeks. At the end of the 4th week (+4 week, see Table 2), the captopril dose was increased to 50 mg b.i.d. for an additional 4 weeks and then it was discontinued (+8 week, Table 2).

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Creatinine clearance (ml/min) ± SEM 5.2 ± 0.8 8 ± 1.8 1 ± 0.08 118 ± 10

Table 2. Evolution of proteinuria, C5b-9, renal function, and blood pressure throughout the study

<table>
<thead>
<tr>
<th>Captopril</th>
<th>−4 week</th>
<th>Baseline</th>
<th>+4 week</th>
<th>+8 week</th>
<th>+12 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (g/day)</td>
<td>8 ± 1.5</td>
<td>8 ± 1.8</td>
<td>6.5 ± 1.6</td>
<td>5.2 ± 1.3</td>
<td>7.3 ± 1.7</td>
</tr>
<tr>
<td>C5b-9 (ng/mg UCr)</td>
<td>89 ± 23</td>
<td>1 ± 0.08</td>
<td>1 ± 0.08</td>
<td>1 ± 0.10</td>
<td>1 ± 0.12</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.07</td>
<td>112 ± 12</td>
<td>118 ± 10.9</td>
<td>105 ± 11.4</td>
<td>115 ± 13.5</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>100 ± 4</td>
<td>103 ± 4.2</td>
<td>97 ± 3.5</td>
<td>96 ± 6.1</td>
<td>96 ± 4.2</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>113 ± 12</td>
<td>118 ± 10.9</td>
<td>105 ± 11.4</td>
<td>115 ± 13.5</td>
<td>113 ± 13.01</td>
</tr>
</tbody>
</table>

*P < 0.05 with respect to baseline values; bP < 0.05 with respect to values at +8 week.

Patients were evaluated every 4 weeks on five occasions (−4 week, baseline, +4 week, +8 week and +12 week). At each examination, body weight and blood pressure were measured and blood was drawn for general biochemistry. Urinary protein, creatinine, sodium, potassium, and urea were measured in a 24-h urine sample, which was collected the day before the visit. At baseline and at +8 week visits, freshly voided urine samples for C5b-9, creatinine, and protein were obtained. Freshly voided urine samples for the same parameters were also obtained from 14 normal controls, with normal blood pressure and without any known renal disease.

Urinary C5b-9 levels were measured using ELISA kits (Quidel Labs, La Jolla, CA, USA). Wells were lined with a mouse monoclonal antibody against human C5b-9. Urine samples were added to the wells and incubated for 1 h. The wells were washed five times and a goat antibody against C6-C7 complement components was added. After 1 h of incubation, the wells were again washed five times. Chromogen was added to the wells and the light absorbance at 405 nm was measured after 30 min. A standard curve was generated using known amounts of C5b-9 provided with the kit. Inter- and intra-assay variabilities for urinary C5b-9 were 7.5% and 5.5% respectively. C5b-9 levels are expressed as nanograms per milligram of urinary creatinine (ng/mg UCr).

Statistical analysis

All data are presented as mean ± standard error. Evaluation of statistical significance was performed with Wilcoxon test for paired data and Mann–Whitney test for unpaired data. Correlations between variables were calculated using the Pearson correlation coefficient. Significance was considered when P < 0.05.

Results

Patients with MGN had significantly higher baseline urinary levels of C5b-9 than the control group of 14 healthy volunteers (89 ± 23 ng/mg UCr versus 3.7 ± 1.4 ng/mg UCr, P < 0.001). Proteinuria decreased from 8 ± 1.8 to 5.2 ± 1.3 g/day after 8 weeks of captopril treatment (P < 0.05) (Table 2). Four weeks after captopril withdrawal (+12 week, Table 2), proteinuria significantly increased to 7.3 ± 1.7 g/day (P < 0.05 with respect to values at +8 week). There were no significant changes in other clinical or biochemical parameters throughout the study.

Mean proteinuria decrease (% of proteinuria reduction at +8 week with respect to baseline values) was 32 ± 8.8%. However, proteinuria decrease was not uniform (Figure 1). Captopril-induced proteinuria decrease did not show any correlation with the clinical or biochemical parameters at baseline. Similarly, no correlation was found between proteinuria decrease and blood pressure changes, urinary urea excretion or urinary sodium throughout captopril treatment. Proteinuria decrease was also independent of baseline urinary C5b-9 levels. As is shown in Figure 1, some patients with high levels of urinary C5b-9 showed an antiproteinuric response to captopril, whereas in other cases proteinuria decrease was poor or absent. On the
other hand, some patients with low or negative urinary excretions of C5b-9 had a poor antiproteinuric response (Figure 1).

Baseline urinary C5b-9 values of patients with MGN showed a significantly positive correlation with baseline proteinuria excretions ($r = 0.60$, $P < 0.05$) (Figure 2). Urinary C5b-9 levels measured after 8 weeks of captopril treatment decreased from $89 \pm 23$ ng/mg UCr to $73 \pm 25$ ng/mg UCr, with no statistical significance (Table 2). However, similar to the baseline values, a significantly positive correlation between C5b-9 levels and proteinuria excretion was found at +8 week ($r = 0.77$, $P < 0.05$) (see Figure 2).

Discussion

Several experimental and clinical studies have shown that urinary C5b-9 levels are frequently increased in MGN [6–12,15]. However, the significance of C5b-9 urinary is unclear. Increased urinary C5b-9 excretion is not specific for MGN; patients with heavy proteinuria secondary to diabetic nephropathy, focal glomerulosclerosis and other renal diseases frequently show increased urinary C5b-9 [9,12]. In some studies no correlation was found between the magnitude of proteinuria and the urinary C5b-9 levels [8,11], while others have shown a positive correlation between both variables [9,12]. We have found that C5b-9 levels showed a significant correlation with the magnitude of proteinuria (Figure 2).

Several studies have reported an antiproteinuric effect of ACE inhibitors in MGN; the magnitude of proteinuria reduction ranged between 27 and 59% of the baseline values [1–4]. In the present study we observed a significant decrease in proteinuria induced by captopril; mean proteinuria decrease was $32 \pm 8.8\%$ with respect to baseline values. After captopril discontinuation, proteinuria increased significantly (Table 2). As in previous studies, the antiproteinuric effect was independent of systemic blood pressure changes and was not accompanied by modifications in renal function.

We found a remarkable variability in the antiproteinuric response to captopril (Figure 1); some patients showed proteinuria reductions higher than 65% of the baseline values, whereas in other patients the antiproteinuric effect was totally absent. The reasons for these discrepancies remain unexplained. A theoretical possibility is that ongoing immune-complex formation would influence the response. If this hypothesis is correct, urinary C5b-9 levels should serve as a predictor of antiproteinuric effect of ACE inhibitors. However, our study clearly shows that some patients with very high urinary C5b-9 levels have marked decreases in proteinuria with captopril (Figure 1) while other patients with negative or very low urinary C5b-9 levels did not respond to this treatment.

In conclusion, our study shows that urinary C5b-9 excretion does not predict the antiproteinuric response to ACE inhibitors.

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References


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