Anti-proteinuric and anti-coagulatory effects of camostat mesilate in azotemic diabetics

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Key words: ACE inhibition, plasma fibrinogen, plasma D-dimer of FDP, urinary FDP, plasma E fragment of FDP

Abstract

The present study was conducted on 8 patients with advanced diabetic nephropathy who showed a significant reduction of proteinuria through ACE inhibition. Camostat mesilate, one of the most potent protease inhibitors developed for oral use, was administered to these patients at a daily dose of 600 mg starting after 4 weeks of ACE inhibitor administration. Laboratory data were obtained 1) just before the ACE inhibition, 2) after 4 weeks of the ACE inhibitor single treatment, and 3) after another 4 weeks of the additional treatment with camostat mesilate. The urinary protein excretion decreased from 1) 10.1 ± 1.3 to 2) 7.3 ± 1.1, and 3) 4.6 ± 0.9 g/day [mean ± SEM; significance of difference 1)–2), p < 0.05; 2)–3), p < 0.01], and the serum total protein values increased from 1) 5.0 ± 0.3 to 2) 5.2 ± 0.2, and 3) 5.4 ± 0.3 g/dl [1)–3), p < 0.05]. The plasma levels of fibrinogen, and of E fragment and D-dimer of FDP changed from 1) 476 ± 43 to 2) 477 ± 41, and 3) 374 ± 33 ng/ml [2)–3), p < 0.01], from 1) 125 ± 19 to 2) 147 ± 27, and 3) 104 ± 30 ng/ml [2)–3), p < 0.05], and from 1) 261 ± 60 to 2) 272 ± 86, and 3) 185 ± 56 ng/ml [2)–3), p < 0.05], respectively. Although the urinary excretions of E fragment and D-dimer of FDP decreased in many patients even during ACE inhibition, their urinary excretion ratio to urinary protein fell significantly only after camostat mesilate was administered.

These results suggest that camostat mesilate may suppress the intravascular over-formation of fibrinogen and fibrin, and exert inhibitory effects on the hypercoagulable state induced by advanced diabetic nephropathy. This anti-coagulatory effect might be closely related to its anti-proteinuric effect in diabetic nephropathy.

Introduction

Camostat mesilate is one of various protease inhibitors developed for oral use. Recently, we reported that this drug has an anti-proteinuric effect not only in patients with primary glomerulonephritis but also in patients with advanced diabetic nephropathy [1, 2]. However, since camostat mesilate exerts multi-inhibitory activities on the kallikrein-kinin, complement, and coagulation systems, as well as on platelet functions [3-5], the mechanism by which it reduces proteinuria in diabetic nephropathy has not yet been fully elucidated. Furthermore, the decrease in plasma fibrinogen and urinary excretion of fibrinogen-fibrin degradation products (FDP) during treatment with camostat mesilate, as reported in our previous study [2], might be caused by improvement of the nephrotic state.

The present study was conducted on 8 patients with advanced diabetic nephropathy who revealed a significant reduction of proteinuria during treatment with angiotensin converting enzyme inhibitor (ACE inhibitor). We added camostat mesilate to these patients to investigate the changes in values of the plasma fibrinogen and FDP, and
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urinary FDP excretion during ACE inhibition and camostat mesilate treatment. Since we could not detect the total plasma FDP levels by the latex agglutination method as reported previously [2], E fragment and D-dimer of FDP were measured instead.

The results obtained provided further important information concerning the effects of camostat mesilate in patients with diabetic nephropathy.

Patients and Methods

Eight patients with advanced diabetic nephropathy (4 males and 4 females, 42 to 70 years old) who showed a significant reduction of proteinuria following ACE inhibitor therapy were selected for this study. They had been diagnosed as having non-insulin-dependent diabetes mellitus for more than 15 years and hypertension of shorter duration. All patients had been treated with insulin regimens at other hospitals, and were transferred to Sendai Shakaihoken Hospital because of the development of complications as shown in Table 1. Clinical diagnosis of diabetic nephropathy was made without renal biopsy in all patients, since proteinuria was first detected in the latter periods of the course of their diabetes, and diabetic proliferative retinopathy and azotemia were found. They were treated by conventional therapy at our center and, after the disease had become stabilized, an ACE inhibitor (Alacepril) was given for 4 weeks. Subsequently, camostat mesilate at a daily dose of 600 mg was added for 4 weeks. The serum creatinine and total protein, plasma fibrinogen, E fragment and D-dimer of FDP, and the urinary excretion of protein and E fragment and D-dimer of FDP over 24 hours were determined 1) just before the treatment with ACE inhibitor, 2) 4 weeks after the ACE inhibitor administration had started, and 3) 4 weeks after the treatment with camostat mesilate had been added.

The urinary protein was measured by the Exton-Rose method, the serum creatinine and total protein with a standard automated analyzer (SMAC-II; Technicon), and the plasma fibrinogen from the thrombin coagulation time. The plasma and urinary E fragment and D-dimer of FDP were determined using an LPIA system and the dimertest-EIA method, respectively.

The normal ranges for the plasma concentrations of fibrinogen, E fragment and D-dimer of FDP are 160 to 400 mg/dl, less than 100 ng/ml,

<table>
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<tr>
<th>Patient no.</th>
<th>Age/Sex</th>
<th>Duration of DM (years)</th>
<th>Reason for admission</th>
<th>Serum Cr&lt;sup&gt;2&lt;/sup&gt; (mg/100 ml)</th>
<th>Serum TP&lt;sup&gt;3&lt;/sup&gt; (g/100 ml)</th>
<th>Administered dose of Alacepril (mg/day)</th>
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<tr>
<td>1</td>
<td>70/F</td>
<td>15</td>
<td>NS&lt;sup&gt;4&lt;/sup&gt;</td>
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<td>1.7</td>
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<tr>
<td>2</td>
<td>53/M</td>
<td>25</td>
<td>PRF&lt;sup&gt;5&lt;/sup&gt;</td>
<td>8.2</td>
<td>9.3</td>
<td>9.7</td>
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<td>3</td>
<td>63/F</td>
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<td>NS</td>
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<td>4</td>
<td>42/F</td>
<td>22</td>
<td>NS</td>
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<tr>
<td>5</td>
<td>50/F</td>
<td>17</td>
<td>CHF&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>3.3</td>
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<tr>
<td>6</td>
<td>61/M</td>
<td>21</td>
<td>NS</td>
<td>2.1</td>
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<tr>
<td>7</td>
<td>48/M</td>
<td>18</td>
<td>PRF</td>
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<td>8</td>
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Mean ± SEM

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<th>4.1 ± 4.2 ± 4.4 ± 5.0 ± 5.2 ± 5.4 ±</th>
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<tr>
<td>Serum Cr&lt;sup&gt;2&lt;/sup&gt;</td>
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<td>Serum TP&lt;sup&gt;3&lt;/sup&gt;</td>
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<tr>
<td>Administered dose</td>
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<td>of Alacepril</td>
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<tr>
<td>(mg/day)</td>
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<tr>
<td>n.s.</td>
<td>p&lt;0.05&lt;sup&gt;7&lt;/sup&gt;</td>
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<sup>1</sup> Diabetes mellitus, <sup>2</sup> creatinine, <sup>3</sup> total protein, <sup>4</sup> nephrotic syndrome, <sup>5</sup> progression of renal failure, <sup>6</sup> congestive heart failure, <sup>7</sup> not significant, and <sup>8</sup> p value.
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and less than 150 ng/ml, respectively. In normal subjects, urinary E fragment and D-dimer of FDP are not detected.

Results

All patients completed the whole study (4 weeks of ACE inhibitor treatment and a further 4 weeks of additional treatment with camostat mesilate) without developing any serious complications such as infections, ischemic vascular diseases, gastrointestinal, or exacerbation of diabetic proliferative retinopathy. During the study period, the insulin regimens and anti-hypertensive drugs were not altered, except in one patient (patient no. 4) who required a slightly increased dose of insulin while receiving the ACE inhibitor single treatment. Although the serum creatinine levels did not change as a whole after the 8 weeks of study, 2 patients (patient no. 2 and 7) who had been transferred to Sendai Shakaihoken Hospital because of progression of renal failure, revealed successive elevations of their serum creatinine despite the treatments with ACE inhibitor and camostat mesilate (Table 1).

The fluctuations in urinary protein excretion and serum total protein level are summarized in Fig. 1 and Table 1. The changes in urinary protein excretion and in serum total protein during the 4 weeks of ACE inhibition were almost the same as those during the 4 weeks of camostat mesilate treatment. However, the values for plasma fibrinogen, E fragment and D-dimer of FDP decreased only after camostat mesilate treatment had been started, as shown in Fig. 2. The urinary values for E fragment of FDP were significantly decreased during the previous treatment with ACE inhibitor alone as well as during the camostat mesilate treatment, and many patients revealed a reduction in urinary D-dimer of FDP even during the ACE inhibitor single treatment. However, the ratios of urinary E fragment and D-dimer of FDP to urinary protein (derived from the following equation: urinary E fragment or D-dimer of FDP (µg/24 hours)/urinary protein (g/24 hours) × 106, fell only after the additional treatment with camostat mesilate had begun (Fig. 3).

Discussion

Camostat mesilate is a derivative of gabaxate mesilate developed for oral use. Its anti-proteinuric effect has been observed in patients with diabetic nephropathy as well as in primary glomerulonephritis [1, 2]. However, since it directly or indirectly inhibits the kallikrein-kinin system, complement system, coagulation system, and platelet functions through its strong anti-proteinase activities, the mechanism whereby camostat mesilate reduces proteinuria in patients with diabetic nephropathy remains unclear. Although we noted decreased values of plasma fibrinogen and total urinary FDP during camostat mesilate treatment in our previous report [2], these changes might have been secondary phenomena through improvement of the nephrotic state. For these reasons, we selected patients with typical advanced diabetic nephropathy who responded

![Fig. 1. Urinary protein values (1) just before ACE inhibitor administration, (2) after 4 weeks of ACE inhibitor single treatment, and (3) after 4 weeks of additional treatment with camostat mesilate.]

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\begin{array}{c|c|c}
\text{Patient no.} & (1) & (2) \\
\hline
1 & 10.1±1.3 & 7.3±1.1 \\
2 & & 4.6±0.9 \\
3 & & \\
4 & & \\
5 & & \\
6 & & \\
7 & & \\
8 & & \\
\hline
\end{array}
\]

\[p < 0.05 \quad p < 0.01\]

Mean ± SEM
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Fig. 2. Plasma fibrinogen (A), and E fragment (B) and D-dimer (C) of FDP (1) just before ACE inhibitor administration, (2) after 4 weeks of ACE inhibitor single treatment, and (3) after 4 weeks of additional treatment with camostat mesilate.

well to ACE inhibitor, and comparative studies were performed during single treatment with the ACE inhibitor and after additional treatment with camostat mesilate. Furthermore, we measured the E fragment and D-dimer of FDP in this study, since we did not detect the changes in plasma total FDP of many diabetic patients in our previous study using the latex agglutination method [2]. To minimize the influence of other diseases on the coagulation system, patients with complications such as infectious episodes, ischemic vascular disease, or a dehydrated state due to gastroenteropathy, or a dehydrated state were excluded from the present study.

The plasma values for fibrinogen, and E fragment and D-dimer of FDP decreased only after the camostat mesilate administration had been started, although the changes in urinary protein excretion and serum total protein during the ACE inhibition were almost the same as those during the additional treatment with camostat mesilate. The urinary excretion of E fragment and D-dimer decreased in many patients even during the treatment with ACE inhibitor alone, but their ratios of urinary excretion to urinary protein fell only after the additional treatment with camostat mesilate had begun.

E fragment of FDP derives from the degraded fragments of fibrinogen, and is also obtained by cross-linked fibrin digestion, while D-dimer is obtained only through the digestion of cross-linked fibrin. Since the observed decrease in plasma fibrinogen during the treatment with camostat mesilate was accompanied by a decrease in plasma E fragment of FDP, this reduction of plasma fibrinogen suggested no increase in the lysis of fibrinogen, but suppression of fibrinogen synthesis. Furthermore, the decrease in plasma D-dimer of FDP might indicate an inhibitory effect of camostat mesilate on fibrin formation.

Five patients, who had been exhibiting markedly increased levels of plasma D-dimer associated with abnormally high values of plasma fibrinogen, revealed a reduction of both plasma D-dimer of FDP and fibrinogen after camostat mesilate had been administered. This suggests that camostat mesilate may suppress the excessive formation of fibrinogen and fibrin. Although the detailed mechanism for such suppression of plasma fibrinogen and fibrin formation remains
unknown, the observed changes in plasma levels appear to indicate that camostat mesilate can improve the hypercoagulable state associated with advanced diabetic nephropathy.

Intraglomerular permselectivity for macromolecules is extremely impaired in diabetic nephropathy. To avoid the influence of its amelioration on urinary FDP, we examined the urinary excretion ratios of E fragment and D-dimer of FDP to urinary protein. The results demonstrated a significant fall in these ratios during camostat mesilate treatment. These data imply that...
the reduction of urinary FDP during camostat mesilate administration could not be attributed to improvement of the permselectivity. These would suggest a direct suppressive effect of camostat mesilate itself. Fibrin and/or fibrinogen deposition in the glomeruli has been demonstrated even in the early stages of diabetic nephropathy [6], and urinary FDP have been confirmed to be derived not only from filtration of plasma fibrinogen and FDP, but also from fibrinolysis of intraglomerular fibrin deposits [7]. Since we observed a reduction of the plasma fibrinogen and FDP during the same period, these changes in urinary FDP levels might indicate a decrease in the process of deposition of fibrin and/or fibrinogen in the glomeruli, and a decrease in the lysis of such deposits. This would also suggest an inhibitory effect of camostat mesilate on intraglomerular coagulation. Further, since the hypercoagulable state is closely associated with vascular permeability to macromolecules and influences the permselectivity of the glomeruli, we presume that such a suppressive effect on the coagulation system caused by camostat mesilate might result in an improvement of the glomerular permselectivity and so reduce the urinary protein excretion.

Although we have demonstrated these anti-coagulable effects of camostat mesilate, no patient in the present study experienced any exacerbation of diabetic retinopathy. The suppressive effects on the coagulation system appeared to present no danger of accelerating hemorrhagic complications.

During the combination treatment with ACE inhibitor and camostat mesilate, the serum total protein values underwent a significant increase due to the concomitant reduction in proteinuria. Both of these drugs might therefore be effective for improving the nephrotic state of diabetic nephropathy. However, 2 patients, who were admitted to Sendai Shakaihoken Hospital because of progression of their renal failure, showed a further increase in serum creatinine levels, although their urinary protein, plasma fibrinogen and FDP, and urinary FDP values were decreased throughout the study period. These facts suggest that combination therapy with ACE inhibitor and camostat mesilate should be started at an early stage of diabetic nephropathy even without signs of serious renal failure in an effort to improve the prognosis of this disease. Further studies are needed to decide at which stage of diabetic nephropathy these treatments should be initiated.

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References