Role of intrarenal coagulation and anticoagulant therapy in the progression of diabetic nephropathy

KAZO KAIZU, KOHEI URIU, OSAMU HASHIMOTO*, EMIKO MORITA*, SUMIYA ETO* and HIDERO SUZUKI*

Kidney Center, 1st Department of Internal Medicine*
University of Occupational and Environmental Health, Japan, School of Medicine, Kitakyushu, Japan

Key words: diabetic nephropathy, intrarenal coagulation, intrarenal fibrinolysis, anticoagulant therapy, fibrinopeptide A, fibrin/fibrinogen degradation products

Abstract

The aim of the present study was to clarify the role of intrarenal coagulation in the progression of renal dysfunction and to assess the efficacy of anticoagulant therapy in diabetic nephropathy patients. Forty-one diabetic patients were divided into 2 groups: group 1 (G-1), 20 patients with nephropathy; and group 2 (G-2), 21 patients without nephropathy. The levels of fibrinopeptide A (FPA) and fibrinopeptide B$_{15-42}$ (FPB$_{15-42}$), fibrin/fibrinogen degradation products-D dimer (FDP-D dimer), and FDP-E products (FDP-E) and FDP, which are sensitive parameters of coagulation and fibrinolysis, were measured by radioimmunoassay, enzyme immunoassay (EIA), and latex photometric immunoassay, respectively, in both the blood and urine. The levels of urinary FPA, FDP-D, FDP-E, and FDP were found to be much higher in G-1 than in G-2. Significant relations were observed among the urinary levels of these four parameters. The renal function in all cases with higher levels of urinary parameters was severely deteriorated.

Following heparin administration to these patients, marked reductions of the urinary FPA, FDP-D, and FDP-E and improvement of nephrotic syndrome were observed. The present data suggest that in diabetic nephropathy: (1) intrarenal coagulation is likely to occur and to induce progression of renal dysfunction; and (2) heparin therapy could be effective in diabetic nephropathy when the patients are selected according to the above parameters of coagulation and fibrinolysis.

Introduction

Diabetic nephropathy is one of the common secondary complications of diabetes mellitus [1]. It has been shown that 40% of patients with insulin dependent diabetes mellitus (IDDM) [2] eventually develop nephropathy with increased proteinuria and a decreased glomerular filtration rate (GFR). However, not only the pathogenesis but also the cause of the progression of diabetic nephropathy is not well understood. Immunoglobulins, such as IgG and IgM, complement, and fibrin/fibrinogen are commonly found in the glomeruli by immunofluorescent techniques [3, 4].

Fibrin deposits are frequently observed in the mesangium, glomerular basement membrane and nodular lesions, suggesting that coagulation and fibrinolysis occur in the glomeruli of the kidneys of patients with diabetic nephropathy. Further, it is well known that a wide variety of vascular complications which frequently occur in diabetes mellitus, such as diabetic retinopathy, myocardial infarction, stroke and microangiopathy, are associated at least in part with defects of the hemostatic system [5]. Alterations of the coagulation and fibrinolytic systems and aggregation of platelets have been widely found in diabetes. It is considered, therefore, that abnormalities of coagulation and fibrinolysis might occur in both
the systematic circulation and the microcirculation including that of the glomeruli. Recently, several molecular markers for evaluating the actual degree of activation of the coagulation and fibrinolytic systems have been investigated. In the present study, we measured the levels of several sensitive molecular markers, such as fibrinopeptide A (FPA), fibrinopeptide B\(\beta_{15-42}\) (FPB\(\beta_{15-42}\)), fibrin/fibrinogen degradation products (FDP), FDP-D and FDP-E in order to assess the systemic and intraglomerular coagulation and fibrinolysis in patients with diabetic nephropathy.

FPA is a small peptide consisting of 16 amino acids, which is cleaved from fibrinogen by the action of thrombin. Determination of the level of FPA per se indicates activation of the coagulation [6]. Since FP\(\beta_{15-42}\) is a peptide which is cleaved from fibrin II by the action of plasmin, FPB\(\beta_{15-42}\) indicates activation of secondary fibrinolysis [7]. FDP, FDP-D and FDP-E reflect activation of fibrinolysis/fibrinogenolysis. The aim of the present study was to clarify the role of intraglomerular coagulation and fibrinolysis in the progression of diabetic nephropathy, and to assess the efficacy of anticoagulant therapy in diabetic nephropathy patients.

**Patients and Methods**

Forty-one patients were divided into 2 groups. Group 1 consisted of 20 patients with diabetic nephropathy who were diagnosed on the basis of persistent macroproteinuria as measured by the sulfosalicylic acid method. Group 2 consisted of 21 patients who did not display persistent proteinuria. The various parameters of coagulation and fibrinolysis, FPA, FPB\(\beta_{15-42}\), FDP, FDP-D and FDP-E, were measured in both the blood and urine. FPA and FPB\(\beta_{15-42}\) were estimated by radioimmunoassay [6, 7]. FDP-D was measured by enzyme immunoassay [8], and FDP and FDP-E were determined by latex photometric immunoassay [9].

The blood samples for measurement of the FPA, FPB\(\beta_{15-42}\) and FDP-D were collected directly by careful venepuncture with a 21-gauge needle into siliconized tubes. They were then inverted 3 or 4 times, and kept at 4°C. The samples for FPA, FPB\(\beta_{15-42}\) and FDP-D determination were mixed with heparin in a ratio of 1 volume of anticoagulant to 9 volumes of blood. The blood samples for obtaining plasma were subjected to 10 min centrifugation at 4°C. The blood samples for obtaining serum were kept at room temperature for more than 1 hour, and then subjected to 30-min centrifugation. All blood samples were stored at −20°C until assay. Aliquots of 24-hour urine collections were centrifuged at 1500 rpm for 10 min at 4°C, and stored at −20°C until assay. Ten hospitalized diabetic nephropathy patients with high levels of urinary FPA, FDP and FDP-D were selected (Table 2). The mean age of the 10 patients was 58.1 ± 10.2 years old. All had non-insulin dependent diabetes mellitus. Seven patients were treated with insulin, 2 with oral hypoglycemic drugs and only one by means of diet. Subcutaneous administration of 7,500 units of long-acting heparin calcium was carried out every 12 hours for 14 days. Blood samples and aliquots of 24-hour urine collections at 4°C were obtained before and at 7 and 14 days after the administration of heparin. Estimations of the amounts of urinary protein and several biochemical parameters, such as the serum total protein and albumin concentration, were performed. In addition, the 24-hour creatinine clearance was measured before and after the heparin administration.

Statistics: All data in the tables and figures are expressed as the means ± SD, and the paired or unpaired Student's t test was used for analysis. p-values of less than 0.05 were considered as statistically significant.

**Result**

The HbA\(_1c\) levels in group 1 were slightly higher than those in group 2, but the fasting blood glucose levels in the 2 groups were not significantly different (Table 1). The blood-glucose control of the patients treated with heparin was good (Table 2). The creatinine clearance in these patients varied from 11.0 ml/min to 48.5 ml/min (mean, 30.6 ± 13.1 ml/min). The amounts of urinary protein was 3.5 ± 1.6 g/day. Fig. 1 illustrates the blood levels of fibrinogen, FPA, FPB\(\beta_{15-42}\) and FDP in groups 1 and 2. The concentrations of plasma FPA and FPB\(\beta_{15-42}\) in the diabetic patients were higher than the normal ranges, although there was no difference in the
levels of serum FDP between the diabetic patients and healthy subjects. The plasma fibrinogen concentration was significantly higher in group 1 than in group 2, but there were no differences in levels of FDP, FPA and FPB_{15-42} in the blood between the 2 groups. Twenty-five out of the 41 diabetic patients (61%) revealed high concentrations of blood FPA of more than 5 ng/ml, which is the upper limit of the normal plasma FPA concentration. All 41 diabetic patients (100%) displayed high concentrations of blood FPB_{15-42}.

Data for the levels of FDP, FPA and FPB_{15-42} in the urine of groups 1 and 2 are summarized in Fig. 2. In group 1, abnormally high concentrations of these urinary fibrinolysis markers were observed in 6 of 20 patients (30%)

Table 1. Clinical profiles of diabetic patients (20 with nephropathy; 21 without nephropathy) whose coagulation and fibrinolysis in the blood and urine were examined

<table>
<thead>
<tr>
<th>Nephropathy</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Urinary protein (mg/dl)</td>
<td>55.1 ± 88.7</td>
<td>0</td>
</tr>
<tr>
<td>24Ccr (ml/min)</td>
<td>68.0 ± 14.3</td>
<td>53.1 ± 24.6</td>
</tr>
<tr>
<td>Fasting blood sugar (mg/dl)</td>
<td>165.8 ± 40.3</td>
<td>147.3 ± 38.9</td>
</tr>
<tr>
<td>Hba_{1c} (%)</td>
<td>7.3 ± 1.6</td>
<td>6.6 ± 1.4</td>
</tr>
</tbody>
</table>

24Ccr, 24-hour creatinine clearance.

Table 2. Clinical profiles of diabetic nephropathy patients who were treated daily with 15,000 units of heparin for 14 days

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Duration of DM (yr)</th>
<th>Hba_{1c} (%)</th>
<th>FBS (mg/dl)</th>
<th>U-Pro (g/day)</th>
<th>24Ccr (ml/min)</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>43</td>
<td>M</td>
<td>13</td>
<td>n.d.</td>
<td>96</td>
<td>4.3</td>
<td>23.9</td>
<td>Insulin</td>
</tr>
<tr>
<td>2.</td>
<td>52</td>
<td>M</td>
<td>6</td>
<td>5.6</td>
<td>105</td>
<td>6.3</td>
<td>47.1</td>
<td>S.U.</td>
</tr>
<tr>
<td>3.</td>
<td>53</td>
<td>M</td>
<td>17</td>
<td>n.d.</td>
<td>133</td>
<td>5.8</td>
<td>36.7</td>
<td>Insulin</td>
</tr>
<tr>
<td>4.</td>
<td>48</td>
<td>M</td>
<td>16</td>
<td>8.6</td>
<td>126</td>
<td>3.0</td>
<td>13.2</td>
<td>Insulin</td>
</tr>
<tr>
<td>5.</td>
<td>54</td>
<td>F</td>
<td>12</td>
<td>5.1</td>
<td>120</td>
<td>3.8</td>
<td>21.8</td>
<td>Diet</td>
</tr>
<tr>
<td>6.</td>
<td>73</td>
<td>M</td>
<td>12</td>
<td>6.1</td>
<td>150</td>
<td>2.5</td>
<td>24.2</td>
<td>Insulin</td>
</tr>
<tr>
<td>7.</td>
<td>65</td>
<td>F</td>
<td>20</td>
<td>n.d.</td>
<td>123</td>
<td>3.7</td>
<td>48.5</td>
<td>Insulin</td>
</tr>
<tr>
<td>8.</td>
<td>55</td>
<td>M</td>
<td>19</td>
<td>6.9</td>
<td>129</td>
<td>1.9</td>
<td>41.6</td>
<td>S.U.</td>
</tr>
<tr>
<td>9.</td>
<td>72</td>
<td>F</td>
<td>15</td>
<td>5.7</td>
<td>130</td>
<td>2.3</td>
<td>13.1</td>
<td>Insulin</td>
</tr>
<tr>
<td>10.</td>
<td>66</td>
<td>M</td>
<td>15</td>
<td>n.d.</td>
<td>87</td>
<td>3.6</td>
<td>11.0</td>
<td>Insulin</td>
</tr>
<tr>
<td>MEAN</td>
<td>58.1</td>
<td></td>
<td>14.9</td>
<td>6.3</td>
<td>119</td>
<td>3.48</td>
<td>30.6</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>10.2</td>
<td></td>
<td>4.1</td>
<td>1.26</td>
<td>18.8</td>
<td>1.63</td>
<td>13.1</td>
<td></td>
</tr>
</tbody>
</table>

FBS, Fast blood sugar; U-Pro, urinary protein; n.d., Not done;  , sulfonylurea.
Fig. 1. Levels of fibrinogen, fibrin/fibrinogen degradation products (FDP), fibrinopeptide A (FPA) and fibrinopeptide B$_{15-42}$ (FPB$_{15-42}$) in the blood of diabetic patients with nephropathy (group 1) and without nephropathy (group 2).

Fig. 2. Levels of urinary FDP, FPA and FPB$_{15-42}$ in diabetic patients with nephropathy (group 1) and without nephropathy (group 2).

for FDP, 6 of 20 (30%) for FPA and 12 of 20 (75%) for FPB$_{15-42}$. On the other hand, group 2 contained no patients whose urinary FDP or FPA levels showed abnormalities, and there were only 2 patients with abnormal findings for in urinary FPB$_{15-42}$. Significant differences in urinary FDP, FPA and FPB$_{15-42}$ existed between groups 1 and 2.

We examined the effects of heparin administration in patients with diabetic nephropathy due to non-insulin dependent diabetes mellitus. As mentioned above, 7,500 units of subcutaneous heparin was administered twice daily to 10 diabetic nephropathy patients for 14 days, and the effects of this heparin on the coagulation and fibrinolysis parameters were evaluated. There were no significant changes in blood FDP, FDP-D and FPA following the heparin administration (Fig. 3). However, the blood FPA did decrease in 3 out of 10 patients who had shown abnormally high con-
Anticoagulant therapy in diabetic nephropathy

Fig. 3. Effects of heparin on the blood FDP, FDP-D and FPA in 10 diabetic nephropathy patients.

Fig. 4. Effects of heparin on the urinary FDP in 10 diabetic nephropathy patients.

Fig. 5. Effects of heparin on the urinary FDP-D in 10 diabetic nephropathy patients.

Fig. 6. Effects of heparin on the urinary FPA in 10 diabetic nephropathy patients.

Centrations of blood FPA. The effects of the heparin administration on the urinary FDP are illustrated in Fig. 4. Although the amounts of urinary FDP were decreased in 7 out of the 10 patients, the difference was not statistically significant. Marked reductions in the amounts of urinary FDP-D and FPA were observed. As shown in Figs. 5 and 6, respectively, such changes occurred in 7 out of the 10 patients in the case of the urinary FDP-D and in 8 out of 9 patients in the case of the urinary FPA at 7 days after the
Fig. 7. Effects of heparin on the urinary protein in 10 diabetic nephropathy patients.

Fig. 8. Effects of heparin on the concentrations of total protein (TP) and serum albumin (Alb) in 10 diabetic nephropathy patients.

Fig. 9. Effects of heparin on renal function in 10 diabetic nephropathy patients. 24Ccr, 24-hour creatinine clearance; PSP, phenolsulphonphthalein test.

heparin administration, and these changes continued for 14 days. The changes in urinary protein were also investigated before and after the heparin administration. As shown in Fig. 7, a significant reduction in urinary protein excretion was observed. The changes in concentrations of both total protein and serum albumin were examined before and after the heparin administration. Significant increases in both parameters occurred. The 24-hour creatinine clearances were unchanged after the therapy (Fig. 9).

Discussion

Groups of diabetic patients (both insulin dependent and non-insulin dependent) are known to exhibit a decrease in blood fibrinolytic activities [10-14]. In addition, coagulation per se is believed to be slightly activated in diabetics [15, 16]. However, there have been few studies comparing the degrees of coagulation and fibrinolysis in patients with and without nephropathy. In the present study, the levels of FPA, FPB, FDP, FDP-D and FDP-E were measured in order to assess the conditions of coagulation and fibrinolysis in both patients with and patients without diabetic nephropathy. FPA is a low molecular weight peptide which is cleaved from fibrinogen by the action of thrombin. It has been inferred therefore that the circulating FPA reflects the thrombin activity in vivo. Our data clearly demonstrated that the blood FPA was high in
Anticoagulant therapy in diabetic nephropathy

diabetes mellitus. An increase in \( \text{FPB}_{\beta 15-42} \) indicated that fibrinolysis was also increased in the diabetic patients. Since no differences in both the blood FPA and \( \text{FPB}_{\beta 15-42} \) were observed between groups 1 and 2, it appeared that the diabetic patients with nephropathy exhibited activation of both coagulation and fibrinolysis. However, the fact that the plasma fibrinogen concentration in group 1 was significantly higher than that in group 2 suggested that the coagulation in group 1 is might be slightly higher than that in group 2. We measured several molecular markers of coagulation and fibrinolysis including FDP, FDP-E, FDP-D and FPA. Such molecular markers in the urine are believed to be better parameters than those in the blood for evaluating the intraglomerular coagulation and fibrinolysis. It is considered, however, that all of such molecular markers excreted into the urine do not always originate from intraglomerular coagulation and fibrinolysis. Some of the molecular markers in the plasma are also excreted into the urine through the glomerular basement membrane with other plasma proteins.

We suggested in a previous paper [17] that a urinary FDP of which the ratio to urinary protein in renal disease patients exceeded 1.0 (mg of urinary FDP/g of urinary protein) might be derived mainly from intraglomerular coagulation and fibrinolysis, because the urinary FDP in such patients declined after heparin administration without any changes in the serum FDP levels. In addition, a urinary FDP of which the ratio to urinary protein was less than 0.5 (mg of urinary FDP/g of urinary protein) might be considered to be derived mainly from plasma fibrinogen and serum FDP, because the urinary FDP in such patients was not reduced by heparin administration. Since the ratio of urinary FDP to urinary protein in all of the diabetic nephropathy patients examined in the present study exceeded 1.0 (mg/g), we believe that the increased amounts of urinary molecular markers in these diabetic nephropathy patients might have originated mainly from intraglomerular coagulation and fibrinolysis, although part of the markers was also excreted into the urine through the glomerular basement membrane with its increased permeability. Significant positive correlations were noted among the urinary FPA, FDP-D and FDP, and both the amounts of molecular markers of coagulation (FPA) and fibrinolysis (FDP-D and FDP-E) were reduced after heparin administration, suggesting that both coagulation and fibrinolysis might have occurred simultaneously in the glomeruli of the diabetic nephropathy patients. Ten diabetic patients were selected to be treated with heparin (Table 2). Although the diabetic control was good in these patients (HbA1c, 6.3 ± 1.26%), the amount of urinary protein was quite large (mean, 3.48 ± 1.63 g/day), and the values of the 24-hour creatinine clearance were decreased (30.6 ± 13.1 ml/min). Subcutaneous administration of 7,500 units of heparin was performed twice daily in order to obtain a continuous antithrombin action for 24 hours. This dose did not induce any bleeding tendency. In fact, no serious side effects were observed in the present study. Blood parameters such as the levels of FPA, FDP-D and FDP were not significantly changed after the therapy, but the plasma FPA levels in 2 patients which were above normal did decline. The levels of FPA and FDP in the urine underwent a marked decrease, although the decrement in the urinary FDP was not significant. Heparin is thus considered to have improved the intraglomerular coagulation and fibrinolysis of the diabetic nephropathy patients. Surprisingly, the amount of urinary protein also decreased, and the concentrations of serum albumin and total protein increased. Although the cause of the decrease in urinary protein remains unclear, improvement of the intraglomerular coagulation and fibrinolysis induced by heparin might restore the permeability of the glomerular capillaries. Since it has been shown, however, that heparin has a capacity for decreasing both the number of anionic sites and the urinary albumin excretion in streptozotocin-induced diabetic rats [18], these effects of heparin in causing a lower urinary protein excretion might differ from the anticoagulant effect. In addition, we must consider that heparin has the effect of reducing the blood pressure [19], increasing the glomerular filtration rate [20], and inhibiting mesangial cell proliferation [21]. Since the duration of heparin therapy in the present study was only 2 weeks, it is not appropriate to infer whether or not heparin has any effect on the prevention of the decrease in glomerular filtration rate. Long-term heparin administration should be undertaken in order to
elucidate the beneficial effects of heparin on renal function.

In summary, measurements of the urinary FDP, FDP-D and FPA could be valuable parameters for determining the presence of intrarenal coagulation and fibrinolysis. Intraglomerular coagulation might be one of the important factors leading to progression of renal dysfunction. Anticoagulant therapy could become effective and probably improve the progression of renal dysfunction in diabetic nephropathy, if the patients are selected using sensitive urinary parameters of coagulation and fibrinolysis. Anticoagulant therapy with heparin could provide a new therapy for treating diabetic nephropathy patients at the phase of macroproteinuria. It is necessary, however, to examine which other anticoagulants, such as antiplatelet drugs, might have similar effects on intraglomerular coagulation.

Reprint requests to: Kazo Kaizu, M.D. Kidney Center, University of Occupational and Environmental Health, I-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807, JAPAN

References
