Antinephritic Effects of PGE₁ and Thiaprostaglandin E₁, TEI-5178 and TEI-6122, on Crescentic-Type Anti-GBM Nephritis in Rats

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Abstract—The antinephritic effects of PGE₁, TEI-5178 and TEI-6122 on crescentic-type anti-glomerular basement membrane (GBM) nephritis in rats were investigated. The test compounds were subcutaneously administered every day for 39 days after the injection of anti-GBM serum. PGE₁ (2.0 mg/kg/day), TEI-5178 (0.25 or 0.5 mg/kg/day) and TEI-6122 (0.25 or 0.5 mg/kg/day) significantly reduced urinary protein by 30 to 50% of that of the control at the late stage of nephritis. These test compounds also suppressed the increase of blood urea nitrogen and the development of alteration in the glomeruli by the 40th day. Both TEI-5178 (0.5 mg/kg/day) and TEI-6122 (0.5 mg/kg/day) significantly suppressed the production of antibody to rabbit γ-globulin in nephritic rats. This was not the case with PGE₁, however. In additional experiments to clarify the antinephritic mechanisms of the test compounds, it was found that 15 min after one subcutaneous injection of PGE₁ (1.0 mg/kg), TEI-5178 (0.5 mg/kg) or TEI-6122 (0.5 mg/kg), systolic blood pressure in the nephritic rats was transiently reduced by 50 to 60%. On the other hand, these test compounds augmented renal blood flow (20–50%) from 45 min after the injection. The relationship between the antinephritic effect and these subsequent findings will be discussed.

In 1977, Zurier et al. (1, 2) reported that prostaglandin E₁ (PGE₁) protected NZB/NZW F₁ hybrid mice against the development of anemia and lupus nephritis, and then prolonged life. They suspected that the effects of PGE₁ might depend on the enhancement of T-cell activity and the suppression of B-cell activity (3–5). We previously demonstrated that PGE₁-α-cyclodextrin remarkably suppressed the development of chronic serum sickness nephritis in rats (6, 7). Rats with this disease showed crescent formation and adhesion of the capillary wall to Bowman’s capsule in the glomeruli in the final stage of the disease (8), indicating hypercoagulation in the microcirculation of the glomeruli. Hypercoagulation and platelet hyperaggregation in the glomeruli are associated with the development of crescentic-type anti-glomerular basement membrane (GBM) nephritis (9). In the present study, we evaluated the antinephritic effects of PGE₁ and thiaprostaglandin E₁, TEI-5178 and TEI-6122, using rats exhibiting crescentic-type anti-GBM nephritis. Hypertension and reduced renal blood flow are manifestations of deterioration in glomerulonephritis (10, 11). It is well-known that PGE₁ lowers blood pressure, dilates blood vessels, and enhances blood flow. Therefore, additional investigation was undertaken regarding the effects of these agents on blood pressure and renal blood flow in nephritic rats.

Materials and Methods

1. Drugs
PGE1 (Funakoshi Yakuhin Co., Ltd.), TEI-5178 and TEI-6122 (Teijin Co., Ltd.) were used in this study (Fig. 1). TEI-5178 and TEI-6122 are 7-thiaprostaglandin E1 derivatives (12, 13). These test compounds were dissolved in absolute ethanol and stored at -20°C. Just prior to administration, the test compounds were diluted with phosphate-buffered saline (PBS) at pH 7.2.

2. Crescentic-type anti-GBM nephritis

Crescentic-type anti-GBM nephritis was induced by the method of Ito et al. (14). Male Sprague-Dawley rats (Nihon SLC Co., Ltd.), weighing 160 to 170 g, were used. The animals were intravenously injected with 0.56 ml/rat of anti-GBM rabbit serum that was produced as previously reported (15). On the next day, the rats were injected with 5 mg/rat of rabbit γ-globulin (Miles) in 0.25 ml of Freund's complete adjuvant (Difco) into the hind foot pads.

3. Administration

To evaluate the antinephritic effect of the test compounds, for 39 days, the animals were subcutaneously given 1.0 or 2.0 mg/kg/day of PGE1, 0.25 or 0.5 mg/kg/day of TEI-5178 or TEI-6122 in a volume of 0.1 ml/100 g body weight. For the administration of PGE1, a dose was injected twice daily, in the morning and the afternoon. The control rats were given 10% ethanol solution in the same manner. Each group consisted of 8 to 10 animals.

To clarify the antinephritic mechanisms of the test compounds, 5 to 6 nephritic rats were subcutaneously injected with 1.0 mg/kg of PGE1, 0.5 mg/kg of TEI-5178 or 0.5 mg/kg of TEI-6122. The experiments were performed on the 40th day after the injection of anti-GBM serum.

4. General condition, body weight and urine volume

The general condition of the rats was observed over the course of the experiments. Body weight was determined every other day and urine volume, every 10 days.

5. Urine, plasma and kidney

Urine samples were obtained every 10 days after the injection of anti-GBM serum. Each rat was orally given 8 ml of tap water through a stomach tube and then kept in an individual metabolic cage for 24 hr without access to food or water. After this period, a urine sample was collected, centrifuged at 3,000 rpm for 15 min at 4°C, and the supernatant utilized for urinalysis. Blood samples were obtained every 10 days after the injection of anti-GBM serum. Blood was drawn from the tail with a heparinized microsyringe or from the abdominal aorta with a microsyringe in the ratio of 9 volumes of blood to 1 volume of 3.13% trisodium citrate; this was then centrifuged at 3,000 rpm for 10 min to obtain plasma. The kidneys were isolated for light microscopy after drawing blood on the 40th day.

6. Determinations

Urinary protein: The protein content in urine was determined by the sulfosalicylic acid method (16) and expressed as mg per 24 hr urine.
Plasma urea nitrogen: Urea nitrogen content was determined by the urease-indophenol method (17) and expressed as mg per 100 ml of plasma.

Antibody titer: The anti-rabbit γ-globulin titer was determined by the passive hemagglutination method (18) using sensitized sheep red blood cells and expressed as log₂ of the highest dilution that caused no visible agglutination of sensitized sheep blood cells.

Blood pressure: The test compounds were given after the control measurement. The animals were warmed for 3 min in a preheating box (60°C), and then systolic blood pressure was recorded by tail plethysmography (KN-209, Natsume, Co., Ltd.).

Renal blood flow: Rats were anesthetized with sodium pentobarbital (32.4 mg/kg, i.p.) (Dainippon Pharmaceutical Co., Ltd.), and the left kidney was exposed through a flank incision. A polaro electrode was superficially implanted into the kidney, and a reference electrode was inserted into the abdomen far from the polaro electrode. Renal blood flow was measured with an electrolytic tissue blood flow meter (RBF-2, Biomedical Science Co., Ltd.). The test compound was given after the control measurement. The result was expressed as ml/min/100 g tissue.

7. Light microscopy

The kidneys were isolated on the 40th day after the injection of anti-GBM serum. They were then dehydrated and fixed by progressively higher concentrations of chilled alcohol diluted with Tris-HCl buffer at pH 7.5. The tissue was then embedded in paraffin and cut into 2- to 3-μm thick sections. The sections were stained with hematoxylin and eosin (HE) and Masson's trichrome. Fifty glomeruli were assessed for the degree of lesions. The index regarding hypercellularity, crescent formation, fibrinoid necrosis and adhesion of capillary walls to Bowman's capsule was calculated by the method of Suzuki et al. (19). Each histopathological parameter was graded as normal (0 points), mild (1 point), moderate (2 points) or severe (3 points) according to the extent of the alteration. The number of glomeruli corresponding to each score was represented as n₀. n₁. n₂ and n₃. Each index was calculated as follows: index=(1×n₁)+(2×n₂)+(3×n₃).

8. Statistical analysis

All data represent the mean±S.D., and the results were statistically evaluated by Student's t-test or Mann-Whitney's U-test. In all comparisons, differences were considered significant at P<0.05, P<0.01 or P<0.001. Inhibitory percentage was calculated as follows:

Inhibitory percentage (%) \[=\frac{\text{Control} - \text{Test drug}}{\text{Control} - \text{Normal}} \times 100\]

Results

1. General condition

No rats died from injection with PGE₁, TEI-5178 and TEI-6122, and no rats showed growth retardation. Similar food consumption was observed in each group. However, the rats transiently suffered from severe diarrhea and somnolence just after the administration of PGE₁, especially at the higher dose. We did not observe any other abnormal behavior.

2. Body weight and urine volume (Table 1)

The nephritic rats, including the compound-administered rats, showed growth retardation as compared with the normal rats. In addition, the test compound-administered rats were of significantly less body weight than the control rats at the end of the experiment. Test compound-administered rats sometimes exhibited greater urine volume than the normal and/or the control groups, suggesting a rebound of renal function.

3. Effects of PGE₁, TEI-5178 and TEI-6122 on biochemical parameters in urine and plasma

Urine protein (Fig. 2): The control group showed a value of about 200 mg/day on the 10th day after the injection of anti-GBM serum. Thereafter, proteinuria was maintained at about this level throughout the experimental period. In contrast, 2.0 mg/kg of PGE₁ caused a significant suppression of protein output: 44% on the 20th day, 76% on the 30th day and 73% on the 40th day. TEIs significantly suppressed protein excretion into the urine in the late stage of the experiment: the administration of TEI-5178 resulted in 41 to 61% inhibition at 0.5 mg/kg/day, while TEI-6122 resulted in 60 to 70% inhibition at 0.5
Table 1. Changes in body weight and urine volume during administration of PGE₁, TEI-5178 or TEI-6122 in crescentic-type anti-GBM nephritis in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>1</th>
<th>20</th>
<th>40 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>B.W.</td>
<td>161.4±7.5</td>
<td>267.4±7.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U.V.</td>
<td>14.5±1.3</td>
<td>15.4±1.5</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>B.W.</td>
<td>162.1±7.2</td>
<td>231.0±5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U.V.</td>
<td>12.1±1.1</td>
<td>14.6±1.9</td>
</tr>
<tr>
<td>PGE₁</td>
<td>10</td>
<td>B.W.</td>
<td>160.7±4.7</td>
<td>229.6±5.2</td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td></td>
<td>U.V.</td>
<td>12.3±1.3</td>
<td>14.3±1.4</td>
</tr>
<tr>
<td>PGE₁</td>
<td>10</td>
<td>B.W.</td>
<td>164.4±3.8</td>
<td>230.2±8.0</td>
</tr>
<tr>
<td>2.0 mg/kg</td>
<td></td>
<td>U.V.</td>
<td>11.8±2.0</td>
<td>17.8±1.6</td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>B.W.</td>
<td>161.0±3.8</td>
<td>218.3±12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U.V.</td>
<td>11.1±3.9</td>
<td>12.4±1.0</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>B.W.</td>
<td>159.7±5.2</td>
<td>212.5±15.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>U.V.</td>
<td>14.4±2.1</td>
<td>15.7±4.6</td>
</tr>
<tr>
<td>TEI-5178</td>
<td>8</td>
<td>B.W.</td>
<td>161.0±8.8</td>
<td>203.5±14.6</td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td></td>
<td>U.V.</td>
<td>13.7±1.3</td>
<td>20.3±1.6</td>
</tr>
<tr>
<td>TEI-5178</td>
<td>8</td>
<td>B.W.</td>
<td>158.8±2.4</td>
<td>190.8±12.5*</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td></td>
<td>U.V.</td>
<td>14.7±2.3</td>
<td>20.3±1.9</td>
</tr>
<tr>
<td>TEI-6122</td>
<td>8</td>
<td>B.W.</td>
<td>158.3±6.1</td>
<td>203.1±7.2</td>
</tr>
<tr>
<td>0.25 mg/kg</td>
<td></td>
<td>U.V.</td>
<td>14.6±1.3</td>
<td>18.3±4.8</td>
</tr>
<tr>
<td>TEI-6122</td>
<td>8</td>
<td>B.W.</td>
<td>159.8±8.3</td>
<td>187.8±13.2</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td></td>
<td>U.V.</td>
<td>14.2±1.4</td>
<td>17.5±2.8</td>
</tr>
</tbody>
</table>

Numbers indicate the mean±S.D. *shows a significant difference from the control at P<0.05. B.W.: body weight (g). U.V.: urine volume (ml).

Fig. 2. Effects of PGE₁, TEI-5178 and TEI-6122 on urinary protein content in crescentic-type anti-GBM nephritis in rats. Each drug was given daily from the day after the injection of anti-GBM serum (the first day) to the 39th day. ○: normal; ●: control; PGE₁: 0.5 mg/kg/day ○, 2.0 mg/kg/day ●; TEI-5178: 0.25 mg/kg/day △, 0.5 mg/kg/day ▲; TEI-6122: 0.25 mg/kg/day ○, 0.5 mg/kg/day □. Each plot denotes the mean±S.D. of 8 or 10 rats. *indicates a significant difference from the control at P<0.05.

Plasma urea nitrogen (Fig. 3): On the 40th day, the control rats had 30 to 35 mg/100 ml of urea nitrogen. This level was significantly higher (50–60%) than that of the normal rats. PGE₁ at 2.0 mg/kg/day could remarkably suppress the increase of urea nitrogen level by about 30% of the control level. The administration of TEI-5178 resulted in a significant suppression of 50 to 90% over the experimental period. However, TEI-6122 showed a significant suppression of 70 to 80% on the 40th day.

Antibody titer (Fig. 4): In the control group,
antibody titer gradually increased with time. PGE\(_1\) could not influence the production of antibody, although the data are not shown here. TEIs at 0.5 mg/kg/day caused a 20 to 30% reduction as compared with that in the control group in the late stage of the experiment.

4. Effects of PGE\(_1\), TEI-5178 and TEI-6122 on histological alteration of glomeruli (Fig. 5)  
PGE\(_1\) at 2.0 mg/kg/day significantly decreased the index of hypercellularity by 48%, the index of crescent formation by 24%, and the index of adhesion by 71%, respectively, in comparison to those of the control. TEI-5178 at 0.5 mg/kg/day significantly decreased only the index of hypercellularity by 32%. TEI-6122 at both 0.25 and 0.5 mg/kg/day significantly lowered the index of hypercellularity by 40% and 27%, the index of crescent formation by 25% and 16%, and the index of adhesion by 54% and 35%, respectively.

5. Effects of PGE\(_1\), TEI-5178 and TEI-6122 on blood pressure in crescentic-type anti-GBM nephritic rats (Table 2)  
Although data are not shown, the normal rats exhibited a systolic blood pressure of about 110 mmHg. The blood pressure in the crescentic-type anti-GBM nephritic rats was significantly higher (about 135 mmHg). PGE\(_1\) and TEIs showed the maximal hypotensive effect (about 50%) 15 min after the injection of the test compounds. Although the decreased blood pressure was gradually restored to the control level, a significant depression of about 20% was observed 90 min after administration.

6. Effects of PGE\(_1\), TEI-5178 and TEI-6122 on renal blood flow in crescentic-type anti-GBM nephritic rats (Table 3)  
The administration of PGE\(_1\) resulted in a significant increase of about 50% in renal blood flow 90 min after administration as compared with the control level. Renal blood flow tended to increase following administration in nephritic rats treated with TEIs.

Discussion  
In the present study, we demonstrated that PGE\(_1\), TEI-5178 and TEI-6122 (TEIs) significantly suppressed the development of crescentic-type anti-GBM nephritis. The course of experimental anti-GBM nephritis is divided
into two phases: the heterologous phase, which appears one day after the injection of anti-GBM serum as shown by the interaction of rabbit anti-GBM antibody with GBM antigen, and the autologous phase, which can be determined by the interaction of rabbit antibody with rat anti-rabbit antibody a week after the antiserum injection. In the current nephritic model, rats were immunized with rabbit r-globulin in Freund's complete adjuvant. Consequently, the immune reaction in the autologous phase was enhanced, and the collapse of the GBM and hypercoagulation in the glomerular capillary lumen could lead to fibrinoid necrosis of the glomeruli and the crescent formation in Bowman's space (15). In the current study, PGE\(_1\) failed to inhibit the production of antibody directed to rabbit \(\gamma\)-globulin. TEIs also could not inhibit antibody production until day 20. Kunkel et al. (20) demonstrated that 15(s),15 methyl PGE\(_1\) significantly suppressed protein ex-
creatinuria and the development of glomerular alteration in rats exhibiting nephrotoxic nephritis, and they also found that rats treated with 15(s),15 methyl PGE1 had a similar amount of anti-GBM antibody deposited in the glomeruli as the control rats. Therefore, this suppression of antibody production seems little attributable to the antinephritic effects of PGE1 and TEIs on anti-GBM nephritis even though both TEIs suppressed antibody synthesis in the late stage of the experiment, and we had demonstrated the suppressive effect of PGE2 on antibody production in serum sickness nephritis (7).

In additional studies, we investigated the effects of the test compounds on systolic blood pressure and renal blood flow in nephritic rats to clarify the antinephritic mechanisms of the test compounds since higher blood pressure and less blood flow in the glomeruli are generally thought to play a role in the deterioration of glomerulonephritis (21). Iversen and Ofstad observed that rats with passive Heymann nephritis with hypertension exhibited greater protein excretion into the urine and less creatinine clearance than nephritic rats without hypertension. From this, they concluded that hypertension increased glomerular damage substantially in rats with immune complex glomerulonephritis (22). In addition, when hypertension was induced in rats with serum sickness, glomerular damage increased (23). In Dahl salt-sensitive rats with mesangial ferritin-antiferritin immune complex disease and hypertension, mesangial injury and glomerulosclerosis were amplified as compared with nephritic rats without hypertension (24).

Table 2. Effects of PGE1, TEI-5178 and TEI-6122 on blood flow in crescentic-type anti-GBM nephritic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PGE1</td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>147.7±12.0</td>
</tr>
<tr>
<td>TEI-5178</td>
<td>133.0±6.0</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>100.0</td>
</tr>
<tr>
<td>TEI-6122</td>
<td>134.3±5.5</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Results are the mean±S.D. obtained from 5 to 6 nephritic rats. Significant difference from the control blood pressure at 0 min: *P<0.05, **P<0.01, ***P<0.001. The number beneath each result shows the percentage of blood pressure using that at 0 min as 100%.

Table 3. Effects of PGE1, TEI-5178 and TEI-6122 on renal blood flow in crescentic-type anti-GBM nephritic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Renal blood flow (ml/min/100 g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>PGE1</td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg</td>
<td>50.2±5.7</td>
</tr>
<tr>
<td>TEI-5178</td>
<td>42.6±3.2</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>100.0</td>
</tr>
<tr>
<td>TEI-6122</td>
<td>54.7±6.0</td>
</tr>
<tr>
<td>0.5 mg/kg</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Results are the mean±S.D. obtained from 5 to 6 nephritic rats. Significant difference from the control renal blood flow at 0 min: *P<0.01. The number beneath each result shows the percentage of renal blood flow using that at 0 min as 100%.
Moyer et al. reported that progressive renal deterioration in patients with hypertension was prevented by lowering the blood pressure (10). Therefore, it is likely from the experimental and clinical evidence that the control of blood pressure with PGE₁ or TEIs prevents the development of renal damage in crescentic-type anti-GBM nephritic rats.

We showed that these agents could increase the renal blood flow and lower blood pressure in the nephritic rats. It is difficult to account for the relationship of the antinephritic effect and the augmentation of renal blood flow with regard to these agents. Neugarten et al., however, demonstrated that two kidney clip induced hypertension enhanced glomerular proliferation and sclerosis in anti-GBM nephritis (25). Although they did not determine the renal blood flow in the nephritic rats during renal artery clipping, we can surmise that it decreased because of this procedure. Renal ischemia causes lipid alteration in the membrane, particularly of the proximal tubular brush border (11). Glomeruli obtained from nephritic rats produce more thromboxane A₂ than those of normal rats (26, 27), and cultured mesangial cells have the ability to contract utilizing thromboxane A₂ (28). Mesangial cells seem to control the blood flow in the glomeruli because of their contractive ability in response to certain substances (29, 30). Therefore, it is likely that in glomerulonephritis, the glomerular blood flow rate may be decreased due to increased production of thromboxane A₂, which may induce aggregation of platelets and contraction of the glomerular capillaries. Consequently, this condition may cause nutritional deficiencies and metabolic changes in the glomeruli. It has been shown that acute arterial obstruction and subsequent necrosis are induced by the injection of lycopodium power suspension or lauric acid solution into the femoral artery (31) and that subcutaneous injection of adrenaline and ergotamine results in a tail peripheral circulation disorder in rats (32). Kawasaki et al. (31) demonstrated that PGE₁ improved considerably a peripheral circulation disorder induced in dog feet, and they suggested that the effect of PGE₁ may be due to its vasodilator and anti-platelet aggregating actions. TEIs strongly inhibit platelet aggregation in the circulation as does PGE₁, so that it is likely that both PGE₁ and TEIs can dilate the glomerular capillary and inhibit platelet aggregation in the lumen, and then wash out the already aggregated platelets from the glomeruli. It is thought that nutritional deficiencies and metabolic alterations in the glomeruli may be prevented by the administration of these compounds. Although we think that the present results regarding blood pressure and renal blood flow can partly explain the antinephritic effect of these compounds on anti-GBM nephritis, further study is required because 1) the current study was performed in a one shot-trial of these compounds, and 2) in the renal blood flow experiment, TEIs exhibited only slight augmentation, and we did not measure RPF (renal plasma flow) using inulin.

References
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