Effects of Endothelin and Angiotensin II on Renal Hemodynamics in Experimental Mesangial Proliferative Nephritis

Key Words
Glomerulonephritis
Endothelin
Angiotensin II
Hemodynamics
Hypertension

Abstract
The renal hemodynamic response to pressor substances in the diseased kidney has been suggested to be different from that in the normal kidney. The aim of this study was to investigate the effects of endothelin and angiotensin II on renal hemodynamics in experimental nephritis induced by the administration of antithymocyte serum in Wistar rats. This model showed mesangiolysis in the glomeruli on day 2 and hypercellular lesions on day 8. Prior to the injection of either endothelin or angiotensin II, the glomerular filtration rate and renal plasma flow were significantly lower in model rats on day 2 or day 8 than in the control rats. The basal glomerular filtration rate and renal plasma flow on day 8 were negatively correlated with the mesangium cell number. The injection of endothelin (0.5 ng/kg BW) led to a decrease in both renal plasma flow and glomerular filtration rate in rats on day 8 which was significantly greater than that in the control rats. Similarly, angiotensin II infusion (0.2 μg/kg BW) reduced both renal plasma flow and glomerular filtration rate in the rats on day 8 and the reductions were significantly greater than those in the control rats. In conclusion, renal hemodynamics in rats with mesangial proliferation of the kidney were more sensitive to both endothelin and angiotensin II than those in the normal kidney.

Introduction
Hypertension frequently coexists with glomerulonephritis and is one of the factors accelerating renal dysfunction [1]. Systemic hypertension per se seems to have little hemodynamic effect on the normal kidney in a chronic hypertensive state, because renal vasculatures autoregulate intraglomerular pressure and flow against the changes of systemic arterial pressure by regulating arteriolar resistance [2].

On the other hand, the inability of the renal vasculature in the diseased kidney to autoregulate intraglomerular pressure has been suggested to lead to renal hyperhemodynamics [3]. Thus, the vascular response to the pressor substances in the diseased kidneys may be different from that in the normal kidneys. Endothelin (ET), which was recently disclosed, is a prominent and long-acting vasoconstrictor [4]. In others, angiotensin II (AII), which is one of the classical pressor substances, regulates renal circulation while reduc-
ing the glomerular ultrafiltration coefficient \(C_f\). Both ET and AIH induce the contractile response of renal arterioles or mesangial cells [6, 7]. In this report, we have shown the effect of vasocostricators such as ET and AIH on renal hemodynamics in the kidney with mesangial proliferation, using a rat model of glomerulonephritis induced by specific immunologic injury to the mesangial cells [8].

**Methods**

Induction of Experimental Glomerulonephritis

Antirat thymocyte serum (ATS) was produced by immunizing New Zealand White rabbits with \(1 \times 10^8\) Wistar rat thymocytes in complete Freund's adjuvant, followed with \(1 \times 10^7\) thymocytes given intravenously 2 and 4 weeks later [9]. Immunization serum was collected from the same animal and used in the control experiments as normal rabbit serum. Glomerulonephritis was induced in Wistar rats (150-200 g BW) by the intravenous administration of \(1 \times 10^6\) ATS/100 g BW. The control animals received the same dose of normal rabbit serum instead of ATS. The rats were killed for a histological examination of the kidney tissue after the studies of inulin and p-aminohippurate. The clearance of inulin and p-aminohippurate was done by the previously mentioned method [10]. Renal vascular resistance was calculated as follows: renal vascular resistance = mean blood pressure/\(C_f\) of p-aminohippurate.

The kidneys were fixed in 10\% neutral buffered formalin and \(C_f\) and p-aminohippurate clearances were determined after \(24 \text{ h}\) and \(48 \text{ h}\) after ATS administration.

**Results**

On day 2 after ATS injection, there was a decrease in the glomerular nucleon counts with mesangiosis (fig. 2), compared to the controls (fig. 1a). In contrast, the glomeruli showed a marked increase in day 8 (fig. 2). Figure 2 shows the number of nuclei per glomerulus in each stage. The nuclei number on day 2 was significantly lower than that of the controls and day 8 was significantly higher than that of the controls and day 2 group (p < 0.005). The serum concentrations of total protein, blood urea nitrogen, creatinine and sodium were not significantly different among all stages. Mean blood pressure and renal hemodynamics before the injection of AIH or ET in all subjects are shown in table 1. Before the injection of ET, there was no difference in the mean blood pressure among all groups. Rats on day 8 showed a reduction of renal function, reflected by the significantly lower levels of renal plasma flow (RPF) of 2.96 ± 0.42 (mean ± SD) ml/min/g kidney and glomerular filtration ratio (GFR) of 0.727 ± 0.079 ml/min/g kidney, and compared with those in the control rats and those of the day 2 group (p < 0.05). The renal vascular resistance (RVR) on day 8 was 55.3 ± 6.9 mm Hg/ml/g kidney, which was significantly higher than that on day 2 (p < 0.05), but not significantly different from the control.

In addition, the filtration fractions (FF) on day 2 and 8 were significantly lower than that of the control (p < 0.05). On day 8, both GFR and RPF were inversely correlated with the number of nuclei in the glomeruli (r = -0.76, p < 0.005 for GFR; r = -0.76, p < 0.05 for RPF), while RVR correlated positively with the nuclei number (R = 0.62, p < 0.05; fig. 5).

Blood pressure began to increase shortly after the injection of ET and remained high after 10-20 min. The degree of blood pressure increase by ET was about 40-45 mm Hg, which was not significantly different from either that of the control, or of the day 2 or day 8 group after ATS injection. After the injection of ET, reductions in GFR and RPF with elevated RVR were observed in all 3 groups (table 2). The changes of RPF and GFR on day 8 were -33.7 ± 6.2 and -30.0 ± 8.1 \%, and significantly lower than those of the control and day 2 group (p < 0.05 in GFR; p < 0.05 in RPF). There was no significant difference in the changes of GFR, RPF and RVR between the control and day 2 group (fig. 4). However, RVR on day 8 was more greatly changed than that in the control or day 2 group (p < 0.05). The decrease in FF did not correlate with the cell number (not shown).

The increase of AIH immediately increased the arterial blood pressure, and its degree of increase was not significantly different among the three 3 groups (table 3). The changes of GFR, RPF and RVR on day 8 were -25.9 ± 4.4, -36.7 ± 9.4 and 133 ± 38\% respectively, which were significantly greater than those in either the day 2 or the control group (p < 0.05; fig. 4).

**Table 1.** Renal hemodynamics in anti-Thy-1 nephritis

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Control</th>
<th>Day 2</th>
<th>Day 8</th>
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<tr>
<td>Mean blood pressure, mm Hg</td>
<td>105.0 ± 11</td>
<td>97.0 ± 10</td>
<td>102.8 ± 8</td>
<td></td>
</tr>
<tr>
<td>GFR, ml/min/g kidney</td>
<td>0.994 ± 0.080</td>
<td>0.830 ± 0.11</td>
<td>0.727 ± 0.079</td>
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<td>RPF, ml/min/g kidney</td>
<td>35.0 ± 10.5</td>
<td>4.4 ± 1.5</td>
<td>6.9 ± 1.4</td>
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<tr>
<td>RVR, mm Hg/ml/g kidney</td>
<td>30.0 ± 4.2</td>
<td>35.2 ± 6.9</td>
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</tr>
<tr>
<td>FF</td>
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Data are presented as the mean ± SD.

\(a\) vs control, \(p < 0.05\) versus control;

\(b\) vs control, \(p < 0.05\) versus control;

\(c\) vs control, \(p < 0.05\) versus day 2.

**Table 2.** Renal hemodynamics in anti-Thy-1 nephritis

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\(c\) vs control, \(p < 0.05\) versus day 2.
glomerular cells in mesangial proliferative nephritis, and All was greater in rats with mesangial proliferation than the glomeruli on day GFR.

Discussion

This study revealed that the basic values reduction of GFR and RPF and RVR correlated well with the number of glomerular cells in mesangial proliferative nephritis, and also that the decrease in GFR by the injection of either ET or All was greater in rats with mesangial proliferation than in those with normal kidneys. These results are consistent with the clinical findings that the fall in GFR in response to All is larger in patients with mesangial proliferation than in healthy subjects [11], suggesting the importance of mesangial proliferation on renal hemodynamics in glomerulonephritis.

Fig. 3. GFR, RPF and RVR in relation to the number of nuclei in the glomeruli on day 8 after ATS injection.

Table 2. Hemodynamic change after ET injection

<table>
<thead>
<tr>
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<th>n = 6</th>
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<tbody>
<tr>
<td>GFR</td>
<td>before</td>
<td>after</td>
<td>control</td>
</tr>
<tr>
<td>RPF</td>
<td>1.02±0.045</td>
<td>1.09±0.068</td>
<td>0.809±0.016</td>
</tr>
<tr>
<td>RVR</td>
<td>3.74±0.72</td>
<td>6.66±0.32</td>
<td>3.420±0.64</td>
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<tr>
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<tr>
<td>RPF</td>
<td>1.24±0.64</td>
<td>6.66±0.32</td>
</tr>
<tr>
<td>RVR</td>
<td>2.84±0.69</td>
<td>2.67±0.25</td>
</tr>
<tr>
<td>n = 12</td>
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<td></td>
</tr>
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</table>

Hemodynamic changes after injection of ET and All. Each column represents the mean value of 6 experiments and vertical bars denote SD.

Hemodynamic Changes with ET

- GFR
- RPF
- RVR

Hemodynamic Changes with All

- GFR
- RPF
- RVR

This model was made by intravenous administration of heterologous ATS, which reacts with a Thy-1-like antigen present in rat glomerular mesangial cells [9, 12]. This led to initial lytic lesions and subsequently proliferative/infiltra-
tive lesions in the mesangium. In the ATS-induced glomerular lesion, specific binding of intravenously administered ATS to mesangial cell-surface has been found, and the cells which are seen in the proliferative stage are mesangial cells and infiltrating monocytes/macrophages particularly in the areas of mesangial ballooning [9, 12]. Because of this selective change in mesangial cells and the similarity to historical lesions in human glomerulonephritis, we chose this model for glomerulonephritis with mesangial proliferati-

In this study, a good correlation existed between the degree of mesangial proliferation and the decrease in GFR or RPF. The mesangial cells are located between capillaries and possess many of the functional properties of smooth muscle cells [3]. The presence of both actin and myosin is well documented and the contractile properties of cultured mesangial cells are well established [14]. The decreased FF in this nephritis may suggest that the mesangial cells could induce a stronger contractility of glomerular capillary walls, which results in the decrease in GFR associated with the reduction of glomerular surface area. Yamamoto et al. [15] also suggested that mesangial cells might contribute either indirectly or directly to the regulation of arteriolar resistance and plasma flow as well as glomerular capillary hydrostatic pressure in this model. The correlation between RVR and the degree of mesangial proliferation indicates the role of mesangial cells for the reduction of renal hemo-
dynamics in anti-Thy-1 nephritis.

Both All and ET have been shown to reduce glomerular flow and glomerular ultrafiltration [5, 6, 16, 17]. The former is caused by vasconstriction of the glomerular arterioles and the latter by apparently acting on contractile elements within the mesangium. The renal hemodynamic response of the kidney with mesangial proliferation to infused ET or All was modified when compared to those of the control kidney. The reduction of GFR following the injection of both pressor substances was greater in the rats on day 8 than in the control rats. This might be explained by the quantitative change of mesangial cells in this nephritis. Illien et al. [5] have previously reported that the All-mediated fall in

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GFR is due to a direct action on the glomerulus, probably on the mesangial cells, but not on either afferent or efferent arterioles. In the present study, the increase in the number of mesangial cells may thus be essential for the enhanced contractility of glomerular capillaries in anti-Thy-1 nephritis. Furthermore, the mesangial cells are closely connected to afferent arterioles by gap junctions through Goormaghtigh’s cells which are thought to work as a syncytium [22, 23]. These morphological characteristics suggest that mesangial cells play a role in regulating afferent arteriolar resistance. The increased mesangial cells might thus elevate the contractility of afferent arterioles after ET or AII infusion in mesangial proliferative nephritis.

In conclusion, renal hemodynamics in response to the pressor substances were augmented in mesangial proliferative nephritis. The increased number of mesangial cells might, therefore, contribute to the enhanced contractility of the renal vasculatures in anti-Thy-1 nephritis.

Acknowledgements
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References

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