Antihypertensive Effect of Interleukin-2 in Salt-Sensitive Dahl Rats

Toshihiko Ishimitsu, Yoshio Uehara, Atsushi Numabe, Hiroki Tsukada, Yoshiichi Ogawa, Shigeru Yagi

Abstract We investigated the effects of interleukin-2, which stimulates the proliferation and maturation of thymus-derived lymphocytes, on hypertension and organ injuries in genetically hypertensive rats. Interleukin-2 (5×10^4 U/kg body wt) was subcutaneously injected into Dahl salt-sensitive rats fed a 4% NaCl diet and spontaneously hypertensive rats once a week for 10 weeks. The effects on blood pressure, cardiovascular hypertrophy, and renal function were evaluated. Interleukin-2 treatment lowered blood pressure in Dahl salt-sensitive rats (162 versus 187 mm Hg, P<.005). This antihypertensive effect was associated with an increase in glomerular filtration rate (589 versus 428 mL/min per 100 g body weight, P<.005) and reduction in cardiac weight (268 versus 305 mg/100 g body weight, P<.005). Interleukin-2 also alleviated the marked glomerular sclerosis in Dahl salt-sensitive rats (glomerular injury score, 151 versus 220; P<.001). In contrast, interleukin-2 did not affect the development of hypertension or organ injuries in spontaneously hypertensive rats. Histologically, glomerular and arterial lesions of the kidney were much less marked in spontaneously hypertensive rats than in Dahl salt-sensitive rats. These data indicate that interleukin-2 ameliorates the development of hypertension and cardiac and renal injuries in Dahl salt-sensitive rats. (Hypertension. 1994;23:68-73.)

Key Words • interleukin-2 • glomerular filtration rate • hypertension, sodium-dependent • rats, inbred strains • heart hypertrophy

Since White and Grollman reported the implications of autoimmunity in hypertension after a renal infarct, an increasing amount of evidence has been accumulated suggesting an association between hypertension and immune dysfunction in both humans and experimental hypertensive animals. Especially in the case of genetic hypertension, it has been suggested that the suppressor thymus-derived (T) lymphocyte dysfunctions and subsequent autoimmunities would bring about vascular injuries and renal damage, both of which are supposed to contribute to the development and maintenance of hypertension. In fact, it has been demonstrated that the periterrinal spaces are infiltrated with inflammatory cells in aged spontaneously hypertensive rats (SHR) and these lesions are resolved by immunosuppressive therapy. Moreover, the arterial damage in salt-induced hypertension could not be produced in nude mice. More recently, the antihypertensive effects of cytokines, humoral immunomediators produced by immunocompetent cells, were examined in several studies; however, the results were conflicting. This controversy may be derived from the differences in cytokines and hypertensive animal models used in the studies.

With regard to hypertensive animal models, most of the earlier studies have been performed using SHR or rat models for secondary hypertension. Little data are available as to the role of the immune system in Dahl salt-sensitive (Dahl S) rats, a genetic model for salt-induced hypertension. It has been reported that Dahl S rats are more vulnerable to vascular injuries, eg, intimal and medial thickening and thrombotic formation with periarterial infiltration of inflammatory cells, and renal damage. These pathophysiological properties strongly suggest the role of the immune system in the genesis of hypertension in Dahl S rats. In the present study, we examined the effects of interleukin-2 on the development of hypertension and organ injuries in two genetically hypertensive rat models, Dahl S rats and SHR. Interleukin-2 is a potent immunomediator and is known to promote proliferation and maturation of T lymphocytes.

Methods

All procedures were in accordance with the institutional guidelines for animal research. Male 6-week-old Dahl S (n=10) and Dahl salt-resistant (Dahl R, n=10) rats were originally obtained from Brookhaven National Laboratories, Upton, NY, and bred by Eisai Co, Ltd, Tokyo, Japan. The rats were fed a high salt diet containing 4% NaCl (wt/wt). Five Dahl S and five Dahl R rats were subcutaneously injected with 5×10^4 U/kg body wt recombinant human interleukin-2 (Toray Industries, Kanagawa, Japan) dissolved in 100 μL of 0.9% isotonic saline once a week; the other rats were injected with 100 μL of isotonic saline alone following the same procedure. The human interleukin-2 was produced in yeast and purified by liquid chromatography. Its purity was more than 95%, and its specific activity was 2.6×10^8 U/mg protein. The first interleukin-2 injection was given at the same time as the start of the high salt diet. Systolic blood pressure was measured by the tail-cuff method every week around 2 PM before interleukin-2 was injected. After 4, 7, 9, and 10 weeks, a 24-hour urine sample was collected, and then the rats were killed under pentobarbital anesthesia (30 mg/kg body wt IP). Blood samples were drawn from the inferior vena cava. The wet tissue weights of
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Fig 1. Light micrographs show representative glomeruli assigned to various severity scores. a: Glomerulus showing normal appearance (score 0, from untreated Dahl salt-resistant rats); b through e: glomeruli showing 0% to 25% (score 1, from treated Dahl salt-sensitive [S] rats), 25% to 50% (score 2, from treated Dahl S rats), 50% to 75% (score 3, from untreated Dahl S rats), and 75% to 100% (score 4, from untreated Dahl S rats) sclerosis, respectively; f: glomerulus showing global sclerosis (score 4, from untreated Dahl S rats). (Periodic acid–Schiff stain, ×300.)

the heart and descending thoracic aorta were measured. The kidney was obtained for histological investigation.

The kidney was fixed with 10% Formalin solution, embedded in paraffin, and 2-μm sections were stained with hematoxylin and eosin and periodic acid–Schiff. Histological evaluation of the kidney was performed by one of the authors (Y.U.) in a blind fashion. The glomerular and arterial lesions were evaluated by light microscopy using semiquantitative scoring methods. Briefly, lesion severity was graded from 0 to 4 for glomeruli and 0 to 3 for intrarenal arteries, and the injury score was calculated by summing the products of the severity score and the percentage of glomeruli or arteries displaying the same degree of severity. Figs 1 and 2 show representative micrographs of glomeruli and intrarenal arteries assigned to each severity score.

Urinary protein concentration was measured using a protein assay kit (Bio-Rad Laboratories, Richmond, Calif). The activity of N-acetyl-β-D-glucosaminidase (NAG) in the urine, as an indicator of tubular injury, was measured using sodio-m-cresol-sulphophthaleinyl N-acetyl-β-D-glucosaminide as its substrate (NAG assay kit, Shionogi Pharmaceutical Co, Osaka, Japan). Urinary and plasma levels of creatinine were measured with a creatinine autoanalyzer (Beckman Instruments Japan, Tokyo, Japan).

Male 6-week-old SHR (n=20) and normotensive control Wistar-Kyoto (WKY) rats (n=14) from the Tokyo University breeding colony were fed a regular laboratory chow, and interleukin-2 (5×10^4 U/kg body wt) was administered in the same manner as in the Dahl rat experiment. After 10 weeks, rats were killed and examined in the same procedure described in the Dahl rat study.

For evaluation of the effect of interleukin-2 treatment on blood pressure by direct measurement, 16 male Dahl S rats aged 6 weeks were fed a high NaCl (4%) diet and treated with interleukin-2 (n=8) or vehicle (n=8) for 10 weeks in the same manner. Then, mean arterial pressure was directly measured through a PE-50 polyethylene catheter inserted into the femoral artery with rats under light ether anesthesia using a pressure transducer coupled to a recorder (AP-600G and WS-682G, Nihon Kohden, Tokyo, Japan). Blood pressure values were read when the rats were quiet but responded to a pinch with forceps.

Values are expressed as mean±SEM. Changes in body weight and blood pressure were analyzed by analysis of variance for repeated measures followed by the Bonferroni method. The two-tailed Student’s t test was used for the comparison of treated with untreated groups. A value of P<.05 was considered significant.

Results

Fig 3 illustrates alterations in systolic blood pressure over the therapeutic period caused by interleukin-2 in Dahl rats. Development of hypertension was significantly tempered in Dahl S rats treated with interleukin-2 compared with the untreated group (F=5.08, P<.04), and the difference was statistically significant after 7 weeks and thereafter. Directly measured mean arterial pressure after 10 weeks was also significantly lower in eight treated Dahl S rats than in eight untreated Dahl S rats (152±2 versus 163±3 mm Hg, P<.03). Interleukin-2 did not influence blood pressure in normotensive Dahl R rats. In contrast to the Dahl S rats, as shown in Fig 4, the development of spontaneous hypertension in SHR was not influenced by the same dose (5×10^4 U/kg body wt) of interleukin-2 (185±2 mm Hg for untreated SHR versus 186±3 mm Hg for
treated SHR at the 10th week of the therapeutic period.

Table 1 summarizes the results of various measurements at the end of the therapeutic period in Dahl rats. Body weights and urine volumes at the end of the therapeutic period were similar in the four groups, Dahl R and S rats with or without interleukin-2 treatment. Urinary excretions of protein and NAG were greater in the untreated Dahl S rats than in the untreated Dahl R rats. However, these parameters were not significantly changed by interleukin-2 treatment when compared with the untreated control group. Urinary protein excretion also did not differ significantly between the treated and untreated Dahl S rats at 4, 7, 8, or 9 weeks (4 weeks, 23±4 versus 22±2 mg/d per 100 g body weight; 7 weeks, 30±3 versus 32±2; 8 weeks, 31±3 versus 29±2; 9 weeks, 32±2 versus 34±2). With regard to renal function, interleukin-2 improved the decreased creatinine clearance rate in Dahl S rats. Cardiac ventricles were heavier in untreated Dahl S rats than in treated Dahl S rats. Interleukin-2 treatment reduced...
this hypertensive cardiac hypertrophy by 12%. The weight of the thoracic aorta per unit area was also greater in Dahl S rats than in Dahl R rats. However, this parameter of vascular wall thickening was not significantly reduced by interleukin-2 treatment.

On the other hand, as indicated in Table 2, SHR also showed cardiovascular hypertrophy at the end of the experimental period compared with control WKY rats. However, the renal injury parameters were not significantly different between SHR and WKY rats. Interleukin-2 treatment did not affect blood pressure or cardiovascular and renal injury parameters in SHR.

Table 3 lists the results of histological evaluation of the kidney in each rat group. After 10 weeks of a high salt diet, control Dahl S rats demonstrated marked glomerular sclerosis and arterial lesions compared with control Dahl R rats. Interleukin-2 treatment brought about highly significant alleviation of this glomerular sclerosis in Dahl S rats, although the extent of arterial lesions was not significantly changed. Control SHR also showed significantly higher glomerular and arterial injury scores than the control WKY rats; however, the injuries were very mild compared with those in Dahl S rats. Interleukin-2 treatment also tempered this mild glomerular sclerosis in SHR.

Discussion

In the present study, weekly administration of interleukin-2 attenuated the progression of hypertension in Dahl S rats. This was accompanied by improvements in cardiac hypertrophy and renal dysfunction. The reduction of cardiac weight seems to provide evidence for the antihypertensive effect of interleukin-2 in Dahl S rats. The alleviation of histological injury of the kidney by interleukin-2 was not marked; however, it seems significant enough to improve creatinine clearance.

The Dahl S rats treated with interleukin-2 showed blood pressure 25 mm Hg lower than the untreated group when evaluated by the tail-cuff method. However, blood pressure reduction in Dahl S rats by the same interleukin-2 treatment was no more than 11 mm Hg in the second experiment in which arterial pressure was measured directly. This variation is thought to be derived from the different conditions under which blood measured directly.
TABLE 3. Effects of Interleukin-2 Treatment on Histological Injury Scores of Glomerular and Intrarenal Arteries In Genetically Hypertensive Rats

<table>
<thead>
<tr>
<th>Rat Group</th>
<th>Glomerular Injury Score (0 to 400)</th>
<th>Arterial Injury Score (0 to 300)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dahl salt-sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>151 ± 9†</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Control</td>
<td>220 ± 5†</td>
<td>65 ± 12†</td>
</tr>
<tr>
<td>Dahl salt-resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>49 ± 5</td>
<td>6 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td>60 ± 4</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>48 ± 5§</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Control</td>
<td>71 ± 8§</td>
<td>14 ± 3§</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>38 ± 1</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td>41 ± 4</td>
<td>6 ± 2</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. Values are mean ± SEM.

§P < .05, *P < .001 vs respective control values in untreated rats.
||P < .05, tP < .01, tP < .001 vs values in untreated normotensive control rats.

pressure was measured. In measurements by the tail-cuff method, rats were restrained and warmed in a conscious state, and the value reflects systolic blood pressure. On the other hand, in the latter experiment, mean arterial pressure was recorded through a catheter inserted into the femoral artery with rats under light ether anesthesia. Moreover, systolic blood pressure values measured by the tail-cuff method at 10 weeks in the second experiment were comparable (167±4 versus 190±3 mm Hg for treated versus untreated, P < .005, a difference of 23 mm Hg). Thus, it is supposed that the repeated experiment showed a similar degree of antihypertensive effect by interleukin-2 treatment in Dahl S rats.

Recently, Given et al. have reported that interleukin-2 did not alter the blood pressure of Dahl S rats. This seems to conflict with the results of our study, but several reasons can be offered to explain this discrepancy. First, Given et al gave 5 x 10^3 U of interleukin-2 per kilogram of body weight to the rats at the beginning of the experiment and the sixth week and observed the effects until the eighth week. We gave a dose 10 times higher every week for 10 weeks. This difference in the dose and frequency of interleukin-2 injection may be responsible for the lack of significant antihypertensive effect in the study of Given et al. Second, the number of rats used in the experiment was smaller in their study than in ours. Considering that the antihypertensive effect of interleukin-2 was modest in our study, the number of rats might not be large enough to show a significant blood pressure change in the study of Given et al. Third, they did not refer to the age of their Dahl S rats, and effects of interleukin-2 may be influenced by the age of the rats used.

On the other hand, interleukin-2 did not affect the blood pressure of the SHR in our study. Tuttle and Boppana have reported that a single subcutaneous injection of 5 x 10^3 U/kg interleukin-2 completely abolished the development of hypertension in SHR. However, the result could not be reproduced by other investigators. In the present study, although we gave a 10 times higher dose repeatedly, the development and progression of hypertension in SHR was not influenced. Thus, our results add a negative view as to the antihypertensive effect of interleukin-2 in SHR.

In the current study, interleukin-2 attenuated hypertension development in Dahl S rats but failed to affect hypertension in SHR. Thus, the antihypertensive effects of interleukin-2 were not constant between the two genetic models of hypertension. Dahl S rats are known to develop vasculitis-like arterial lesions, eg, intimal and medial hyperplasia and thrombotic formation with periarterial massive infiltration of inflammatory cells, in the kidney and mesentery after a few months of a high salt diet. Such histological findings suggest the involvement of an immunological mechanism in the development of hypertension and organ injuries in Dahl S rats, although the immune system of Dahl rats has been investigated rarely. On the other hand, in SHR, such vasculitis-like lesions do not occur until a much later stage, at a year or later. In the current study, parameters of renal function did not differ between SHR and WKY rats, whereas Dahl S rats showed reduced renal function. Histological examination also showed much milder glomerular and arterial lesions in SHR than in Dahl S rats. Therefore, it is speculated that interleukin-2 is likely to exhibit an antihypertensive effect when immunologically evoked organ injuries are marked.

Interleukin-2 is supposed to stimulate proliferation and maturation of T lymphocytes and make up for the decreased T lymphocyte function in genetically hypertensive rats. Assuming that an autoimmune mechanism is involved to some extent in the formation of arterial lesions in hypertensive rats, restoration of the suppressor T lymphocyte function by interleukin-2 treatment would be expected to reduce such vascular injuries and thereby may lower the blood pressure. The improvement of renal function by interleukin-2 may have been brought about by participation of the same mechanism in the kidney.

In summary, we demonstrated that interleukin-2 treatment attenuated the development of hypertension as well as reduced cardiac hypertrophy and improved renal dysfunction in Dahl S rats. However, interleukin-2 did not demonstrate an antihypertensive effect in SHR.

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References


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