Interleukin-2 Does Not Attenuate Hypertension in Spontaneously Hypertensive Rats

David W. Pascual, Hongkui Jin, Kenneth L. Bost, and Suzanne Oparil

It was recently reported that interleukin-2, when administered as a single bolus injection (5,000 units/kg), could prevent the development of hypertension in young spontaneously hypertensive rats and lower blood pressure to normotensive levels in spontaneously hypertensive rats with established hypertension. Consequently, efforts were made to duplicate this finding. Male spontaneously hypertensive rats (35 days old) were injected subcutaneously with 50,000 units/kg (3,500 units/rat) of recombinant interleukin-2 (Amgen) and had systolic blood pressure measured twice weekly by the tail-cuff technique. Systolic blood pressure in the interleukin-2–treated group was not significantly different from the vehicle-treated control group at any time point over 32 days of follow-up. A second injection of recombinant interleukin-2 (5,000 units/kg) was administered 32 days after the first injection. Again, no reduction in blood pressure was observed in the interleukin-2–treated group over an additional 38 days. Mean arterial pressure (±SEM) measured via intra-arterial cannula in conscious rats at age 105 days (38 days after the second treatment) was 168.5±3.5 mm Hg in interleukin-2–treated spontaneously hypertensive rats and 170.3±3.6 mm Hg in vehicle-treated controls. Both recombinant interleukin-2 preparations conformed to their respective manufacturer’s indicated specific activity as determined by the ability of the interleukin-2 to induce proliferation of the interleukin-2–dependent cell line HT-2. Thus, this study demonstrated that interleukin-2 was ineffective in preventing or attenuating hypertension in spontaneously hypertensive rats.

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In a recent report,1 it was suggested that a single injection of recombinant interleukin-2 (IL-2) could prevent or reduce hypertension in spontaneously hypertensive rats (SHR). More recently, the same group has also suggested that gamma interferon could induce similar antihypertensive effects in SHR.2 However, in both cases the authors failed to delineate a mechanism to explain how a single bolus subcutaneous injection could have such enduring antihypertensive effects. Theoretically, these immune cell modifiers could be considered as alternatives in the treatment of hypertension, as there is evidence to suggest that hypertension may be an autoimmune disorder.3 T cell–related immunodeficiency has been shown in SHR,4–7 and elevated serum antibodies8 and autoantibody9 levels have been demonstrated in some patients with essential hypertension. Taking into consideration these findings and the implications of the IL-2 study,1 this report addresses the capability of IL-2 as an antihypertensive agent.

Methods

Twenty male salt-sensitive SHR (SHR-S) were obtained from Taconic Farms, Germantown, N.Y., at 4 weeks of age and maintained on Purina rat chow and water ad libitum throughout the study. Systolic blood pressure was measured 1–2 times weekly in conscious, prewarmed, restrained rats by the tail-cuff method using an electrosphygmomanometer and physiograph recorder (Narco Bio-Systems, Houston, Tex.). For each rat, five measurements were made and the median value was taken as systolic blood pressure at any given time point. Body weight determinations were also performed on the same day as the blood pressure measurement. Forty-eight hours before termination of the study, rats were anesthesi-
tized with ether, and cannulas (polyethylene PE-10 fused with PE-50) were implanted into the abdominal aorta through the right femoral artery. Forty-eight hours after surgery, the arterial catheter was connected to a Model CP-01 pressure transducer (Century Technology Company, Inglewood, Calif.) coupled to a Grass Model 7 polygraph (Grass Instruments, Quincy, Mass.). Mean arterial pressure and heart rate were measured simultaneously.

Human recombinant IL-2 (natural sequence) was obtained from Amgen Biologicals, Thousand Oaks, Calif. The 20 SHR were divided into two groups: one group (n=10) received 50,000 units/kg (3,500 units/rat) IL-2 diluted in 0.9% saline and administered via subcutaneous injection between the shoulders (0.3 ml), and the second group (n=10) received 0.3 ml 0.9% saline alone administered in a similar fashion. A second injection of human recombinant IL-2 (5,000 units/kg, Cellular Products, Buffalo, N.Y.) diluted in 0.9% saline was given 32 days later to the group previously treated with IL-2 by the route previously stated. The vehicle group again received vehicle only.

The recombinant IL-2 obtained from both companies was tested for activity using the IL-2-dependent cell line HT-2. Recombinant mouse IL-2 (a gift from Dr. Ken W. Beagley, Department of Medicine, University of Alabama at Birmingham) was used to standardize the HT-2 proliferation assay. Then 2x10^6 HT-2 cells/well in RPMI-1640 (Whittaker Bioproducts, Walkersville, Md.) with 10% fetal calf serum and 2x10^4 HT-2 cells/well in RPMI-1640 (Whittaker Bioproducts, Walkersville, Md.) plus nonessential amino acids (0.1 mM), L-glutamine (2.0 mM), sodium pyruvate (0.1 mM), HEPES (10 mM), penicillin (100 units/ml), and streptomycin (100 /µg/ml) supplements (GIBCO, Grand Island, N.Y.) were aliquoted into 96-well, flat-bottom microtiter plates (Costar, Cambridge, Mass.) and cultured in the presence of varying dilutions of IL-2 for 24 hours with a final volume of 0.1 ml. The HT-2 proliferative response was determined using a colorimetric assay. Briefly, 25 µl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide (MTT, Sigma Chemical Co., St. Louis, Mo.) was added to each well and incubated with the cells for 2–3 hours at 37° C. Subsequently, cells were lysed with 20% sodium dodecyl sulfate solution in 50% N,N-dimethylformamide (high-performance liquid chromatography grade, Aldrich Chemical Co., Milwaukee, Wis.). After overnight incubation at 37° C, the absorbancies were measured (test wavelength at 570 nm and reference wavelength at 630 nm) using Kinetics Reader Model EL312, Bio-tek Instruments, Winooski, Vt.). The extent of proliferation by IL-2-stimulated HT-2 cells is proportional to the amount of MTT uptake and is reflected by increases in color generation, whereas unstimulated HT-2 cells produce minimal color change. One unit of IL-2 activity was defined as the amount of IL-2 that induced 50% of maximal proliferation in 100 µl culture.

Results

To test the effectiveness of IL-2 as an antihypertensive agent, recombinant human IL-2 was administered at a dose 10-fold higher than that used in the Turtle and Boppana study via a subcutaneous injection into 35-day-old male SHR (n=10) with a mean systolic blood pressure of 128±3.0 mm Hg. The IL-2-treated SHR failed to demonstrate any change in blood pressure compared with the vehicle control group (Figure 1). Both groups continued to show steady increases in blood pressure with time. Because there was no significant difference between groups in blood pressure after 32 days of treatment (Figure 1), the IL-2–treated SHR were given a second injection of recombinant human IL-2 subcutaneously at one tenth the initial dose, and the vehicle control group received 0.9% saline only. The IL-2–treated SHR at this time was the same (source, dose, and route of administration) as in the Turtle and Boppana study. Blood pressures were monitored weekly for an additional 36 days. Again, no significant change in blood pressure was observed between groups (Figure 1). Mean arterial pressure measured via an intra-arterial cannula at age 105 days was 168.5±3.5 mm Hg in the IL-2–treated SHR and 170.3±3.6 mm Hg in the vehicle-treated control group (Figure 2). Body weight in the groups was nearly identical throughout the study (Figure 3).

The lack of effect of recombinant IL-2 on blood pressure in the SHR was not due to absence of biological activity. IL-2 from each source was tested for its ability to induce proliferation of an IL-2–dependent cell line, HT-2. Both IL-2 preparations stimulated the HT-2 cells to proliferate using the colorimetric MTT assay, and the level of activity stated by each manufacturer matched the values obtained in this assay (Table 1). Consequently, this
study demonstrated that active recombinant human IL-2 did not reduce blood pressure in SHR.

Discussion

In the current study, IL-2 obtained from two different commercial sources did not alter blood pressure in young male SHR compared with age-matched vehicle controls. These results differ from those of Tuttle and Boppana, who completely prevented the development of hypertension in SHR from the same supplier by injecting 5,000 units/kg of IL-2 (Cellular Products) subcutaneously into 35-day-old animals. In both studies, the route and site of injection were identical. Standard bioassay for IL-2 activity revealed that both preparations administered in the current study were fully active (met manufacturer's standards). The initial dose of IL-2 administered in the current study was 10 times that given by Tuttle and Boppana; the subsequent dose administered 32 days later was identical to their dose. Although the initial dose of IL-2 given in the present study was tenfold higher, the actual amount given to each animal represented only 3,500 units, which raises the question of how a 10-fold lower dose can elicit such dramatic antihypertensive effects. The findings of Tuttle and Boppana are remarkable in another respect. The systolic blood pressure values reported after IL-2 treatment were far lower than would be expected for SHR, even after "successful" antihypertensive treatment, calling into question the accuracy of their blood pressure determination.

The concept of investigating the role of IL-2 and other immune cell modulators in the pathogenesis of hypertension is of great scientific interest and potential therapeutic usefulness, as previous studies have demonstrated that SHR have depressed T cell function and fewer thymic T cells than normotensive allogeneic strains. A significant reduction in blood pressure has been observed in SHR with established hypertension given Wistar-King-Aptekman or Wistar thymic implants. This attenuation of hypertension obtained by thymic implantation was dependent on multiple implants, and in one study, this attenuation was transient. However, partial restoration of T cell function was observed in SHR after thymic transplant. This evidence suggests that spontaneous hypertension may have, at least in part, an immunological basis. Although there was partial restoration of immunological function in SHR as demonstrated by plaque-forming cell responses and mitogen responses, the evidence thus far is mechanistically inconclusive on how thymic implantations or thymic products can attenuate hypertension. Furthermore, the absence of antihypertensive action of IL-2 in the current study calls into question the blood pressure–lowering capability of one-time or intermittent administration of IL-2 in SHR. Further study is needed to elucidate the mechanisms by which IL-2 or other cytokines may effect blood pressure control.

<table>
<thead>
<tr>
<th>IL-2 source</th>
<th>IL-2</th>
<th>IL-2* activity (units/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Manufacturer determination</td>
</tr>
<tr>
<td>Amgen</td>
<td>recombinant human</td>
<td>79,800</td>
</tr>
<tr>
<td>Cellular products</td>
<td>recombinant human</td>
<td>10,000</td>
</tr>
<tr>
<td>UAB</td>
<td>recombinant mouse</td>
<td>3,000</td>
</tr>
</tbody>
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The interleukin-2 (IL-2) activity was standardized such that the total IL-2 acquired is expressed as units per milliliter. One unit of IL-2 activity was defined as the amount of IL-2 that induced 50% maximal proliferation in 100 μl culture.

*Gift provided by Dr. Ken Beagley, Department of Medicine, University of Alabama at Birmingham (UAB).
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References


**KEY WORDS** • interleukin-2 • blood pressure • essential hypertension • spontaneously hypertensive rats
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