Interleukin-2 and Spontaneous Hypertension

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There are conflicting reports with regard to the antihypertensive effectiveness of interleukin-2 in the spontaneously hypertensive rat. Recently, the original claim of a normalization of arterial pressure in the spontaneously hypertensive rat after a single administration of interleukin-2 has been disputed. Therefore, the present study was performed to determine whether the administration of interleukin-2 was effective in attenuating both the development and maintenance of hypertension in the spontaneously hypertensive rat. Both young prehypertensive spontaneously hypertensive rats and adult spontaneously hypertensive rats with established hypertension received a single subcutaneous dose of 5,000 units/kg human recombinant interleukin-2. Arterial pressure was monitored at weekly intervals in both control and treated animals by the tail-cuff technique. Interleukin-2 administered as a one time single injection had no effect on the development of hypertension in the young animals or on the maintenance of hypertension in the adult animals. Interleukin-2 also was administered as a continuous infusion via osmotic minipumps at dose levels of 5,000 and 50,000 units/kg/wk to both young and adult spontaneously hypertensive rats. Continuous administration of interleukin-2 also had no effect on the development or maintenance of spontaneous hypertension. Therefore, this study firmly demonstrates that interleukin-2 has no effect on the onset or maintenance of hypertension in the spontaneously hypertensive rat. (Hypertension 1991;18:171-175)

There is a large body of evidence that suggests that alterations in immune function may contribute to the pathogenesis of hypertension in the Okamoto spontaneously hypertensive rat (SHR). The SHR has a defect in the functional capability of T-lymphocytes. Therapeutic modalities that target various immunologic functions can ameliorate the spontaneous hypertension in this model. Recently it was reported that the immunomodulatory lymphokine interleukin-2 (IL-2), administered either as a single injection or by continuous infusion, resulted in the attenuation of hypertension in adult SHRs and prevented the development of hypertension in young SHRs. These results have been disputed in a study by Pascual et al. These investigators reported that the administration of IL-2 had no effect on the development of hypertension in the SHR. Therefore, we examined the effect of both single injection and continuous administration of human recombinant IL-2 on the development and maintenance of hypertension in the SHR.

Methods

All rats used in this study were obtained from Taconic Farms, Germantown, N.Y. The animals were offered Purina Rat Chow and water ad libitum and maintained on a 12-hour light/dark cycle.

Effect of Interleukin-2 on Spontaneous Hypertension

To examine the effect of IL-2 on the development of spontaneous hypertension, eight 6-week-old male SHRs were given a single subcutaneous injection of 5,000 units/kg human recombinant IL-2 (Cellular Products, Buffalo, N.Y.). The IL-2 was diluted with 0.9% saline and administered subcutaneously between the scapulae. A second group of eight 6-week-old male SHRs were given saline in an identical manner and served as controls.

To examine the effect of IL-2 on the maintenance of spontaneous hypertension, eight 15-week-old SHRs with established hypertension were given IL-2 (5,000 units/kg) as a single subcutaneous injection in a manner identical to that in the younger animals. Eight age-matched SHRs were injected with saline and served as the control group.

The effect of a single subcutaneous injection of 5,000 units/kg IL-2 on the arterial pressure of both young and adult Wistar-Kyoto (WKY) rats was also examined. IL-2 was administered subcutaneously between the scapulae to eight 6-week-old and eight 15-week-old WKY rats. Saline was administered in a
like manner to eight 6-week-old and eight 15-week-old WKY rats. These animals served as the controls.

The effect of continuous administration of IL-2 on the development and maintenance of spontaneous hypertension was also examined. IL-2 was administered at doses of 5,000 and 50,000 units/kg/wk to both young (6-week-old) and adult (13-week-old) SHRs via osmotic minipump (Alzet model 2001, Alza Corp., Palo Alto, Calif.). For the continuous infusion experiments, IL-2 (Cetus Corp., Emeryville, Calif.) was diluted with phosphate-buffered saline containing 0.1% sodium dodecylsulfate and 0.1% bovine serum albumin. Minipumps were placed subcutaneously in the midscapular region. All animals were lightly anesthetized with methoxyflurane during the placement of the minipumps. The minipumps were replaced at weekly intervals throughout the experiment. The minipumps were weighed before placement of the devices and again after removal from the animal to monitor IL-2 delivery. Control animals were lightly anesthetized with methoxyflurane at weekly intervals but minipumps were not placed.

Measurement of Arterial Pressure

Arterial pressure was determined by tail-cuff sphygmomanometry at weekly intervals in all animals. Rats were placed in individual Plexiglas restrainers and subsequently housed inside an environmentally controlled chamber (30°C) during the tail-cuff pressure determinations. Tail-cuff pressure was measured using programmed eleetrosphygmanometer with photoelectric detection (IITC, Inc., Woodland Hills, Calif.). Recorded pressures were the average of at least three determinations made on each animal during the arterial pressure measurement session.

Tail-cuff pressure measurements were verified at the end of the experimental period in both the control and IL-2-treated animals via direct intraarterial pressure recording. Rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.). A polyethylene catheter (PE-90) with a tip stretched approximately to the size of PE-10 was inserted into the lower abdominal aorta via the left femoral artery. The catheter was tunneled subcutaneously, was exteriorized between the scapulae, and subsequently was filled with heparin (1,000 units/ml). The animals were allowed to recover. Mean arterial pressure (MAP) was recorded the next day in conscious, freely-moving rats using pressure transducers (Cobe Laboratories, Inc., Lakewood, Colo.) coupled to a recorder (model 711, Beckman Instruments, Inc., Palo Alto, Calif.). The analog signals from the recorder were digitized and analyzed using an HD-4 Hemodynamic Analysis and Archives System (Po-Ne-Mah, Inc., Storrs, Conn.). During the mean arterial pressure measurements, the rats were housed in a Plexiglas cage on a bed of wood shavings. The reported pressures represent the average MAP during a 1-hour recording session.

Results

The effect of a single subcutaneous injection of IL-2 on the development of hypertension in young SHRs is illustrated in Figure 1. IL-2 at a dose of 5,000 units/kg had no effect on the development of hypertension in the SHR. Pretreatment tail-cuff pressure of 6-week-old SHRs was 109±6 mm Hg (mean±SEM) in the IL-2-treated group and 116±4 mm Hg in the control group. Arterial pressure increased with age in both the IL-2–treated and the control animals. Tail-cuff pressure of 12-week-old IL-2–treated SHRs was 204±3 mm Hg and 187±8 mm Hg in controls at this time. IL-2 administration had no effect on the growth rate of the SHR. Body weight at 12 weeks of age in the IL-2–treated animals was 270±3 g as compared with 275±7 g in the control group.

The effect of a single injection of IL-2 on the maintenance of hypertension in the SHR is illustrated in Figure 2. IL-2 had no effect on the arterial pressure of adult SHRs with established hypertension. Pretreatment tail-cuff pressure of 15-week-old SHRs was 189±5 mm Hg in the IL-2 group and 182±5 mm Hg in the control animals. Seven weeks...
TABLE 1. Mean Arterial Pressure of 13- and 22-Week-Old Spontaneously Hypertensive Rats After Single Subcutaneous Injection of Interleukin-2

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-wk-old SHR</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>156±7</td>
</tr>
<tr>
<td>IL-2 treated*</td>
<td>161±6</td>
</tr>
<tr>
<td>22-wk-old SHR</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>176±5</td>
</tr>
<tr>
<td>IL-2 treated†</td>
<td>184±3</td>
</tr>
</tbody>
</table>

Values reported are the mean±SEM. MAP, mean arterial pressure; SHR, spontaneously hypertensive rats; IL-2, interleukin-2.

*5,000 units/kg IL-2 at the age of 6 weeks.
†5,000 units/kg IL-2 at the age of 15 weeks.

after IL-2 administration, tail-cuff pressure was 202±6 mm Hg in the treated animals and 202±5 mm Hg in the control animals. IL-2 had no effect on the body weight of the adult SHR. Body weight of 22-week-old IL-2–treated rats was 344±4 g and 340±5 g in the controls.

Tail-cuff pressure measurements were verified in both the young and adult SHRs at the end of the experimental protocol (Table 1). MAP of SHRs administered a single injection of IL-2 at the age of 6 weeks was 156±7 mm Hg. MAP of the age-matched untreated animals was 161±5 mm Hg. MAP of the adult SHRs administered IL-2 was 184±3 mm Hg and the MAP of the age-matched controls was 176±5 mm Hg at this time.

IL-2 had no effect on the arterial pressure or growth rate of either young or old WKY rats (Figures 3 and 4).

The effect of a continuous subcutaneous infusion of IL-2 (dose levels of 5,000 or 50,000 units/kg/wk) on the development and maintenance of hypertension is illustrated in Figures 5 and 6. Continuous administration of IL-2 had no effect on the development of hypertension in the SHRs (Figure 5). Arterial pressure increased in both the 5,000 and 50,000 units/kg/wk groups in a manner similar to that in the control group. Arterial pressure was slightly lower in the SHRs receiving 5,000 units/kg/wk during the initial phase of the experiment. However, these differences were not statistically significant (analysis of variance for repeated measures).

Continuous infusion of IL-2 had no effect on established hypertension in adult SHRs (Figure 6). There was no difference between the arterial pressure of the untreated animals and those receiving...
either 5,000 or 50,000 units/kg/wk of IL-2 (analysis of variance for repeated measures). A transient lowering of arterial pressure after the initiation of continuous infusion was seen. However, arterial pressure returned to preinfusion levels the following week.

Continuous administration of IL-2 at either 5,000 or 50,000 units/kg/wk had no effect on the growth rate of young SHRs or the maintenance of body weight in adult SHRs.

Discussion

Hypertension in the SHR may result, in part, from immune dysfunction. This hypothesis stems from several observations. Takeichi et al. reported that the functional capacity of T-lymphocytes was depressed in the SHR. Ba et al. demonstrated that restoration of T-lymphocyte function in the SHR by transplantation of thymus tissue from the Wistar-King strain was associated with a normalization of blood pressure. Bendich et al. reported that treatment with anti-thymocyte serum resulted in a lowering of arterial pressure in the SHR. In a similar study, Strausser demonstrated that the administration of thymosin fraction 5, an immunomodulatory extract of calf thymus, resulted in a decline in arterial pressure in the SHR. Khraibi et al. were able to attenuate the development of hypertension in the SHR by immunosuppression with cyclophosphamide. Norman et al. reported a long-term attenuation of the hypertension after implantation of Wistar thymus tissue into neonatal SHRs.

IL-2 is a lymphokine produced by T-lymphocytes in response to stimulation by antigen and activated macrophages. IL-2 mediates the growth and functional activity of T- and B-lymphocytes and enhances lymphocyte proliferation in vitro. Administration of IL-2 in vivo enhances T-cell-mediated immune responses and can restore the ability of immunodeficient animals to respond to an antigenic challenge.

The immunologic dysfunction in the SHR and the immunoregulatory role of IL-2 provided the rationale for examining the effect of this lymphokine on the development and maintenance of hypertension in these animals.

In the present study, IL-2 had no effect on the onset or maintenance of hypertension in the SHR. These findings are at odds with those reported by Tuttle and Boppana, but extend the observations of Pascual et al. Tuttle and Boppana reported that a single injection of IL-2 at a dose of 5,000 units/kg administered to adult SHRs with established hypertension normalized the arterial pressure within 4 weeks. The same dose of IL-2 administered to prehypertensive young SHRs completely prevented the development of hypertension in these animals. These investigators also reported that the continuous administration of IL-2 at a dose of 5,000 units/kg/wk via osmotic minipumps prevented the development of hypertension in young SHRs.

In the study by Pascual et al., 35-day-old SHRs received a single injection of IL-2 at a dose of 50,000 units/kg. The animals received a second injection of IL-2 at a dose of 5,000 units/kg 5 weeks later. IL-2 administered by this regimen had no effect on the development of hypertension in the treated animals.

In the present study, both young (6-week-old) prehypertensive SHRs and adult (15-week-old) SHRs with established hypertension received a single injection of human recombinant IL-2 at a dose of 5,000 units/kg. IL-2 administered as a single dose had no effect on the development of hypertension in the young animals or on the maintenance of hypertension in the adult animals. IL-2 was also administered as a continuous infusion at two dose levels to both young and adult SHRs. Continuous infusion of IL-2 at either 5,000 or 50,000 units/kg/wk had no effect on the hypertension in the SHR. IL-2, whether administered as a single injection or as a continuous infusion, was not effective in lowering arterial pressure. Therefore, it can be firmly concluded from the present study that IL-2 has no effect on the onset or the maintenance of hypertension in the SHRs.

The fact that both the present study and that of Pascual et al. found no antihypertensive effect of IL-2 in the SHR prompts one to question the results reported by Tuttle and Boppana. Perhaps the answer lies in the accuracy of the tail-cuff pressure measurements reported by these investigators. In the original study examining the effect of IL-2 on spontaneous hypertension, tail-cuff pressures were reported to be approximately 60 mm Hg before and after the initial treatment with IL-2 in the prehypertensive SHRs. Tail-cuff pressure remained at this level as the animals aged. Values of this magnitude for tail-cuff pressure are low even for a normotensive rat. Therefore, the reported antihypertensive effect of IL-2 in the original study may have been due to an inaccuracy in the tail-cuff pressure measurement.

The inability of IL-2 to lower arterial pressure in the SHR should not cast doubt into question the substantial body of evidence that links altered immunologic reactivity to the development and maintenance of spontaneous hypertension in the SHR. Investigations from many laboratories, including our own, have provided a firm basis for this hypothesis. However, further study is necessary to define further the role of the immune system in the onset and maintenance of hypertension in this model.

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References


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