Antihypertensive Effect of Interleukin-2

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Recent reports indicate that cyclooxygenase inhibitors such as aspirin may facilitate the release of interleukin-2 from thymic T cells. We have previously reported that aspirin has antihypertensive effects in the standard animal model of essential hypertension, the spontaneously hypertensive rat (SHR). Because the SHR has been reported to be deficient in T cells, it was of interest to determine whether the course of hypertension in this model could be altered by interleukin-2, the T cell growth factor. A single bolus of interleukin-2 (5,000 units/kg s.c.) prevented the increase of blood pressure in the young SHR and lowered pressure to normotensive levels in the well-established hypertensive adult SHR. In the latter, the effects of a single dose have been found to persist for at least 6 months with no toxic or untoward effects apparent. Blood pressure in Goldblatt, single-kidney Wistar-Kyoto rats, a model of renal hypertension, was not affected by interleukin-2. (Hypertension 1990;15:89-94)

There is some evidence that the immune system might be involved with the appearance of hypertension in the spontaneously hypertensive rat (SHR). The thymus gland is not as well developed in the SHR as in the Wistar-Kyoto (WKY) rat, and there are fewer T cells.1-3 Allografts, or extracts, of thymus tissue from the WKY rat can normalize blood pressure in the SHR, but not in the Goldblatt rat.24 Immunosuppressant drugs, such as cyclosporine, can lower blood pressure in the SHR but their toxicity qualifies this effect.2 Serum Immunoglobulin G (IgG) levels have been reported to be elevated in humans in association with high blood pressure6-8 as well as other conditions. If hypertension is associated with a defect in the immune system, the question remains as to whether it is the cause or the effect of hypertension.

Previous work from our laboratory has shown that aspirin produces antihypertensive effects in the young SHR that dissipate as the rats approach maturity.9 We were unable to provide a mechanism to explain the antihypertensive effect of aspirin in the SHR at the time.9,10 Recent studies by others have suggested that cyclooxygenase inhibitors may facilitate the synthesis or expression of interleukin-2 (IL-2) by activated thymic T cells. This second messenger could in turn affect the differentiation of both suppressor and helper T cells.11,12 Because a reduction in thymic T cell number has been one factor associated with the SHR and because aspirin has a short-term antihypertensive effect in the SHR, it was of interest to us to determine whether IL-2 modifies the state of hypertension in the SHR. The Goldblatt, single-kidney WKY rat model of hypertension was incorporated in the study to establish whether the effects of IL-2 were directed at pressure per se or at some factor particular to the SHR that is responsible for the increase in pressure.

Methods

Rats

One hundred and seven rats, 67 SHR and 28 WKY rats that were 28 days old and 12 SHR that were 90 days old, were obtained from Taconic Farms (Germantown, New York) and were placed in individual cages with Purina rat chow and water ad libitum. Twelve one-kidney, Goldblatt WKY rats and an equal number of sham-operated WKY rats and SHR were obtained at 28 days of age (Charles River Labs., Inc., Wilmington, Massachusetts) and placed in individual cages with free access to food and water.

Blood Pressure

Systolic blood pressures were determined each week by the tail-cuff method assisted by an electronic plethysmograph (Technilab, Pequannock, New Jersey). Three consecutive blood pressure determinations were recorded while the rats rested quietly in a heated (35°C) acrylic chamber. Whenever feasible, blood pressures were recorded at the same time of day and by the same investigator. When the rats were killed, blood pressures were recorded in randomly selected rats by the direct, intracarotid method and compared with the tail-cuff method. Differences between the two methods did not exceed ±8.0%.

Immunoglobulin G Assay

Serum IgG and IgG1 levels were determined by the method of radial immunodiffusion (RID) during the
first week, and for each of the 4–8 weeks thereafter. Whole blood (0.1 ml) from ether-anesthetized rats was withdrawn by arteriopuncture from the medial tail artery and then centrifuged at 5,000 rpm for 5 minutes. Serum was used immediately. Duplicate samples of unknown (5.0 µl) and of three standards were spotted in prepunched RID plates (The Binding Site, Inc., San Diego, California). The plates were read and calibrated by one person. Ring size for standards and unknowns were averaged and serum IgG and IgG1 concentration (mg/10 ml) determined from standard-derived curves. The use of RID plates incorporates a potential 10% error between plates and a 5% error within the same plate. Sufficient wells were normally present to do a complete assay of standard, control, and drug rats on one plate for each age point. Different plates were used for different age points so that the error resulting from comparing serum IgG values in rats of different ages could be as much as 25%.

Statistics
The average of three blood pressure measurements and duplicate IgG and IgG1 values were pooled with those from rats of the same age and protocol. The Student’s t test was used to determine significance between unpaired observations when appropriate. Tests of variance and two-sided tests were performed throughout by an in-house analysis of variance program. Significance was established at a p value of <0.05.

Protocol: Aspirin and Metoprolol
SHR and WKY rats 28 days old were divided into four groups of 5–7 SHR and WKY rats per group. The first and second groups, which served as controls, received only plain water and food until they were killed at 80 or 100 days of age. The third group received aspirin (100 mg/kg) in their water beginning at 35 days old and continuing until they were 100 days old; the fourth group received metoprolol (1.0 mg/kg) for the same period. Water consumption was monitored and the concentration of each drug adjusted accordingly to provide the above mentioned dose levels. There were no deaths or overt signs of illness in these rats.
Six single-kidney, Goldblatt WKY rats and an equal number of sham-operated SHR and WKY rats, all 28 days old, received no further treatment, except for blood pressure and serum IgG determinations, until 110 days of age.

Interleukin-2
Human recombinant IL-2 (10,000 units/ml) was obtained from Cell Products, Inc. (Buffalo, New York). Initial pilot studies were done with recombinant IL-2 obtained from Sigma Chemical Co. (St. Louis, Missouri). The body of the present study was carried out with IL-2 provided by Cell Products, Inc. The product is identified by the manufacturer as “culture grade” with a purity of more than 99%.

After thawing at room temperature, a working solution was prepared by dilution of the stock 1:10 with a carrier solute (0.9% saline and dextrose solution, Pharmacia Labs., Piscataway, New Jersey).

Protocol: Interleukin-2
Thirty two prehypertensive SHR 28 days old were divided into two groups of 16 rats each. At 42 days of age, one group received a single, subcutaneous bolus injection of 5,000 units/kg of IL-2, or carrier. The volume of the injection did not exceed 0.3 ml. The second group of 16 SHR 49 days old were anesthetized with ether and miniosmotic pumps were implanted subcutaneously on the dorsum at the level of the shoulders. The pumps (Alza Corp., Palo Alto, California) were designed to deliver 5,000 units/kg of IL-2, or carrier alone, at a constant rate over 14 days. Fresh pumps were installed at 63 and 77 days of age. Blood pressures and serum IgG were determined each week for a month. Some of the rats were then killed. Those maintained in the colony were monitored for blood pressure on a biweekly basis.
Six Goldblatt WKY rats, six sham-operated WKY rats, and six sham-operated SHR received a single bolus (5,000 units/kg) of IL-2 at 35 days of age and no further treatment for the remaining 60 days.

Results
Blood pressure and serum IgG and IgG1 levels generally increased with age in the SHR and WKY rats (Figures 1 and 2). Serum IgG and IgG1 levels increased in most, but not all, studies with maturation of each strain of rat, but at all ages beyond 65 days, the serum IgG values in the SHR exceeded those in the WKY rat. The significance of the difference between the SHR and WKY rat beyond 65 days of age varied from protocol to protocol. In Figure 1, at the end of 80 days, mean serum IgG level in the SHR was 162±12 compared with 107±7 mg/10 ml in the WKY rat (p<0.01). In Figure 2, at the end of 100 days, mean serum IgG was 201±4 for the SHR and 179±7 mg/10 ml for the WKY rats (p<0.05).

Antihypertensive Drugs
Spontaneously hypertensive rats. Blood pressure increased progressively through 100 days of age in the untreated SHR and WKY rats but not in those receiving aspirin or metoprolol. The antihypertensive effect of aspirin, but not of metoprolol, began to lessen after 65 days of age and was much diminished by 100 days (Figure 2).
Serum IgG levels did not increase with age in SHR exposed to metoprolol, but like blood pressure, serum IgG levels in the aspirin-treated group began to
FIGURE 1. Line graphs showing blood pressure and serum Immunoglobulin G (IgG) and IgG1, in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats from 35 through 80 days of age. Mean values±SEM for five WKY rats are denoted by ○ and ● for the same number of SHR. *Denotes a significant difference (p<0.01) between strains of the same age. † Denotes significant difference (p<0.01) within one strain and the preceding age.

increase after 65 days of age, returning toward control values by 100 days of age.

Goldblatt rats. Blood pressure in the Goldblatt WKY rats initially increased more rapidly than pressure in the sham-operated SHR, but no significant difference could be discerned between 50 and 70 days (Figure 7A). Blood pressures in both were significantly higher than in the sham-operated WKY rats. Serum IgG levels appeared little affected by the state of blood pressure in these rats. All rats appeared to be healthy at the end of the protocol.

Interleukin-2

Interleukin-2 appeared to cause neither distress nor discomfort in the SHR or Goldblatt rats. They remained alert, eating and drinking with regular frequency, and their gain in body weight matched that of rats receiving only carrier (Figure 3).

Young rats. Hypertension did not develop in 42-day-old prehypertensive SHR that received a single injection of IL-2, but did develop in those that received only carrier (Figure 4). The IL-2–treated rats remained normotensive throughout the duration of the experimental protocol. Serum IgG increased in parallel with blood pressure in the control SHR, but no increase occurred until 83 days of age in SHR receiving IL-2. At 100 days of age, serum IgG in the IL-2–treated SHR was not different from that in the control rats.

When SHR of the same age received IL-2 by osmotic pump, their blood pressures increased until 1 week after implantation of the first pump when pressure stabilized. After implantation of the second pump, pressure dropped slightly (Figure 5). Blood pressure continued to increase in SHR exposed to carrier alone. When administered by pump, IL-2 (5,000 units/kg) did not decrease serum IgG below the level of the control rats. The difference at 82 days of age is due to the slightly earlier (10 days) increase in serum IgG in the SHR as compared with WKY rats.

Mature rats. The effect of bolus injection of IL-2 in older SHR was similar to the effect in the younger SHR. A single bolus injection of IL-2 at 94 days of age reduced blood pressure in 1 week from 132±3.0 to 116±4.0 mm Hg (Figure 6). Pressure continued to fall over the next 3 weeks, eventually reaching 94±2 mm Hg. Five of the IL-2–treated SHR were monitored beyond the first month; all remain normotensive (85±5 mm Hg) 6 months later and continue to be monitored. Throughout this period, blood pressures in the control SHR remained high.

Serum IgG declined from a high of 410±5 mg/10 ml at 94 days of age to 133±26 mg/10 ml at 100 days of age, and 41±3 mg/10 ml at 108 days of age, respectively. The increase in serum IgG at 115 days of age was not associated with any apparent change in the status of these rats.

Goldblatt rats. Single bolus injection of IL-2 in Goldblatt WKY rats at 35 days of age did not prevent the increase in blood pressure (Figure 7). Blood pressure increased to 155±2 mm Hg at 105 days of age. Sham-operated SHR and WKY rats, after receiving a single bolus of IL-2, remained normotensive for the duration of the experiment. Serum IgG levels were not different in the three groups after exposure to IL-2.

Discussion

Serum Immunoglobulin G Levels and Blood Pressure

Serum IgG levels in the SHR and WKY rat increased with age and blood pressure. The industry standard value for serum IgG level in the rat is 70
mg/10 ml (personal communication, Dr. William Shek, Charles River Co., Wilmington, Massachusetts). In our rats, depending on age and strain, serum IgG levels ranged from 80 to 300 mg/10 ml. The highest values were seen in older SHR. Overlap in the values for the SHR and WKY rat occurred between 35 and 65 days of age after which the levels in the SHR consistently exceeded those in the WKY rat. The variation in IgG between study groups, mainly due to the large margin of error inherent in the RID technique, necessarily limits the extent to which IgG differences can be invoked to suggest an association between hypertension and autoimmune disease. A number of factors other than autoimmune disease, including infection, tumors, and defects in catabolism

**FIGURE 3.** Line graph showing body weights (g) in relation to age (in days) in spontaneously hypertensive rats (SHR) receiving interleukin-2 (IL-2) (5,000 units/kg) by single bolus (●), minipump (○), or carrier alone by both routes (○). Each point represents mean±SEM of six to eight rats.

**FIGURE 4.** Line graphs showing blood pressures (top panel) and serum Immunoglobulin G (IgG) (lower panel) in spontaneously hypertensive rats (SHR) receiving interleukin-2 (●) by single bolus injection at 42 days of age or carrier alone (○). Each point represents mean of five to six rats. Blood pressures were significantly different from 44 days onward (p<0.001) and * in lower panel denotes a significant difference (p<0.01) between pairs of same age. Standard error bars have been omitted in this figure as well as in Figure 5 because most approximated limits of circles.
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Interleukin-2 and SHR

Line graphs showing blood pressures (top panel) and serum Immunoglobulin G (IgG) (lower panel) in spontaneously hypertensive rats (SHR) receiving interleukin-2 (IL-2) (5,000 units/kg/2 weeks) by mini osmotic pump at 49, 63, and 77 days of age. ○ represent the mean of five to six SHR receiving only carrier. ● represent mean of five to six SHR receiving IL-2. * Denotes significant difference (p<0.01) between pairs of same age.

of IgG, are known to lead to increases in serum IgG levels. More demanding immunological evidence is required before the conclusion can be made that the increases in serum IgG are related to blood pressure, much less to an autoimmune disease. The complexity of this question is demonstrated in the studies with the Goldblatt rat where no relation between serum IgG levels, age, or blood pressure was seen.

The results obtained with IL-2 may be more persuasive in implicating the immune system in the genesis of hypertension in the SHR. This lymphokine

Line graphs showing blood pressures and serum Immunoglobulin G (IgG) in spontaneously hypertensive rats (SHR) 94–122 days old before and after single bolus injection of interleukin-2 (IL-2) (5,000 units/kg) (●) or carrier alone (○). Each point is mean±SEM of six rats. *Denotes significant difference (p<0.01) between carrier and IL-2 in SHR of same age.

Line graphs showing effect of interleukin-2 (IL-2) (5,000 units/kg) on blood pressure (top panel) and serum Immunoglobulin G (IgG) (lower panel) in six each: Goldblatt Wistar-Kyoto (WKY) rats (○), sham-operated WKY rats (○), and sham-operated spontaneously hypertensive rats (SHR) (●). IL-2 was given by single bolus at 35 days of age. Each point represents mean±SEM of six rats. *Represents significant difference (p<0.01-0.001) between the Goldblatt WKY rats and the sham-operated SHR of same age.
plays an important role in the proliferation and differentiation of the T cells, a component of the immune system that may be deficient in the SHR.1,2,4

The remarkable effect of low dose IL-2 on hypertension and serum IgG levels remains to be explained. A number of conclusions are justified from the existing data. A single subcutaneous injection of IL-2 produces antihypertensive effects at dose levels two to three orders of magnitude lower than those used in cancer or AIDS chemotherapy.11 No toxic effects or signs of discomfort are apparent at these doses, and blood pressure never approaches shock levels. The antihypertensive effect of a single injection persists for months.

Questions remain concerning the most effective dose and incidence of side effects with higher doses of IL-2. These are subjects of active research, but there is general agreement that the half-life of IL-2 in vivo is short, less than 10 minutes. A wide range of single and repeated doses (50,000–100,000 units/kg) have been used in humans in the course of treatment of cancer and AIDS.11 Hypotension is one side effect of repeated high dose administration of IL-2, which reverses rapidly with the cessation of IL-2 treatment. The 5,000 units/kg used in our study was in the range of that used by others in mice for pharmacodynamic studies,13 and less than that used in studies of wound healing in the rat.14 Both studies report no adverse effect of IL-2.

Considering the short half-life of IL-2 in vivo and the enduring effects on blood pressure, it seems reasonable to assume that IL-2 produces some basic change in the hemodynamics of the SHR. Because the reduction in serum IgG is relatively short lived compared with the effect on blood pressure, it is doubtful that rheological factors related to changes in serum IgG alone could effect the decline in blood pressure. Additional studies of IL-2 and the SHR will likely involve a detailed examination of the immune system to assess the mechanism for the antihypertensive effect of IL-2. Evidence at this stage of the investigation suggests that IL-2 is not effective in all models of hypertension because blood pressure in the single-kidney, Goldblatt WKY rat was not lowered by IL-2. This suggests that IL-2 may have a relatively specific effect on essential hypertension and that the depression of cardiac or vascular smooth muscle by IL-2 is probably not an important factor in its antihypertensive effects.

References


KEY WORDS • immunoglobulins • interleukins • Goldblatt hypertension • spontaneously hypertensive rat
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