Effect of Erythropoietin on Hematocrit and Blood Pressure in Normotensive and Hypertensive Rats

Martin Muntzel, Thierry Hannedouche, Bernard Lacour, and Tilman Drüke

ABSTRACT

Treatment with recombinant human erythropoietin (rHuEPO) successfully reverses anemia in uremic patients. Of major concern, however, are blood pressure (BP) increases during rHuEPO therapy, observed particularly in persons with a history of hypertension. To determine whether preexisting hypertension enhances BP increases with rHuEPO, BP responses to 2 wk of rHuEPO or placebo were observed in spontaneously hypertensive rats (SHR) and their normotensive genetic controls (Wistar-Kyoto (WKY) rats). In addition, the role of endothelial-released nitric oxide (NO) in BP alterations caused by rHuEPO through i.v. infusions of endothelium-dependent and independent vasoactive agents were indirectly examined. At trial completion, rHuEPO elevated hematocrit, hemoglobin, and mean cell volume more in SHR than in WKY rats (P < 0.001). Despite the considerable increase in hematocrit, rHuEPO did not alter BP in either strain. An infusion of Nω-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NO formation, elevated BP more in rHuEPO-treated SHR than in identically treated WKY rats (P < 0.05). Further, the administration of L-arginine caused a greater decrease in blood pressure in SHR than in WKY rats, independent of treatment condition (P < 0.01). Because changes in BP with endothelium-independent agents were similar across groups, responses to L-NMMA and L-arginine were specific to the endothelium and probably independent of basal BP. Thus, rHuEPO provoked greater erythropoiesis in SHR than in WKY rats but did not elevate BP. L-NMMA stimulated higher BP in SHR treated with rHuEPO, suggesting a compensatory increase in vasodilatory NO synthesis to protect against a hypertensive effect of the drug in SHR. The hypertensive effect may be ascribed to the exaggerated increase in hematocrit and perhaps viscosity in the SHR strain.

Key Words: Erythropoiesis, L-arginine, Nω-monomethyl-L-arginine, spontaneously hypertensive rats, Wistar-Kyoto rats

Recombinant human erythropoietin (rHuEPO) successfully reverses anemia in uremic hemodialysis patients (1). A major side effect, however, has been the development of hypertension in 20 to 30% of rHuEPO-treated patients, requiring the institution or augmentation of antihypertensive medication (1,2). Elevations in blood pressure by rHuEPO are not limited to the reversal of anemia, because 2 wk of rHuEPO treatment increased blood pressure in normal, nonanemic rats (3,4).

A determinant of hypertensive episodes with rHuEPO therapy may be the presence of preexisting hypertension (2). Consistent with this possibility, both blood transfusion and isotonic saline loading increased blood pressure in anemic patients with a history of hypertension but did not alter pressure in anemics with normotension (5,6). In addition, rHuEPO treatment necessitated the reinforcement of antihypertensive medication in eight hypertensive dialysis patients, yet did not influence pressure in six normotensive dialysis subjects (7). Laboratory studies supported these findings, because experimental manipulations that reduced hematocrit lowered blood pressure in spontaneously hypertensive rats (SHR), but similar alterations in hematocrit had no effect on blood pressure in normotensive Wistar-Kyoto (WKY) rats (8).

The mechanism of blood pressure elevation with rHuEPO is incompletely understood but has been ascribed to increased blood viscosity consequent to increasing hematocrit, to a loss of hypoxic vasodilation, and to direct vasoconstrictor actions of the drug (1,2,9). An additional explanation, proposed by Martin and Moncada (10), implicates alterations in nitric oxide (NO), recently identified as one of the endothelial-derived relaxing factors (11). According to those authors, increases in hemoglobin after rHuEPO therapy may bind NO, thereby favoring the contraction of vascular smooth muscle and the elevation of arterial pressure (10).

To determine whether the blood pressure-raising...
effects of rHuEPO are enhanced by preexisting hypertension in nonanemic rats, we observed pressure responses after chronic rHuEPO therapy in normotensive (WKY) and hypertensive (SHR) rat strains. In addition, we explored the role of NO in blood pressure alterations induced by rHuEPO.

MATERIALS AND METHODS

Twenty male WKY rats and 20 male SHR, weighing 220 to 240 g, were obtained from IFFA CREDO (L’Arbresle, France). The animals were housed in groups of three in a temperature-controlled colony room, illuminated on a 12:12 light-dark cycle. Throughout the experiment, the animals were given continuous access to tap water and standard lab chow. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

After 4 days of habituation, baseline systolic blood pressure (SBP) were recorded from the tail in conscious, loosely restrained rats with a programmed electrophysgmonanometer (NARCO Bio-Systems, Houston, TX). On the same day, venous blood was collected from a subclavian venipuncture in anesthetized rats for the determination of hematocrit.

After baseline measurements, SHR and WKY rats were randomly divided into control (N = 10) and rHuEPO (N = 10)-treated groups. Rats assigned to rHuEPO therapy received s.c. injections of rHuEPO (100 U/kg) in a volume of approximately 0.2 mL (CILAG Company, Levallois, France), once every 2 days for 2 wk. Control rats received injections of the vehicle for rHuEPO, containing (in milligrams per milliliters): 2.5, human serum albumin; 5.8, sodium chloride; 5.8, sodium citrate; and 0.06, citric acid anhydrous, in 1.0 mL of water. During the 2 wk of treatment, SBP and hematocrit were collected approximately once each week for the duration of the experiment. At the end of the second week, hematologic parameters were determined by Coulter analysis and serum iron was assessed with a colorimetric kit with ferrozine (Roche Monarch, Lexington, MA).

On the following day, direct blood pressure measurements were obtained from all animals. The rats were anesthetized with pentobarbital (50 mg/100 g body wt) and placed on a warming table regulated at 38°C. A tracheal cannula was inserted. Polyethylene catheters (Clay Adams, Parsippany, NJ) were inserted in the right femoral vein for drug infusion and in the left femoral artery for blood pressure monitoring. The signal from the arterial catheter was recorded with a transducer (P23DB; Gould-Statham, Cleveland, OH) connected to an amplifier and a recorder (Gould Instruments, Ballainvilliers, France).

After arterial pressure had stabilized, 5 min of blood pressure was recorded for the determination of baseline values. Drug infusions were then begun to assess the role of NO-mediated vasodilation in blood pressure alterations caused by rHuEPO. Nω-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of NO formation, and L-arginine, the endogenous substrate for endothelial NO, were given in doses designed to produce moderate pressor and depressor responses, respectively (12,13). Sodium nitroprusside (SNP; Sigma Chemical Co., St. Louis, MO) and angiotensin II (All; Sigma) were given to determine blood pressure responses to two endothelium-independent vasoactive agents (13,14). Doses of SNP and All were tailored to produce blood pressure responses equivalent to those produced by L-arginine and L-NMMA. Responses to drugs were calculated as peak change in blood pressure from baseline.

L-Arginine, SNP, and All were given as bolus i.v. injections, in random order, approximately 5 min apart from one another. L-Arginine (62.4 mg/mL) was administered as a hydrochloride solution (pH = 5 to 6) in a dose of 100 mg/kg (Veyron-Froment Laboratories, Marseille, France). L-Arginine was injected twice so that both depressor responses could be averaged to give one value for each rat. SNP was given as 0.5 μg/kg, and All was given as 36 ng/kg. L-NMMA (Sigma) was given as the final bolus injection in a dose of 10 mg/kg. L-NMMA was administered as the last injection because the drug stimulates long-lasting (20 to 40 min) increases in blood pressure.

The data were analyzed by appropriate single or repeated measures analysis of variance. Differences between experimental groups were considered significant at the P < 0.05 level.

RESULTS

The evolution of hematocrit and SBP during the study is depicted in Figures 1 and 2, respectively. SHR had slightly greater hematocrits than did WKY

![Figure 1: Effects of 12 days of treatment with placebo or rHuEPO on hematocrit in WKY rats and SHR. Values are means ± SE; N = 10 animals per group. The administration of rHuEPO increased hematocrit in both strains (P < 0.0001). In addition, SHR exhibited higher hematocrits than did WKY rats (P < 0.01).](image)
rats, regardless of treatment with rHuEPO ($P < 0.01$). In addition, the administration of rHuEPO generated expected elevations of hematocrit in both strains ($P < 0.0001$). The administration of rHuEPO did not alter SBP in WKY rats nor in SHR (Figure 2).

Hematologic parameters measured by Coulter analysis are presented in Table 1. Similar to the findings reported above, hematocrit was elevated in rHuEPO-treated groups at trial completion ($P < 0.0001$). Furthermore, this elevation was greater in SHR than in WKY rats ($P < 0.01$). The drug also increased blood hemoglobin more in SHR than in their normotensive counterparts ($P < 0.01$). The elevations in hematocrit and hemoglobin were partly explained by increases in red blood cell counts ($P < 0.0001$). However, this increase was similar in both strains and did not explain higher elevations of hematocrit and hemoglobin in rHuEPO-treated SHR. Rather, the higher elevations may be accounted for by increases in red blood cell volume in rHuEPO-treated SHR ($P < 0.01$) that were not observed in rHuEPO-treated WKY rats. Treatment with rHuEPO elevated the white blood cell (WBC) count equally in both strains ($P < 0.05$). Finally, plasma iron concentrations were significantly lowered by rHuEPO treatment ($P < 0.01$), with no detectable influence of strain. In the placebo-treated control groups, red blood cell counts were greater in SHR than in the normotensive strain ($P < 0.01$). WBC counts were also higher in SHR than in WKY rats ($P < 0.0001$), and rHuEPO increased WBC in both strains ($P < 0.05$).

Direct blood pressure measurements before drug infusion revealed no effect of rHuEPO on SBP in either strain. An analysis of diastolic blood pressures showed similar outcomes.

A representative tracing depicting blood pressure responses to L-arginine, SNP, All, and L-NMMA is shown in Figure 3. Mean changes in SBP elicited by the drug infusions are presented in Figure 4. Blood pressure declined within a few seconds after bolus administrations of L-arginine and quickly returned to baseline at the end of infusion. This decrease in pressure was approximately three times greater in SHR than in WKY rats ($P < 0.0001$). Infusions of SNP resulted in hypotensive responses that were not different between any of the experimental groups. Bolus infusions of All stimulated short-lasting pressor responses that also did not differ between groups. L-NMMA generated blood pressure increases that lasted 10 to 20 min in all rats (Figure 3 shows only a portion of the response). Maximal SBP responses to L-NMMA were similar between control SHR and WKY rats. However, treatment with rHuEPO potentiated the blood pressure increase in SHR ($P < 0.05$), but not in WKY rats.

![Figure 2. Effects of 12 days of treatment with placebo or rHuEPO on SBP in WKY rats and SHR. Groups are the same as those in Figure 1. SBP was higher in SHR than in WKY rats ($P < 0.0001$).](image-url)

**Table 1.** Hematologic parameters and plasma iron of WKY rats and SHR treated with placebo or rHuEPO for 3 wk.

<table>
<thead>
<tr>
<th>Measure</th>
<th>WKY Rats</th>
<th>SHR</th>
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<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>rHuEPO</td>
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<tr>
<td>Hct (%)</td>
<td>44.8 ± 0.6</td>
<td>50.4 ± 1.0</td>
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<tr>
<td>Hb (g/100 mL)</td>
<td>16.2 ± 0.2</td>
<td>18.2 ± 0.2</td>
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<tr>
<td>RBC ($\times 10^6$/mm$^3$)</td>
<td>8.1 ± 0.09</td>
<td>9.0 ± 0.10$^b$</td>
</tr>
<tr>
<td>MCV ($\mu^3$)</td>
<td>55.1 ± 0.2</td>
<td>55.5 ± 0.2</td>
</tr>
<tr>
<td>WBC ($\times 10^3$/mm$^3$)</td>
<td>7.3 ± 0.2</td>
<td>9.2 ± 0.4$^b$</td>
</tr>
<tr>
<td>Platelets ($\times 10^3$/mm$^3$)</td>
<td>600 ± 32</td>
<td>715 ± 44</td>
</tr>
<tr>
<td>Plasma iron ($\mu$mol/L)</td>
<td>24.5 ± 4.5</td>
<td>8.3 ± 2.2$^b$</td>
</tr>
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$^a$ Values are means ± SE. N = 10 animals/group. Hct, hematocrit; Hb, hemoglobin; RBC, red blood cell count.

$^b$ $P < 0.05$ relative to corresponding placebo condition.

$^c$ $P < 0.05$ relative to corresponding WKY group.
DISCUSSION

The chronic administration of rHuEPO raised hematocrit but did not elevate blood pressure in normotensive WKY rats or in SHR with genetic hypertension. These findings contrast with those of other groups describing blood pressure increases after rHuEPO treatment in normotensive rats (3) and in SHR (4). The disparity in blood pressure results may be related to the smaller dose of rHuEPO administered in this report (100 U/kg) compared with those given in earlier studies (150 U/kg [3,4]).

rHuEPO stimulated greater hematocrit and hemoglobin increases in SHR than in WKY rats. This difference was not due to variations in iron availability because plasma iron concentrations were equivalent in SHR and WKY control groups and be-

cause rHuEPO treatment diminished iron comparably in both strains. Neither was the enhanced responsiveness in SHR due to greater stimulation of red blood cell numbers because increases in this parameter were similar in SHR and WKY rats. Rather, rHuEPO augmented mean cell volume (MCV) in SHR but not in WKY rats. Elevations in mean red blood cell volume by rHuEPO were predicted because the accelerated mitotic frequency of late erythroid precursors in bone marrow should decrease the average number of mitoses, resulting in the expulsion of larger immature red blood cells (15). The absence of increased MCV in WKY rats may reflect a less-powerful stimulation of erythropoiesis and a slower acceleration of mitosis.

A hypersensitivity of SHR to rHuEPO may explain previous reports of elevated hematocrit and erythrocytosis in this strain (16–18). SHR have also been characterized by erythroid hyperplasia in bone marrow, increased numbers of erythroid progenitors, and elevated iron incorporation into red blood cells (16,17). Erythrocytosis and high hematocrit in SHR increases blood viscosity and may contribute to the development of hypertension (18). Because earlier studies reported increased endogenous erythropoietin in SHR, this anomaly was thought to be the proximal cause of erythroid hyperplasia in these rats (16,17). The findings presented here suggest that hyperplasia in SHR may also be due to the increased responsiveness to erythropoietin. Although the mechanism of increased responsivity in SHR is unclear, Tepel et al. (19) reported that rHuEPO stimulated greater increases in cytosolic free calcium in platelets from SHR than in those from WKY rats. Importantly, an increase in intracellular calcium appears to be the signal that mediates the proliferative effect of rHuEPO in erythroid precursor cells (20).

In addition to alterations in red blood cell parameters, we also observed a higher WBC count in SHR compared with that in WKY rats and a stimulation of the WBC by rHuEPO in both strains. The fact that rHuEPO stimulates an increase in WBC has been known for some time (21). In addition, the finding of increased WBC in SHR has also been described (22) and corresponds with reports of elevated WBC in human hypertensives (23).

Bolus injections of L-NMMA increased blood pressure in all animals. These findings, in agreement with those from earlier reports (24,25), indicate that NO release from the endothelium is a determinant of basal vascular resistance in rats (24,25). In the groups treated with rHuEPO, the inhibition of NO synthesis with L-NMMA elevated blood pressure more in SHR than in WKY rats. This implies a compensatory increase of NO synthesis to protect against a hypertensive effect of rHuEPO in SHR, which, when disrupted by L-NMMA, results in greater blood
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pressure increases (24). The blood pressure-elevating effect of rHuEPO may be ascribed to the exaggerated increase in the hematocrit and blood viscosity in rHuEPO-treated SHR (26). Although similar NO-mediated vasodilatory compensation would be expected in rHuEPO-treated WKY rats, this group showed only a small increase in hematocrit. In addition, the nonlinear relationship between hematocrit and blood viscosity (27) suggests that viscosity changed very little in WKY rats treated with rHuEPO.

It should be noted that our data do not support the hypothesis of Martin and Moncada (10), at least in the present range of hematocrit increase. Those authors claimed that rHuEPO-induced increases in hematocrit and hemoglobin may trap NO, thus reducing NO-mediated vasodilation. Decreased NO availability in rHuEPO-treated groups should have been reflected as attenuated increases in blood pressure after the disruption of NO synthesis by L-NMMA. We, however, observed normal and enhanced blood pressure increases after L-NMMA in rats treated with rHuEPO.

L-Arginine elicited greater blood pressure reductions in SHR than in WKY rats. Because hypertensive responses to endothelium-independent SNP were similar across both strains, the variation in response to L-arginine was probably independent of baseline blood pressure. This argument, however, does not exclude the possibility that greater decreases in blood pressure to L-arginine in SHR were a consequence of higher baseline blood pressures. In human studies, L-arginine elicited mild hypotension, leading the authors to suggest that the decrease in blood pressure was caused by increased NO production (12). Opposing this hypothesis, other studies found the enzymatic conversion of L-arginine to NO to be saturated and not rate limiting under normal conditions (28). The current finding of enhanced hypotension to L-arginine in SHR suggests either inadequate endogenous L-arginine or increased activity of NO synthase. Further studies will be necessary to determine specificity to the NO system.

To summarize, chronic rHuEPO therapy elicited greater increases in hematocrit and hemoglobin in SHR than in WKY rats but did not stimulate hypertension in either strain. L-NMMA induced a moderate increase in blood pressure in all animals. However, SHR treated with rHuEPO exhibited a greater increase in blood pressure than did controls or WKY rats treated with rHuEPO. This indicates a blood pressure-elevating effect of rHuEPO in SHR that is negated by a compensatory increase in the steady-state release of NO from the vascular endothelium.

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