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Anemia lessens and its prevention with recombinant human erythropoietin worsens glomerular injury and hypertension in rats with reduced renal mass

(glomerular function/proteinuria/chronic renal failure/glomerular sclerosis/blood viscosity)

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ABSTRACT **Chronic renal disease is frequently characterized by anemia, which may modify systemic and renal hemodynamics. In adult Munich–Wistar rats, the mild anemia (hematocrit, ≈42 vol/dl) that accompanies five-sixths nephrectomy was either made more severe (≈30 vol/dl) by feeding a low iron diet or prevented (≈50 vol/dl) by administration of recombinant human erythropoietin (r-HuEpo). In functional studies performed 4 weeks after renal ablation, untreated rats exhibited mild anemia with systemic hypertension and elevation of the single nephron glomerular filtration rate due to glomerular capillary hyperperfusion and hypertension. Preventing anemia with r-HuEpo worsened systemic and glomerular hypertension, effects largely obviated by induction of more marked anemia with the low iron diet. Untreated rats followed for 6 weeks postablation exhibited progressive proteinuria and sclerosis involving 12% of glomeruli, contrasted with 33% in rats given r-HuEpo. Even after 12 weeks, sclerosis involved only 6% of glomeruli in rats with more severe anemia but progressed to 30% in untreated rats. Thus, anemia limits systemic and glomerular hypertension and glomerular injury, whereas its prevention by r-HuEpo severely accelerates hemodynamically mediated glomerular injury in this model. These results suggest that anemia is a hemodynamically favorable adaptation to chronic renal disease and that its overly vigorous correction may have adverse renal hemodynamic and structural consequences.**

Hope of slowing the progression of renal disease has stimulated investigation into the mechanisms responsible for ongoing glomerular injury. Removal of >70% of the renal mass leads to systemic hypertension, progressive proteinuria, and glomerular sclerosis (1–4). Extensive renal ablation is associated with a compensatory increase in the single nephron (SN) glomerular filtration rate (GFR) in the remnant kidney (2–4). Vascular resistance is reduced in afferent and efferent arterioles, allowing an increase in the glomerular capillary plasma flow rate, Q_A. Because the decrease in afferent arteriolar resistance (R_A) is proportionately greater than that in efferent arteriolar resistance (R_E), the glomerular capillary hydraulic pressure ($\bar{P}_{GC}$) and therefore the glomerular transcapillary hydraulic pressure gradient ($\overline{\Delta P}$) rise (2–4). Interventions that attenuate these hemodynamic changes slow the development of glomerular sclerosis. Dietary protein restriction, which lowers SNGFR by reducing Q_A and $\overline{\Delta P}$, affords long-term structural protection in numerous models of progressive renal disease (1, 5, 6). Alternatively, selective reduction of $\overline{\Delta P}$ with angiotensin I converting enzyme inhibitor therapy slows the development of glomerular sclerosis even in the presence of continued hyperfiltration and hyperperfusion (3, 4, 7), suggesting that glomerular capillary hypertension is the crucial hemodynamic determinant of the progression of renal disease. Not surprisingly, factors that aggravate glomerular hypertension enhance the risk of progression of chronic renal failure. For example, potent renal vasodilators such as glucocorticoids, which augment glomerular capillary flows and pressures, worsen glomerular lesions produced by ablation of renal mass in the rat (4).

Thus, conditions that modify glomerular hemodynamics may alter the pace of progression. One such condition is anemia, which is nearly always present in humans once the GFR falls below 40% of normal (8). Indeed, the hematocrit (Hct) falls in inverse proportion to the rise in serum creatinine concentration (9). Chronic anemia leads to an increase in cardiac output, primarily mediated by a reduction in peripheral vascular resistance that results from vasodilatation and reduction in blood viscosity (10–12). Acute lowering of Hct in dogs and rats increases renal plasma flow without affecting GFR, thereby decreasing filtration fraction (13, 14). Evidence suggests that anemia serves to moderate the hypertension that also characterizes clinical renal disease. Raising the Hct from 19 to 43 vol/dl in such patients with transfusion therapy results in significant increases in mean and diastolic blood pressures (12).

Experimental studies confirm the importance of Hct in governance of renal function. Previous micropuncture studies in the normal rat indicate that an acute reduction in Hct on average from 51 to 20 vol/dl results in acute increases in Q_A more than SNGFR, thereby decreasing filtration fraction, and that R_A and R_E both fall, resulting in a reduction in $\overline{\Delta P}$. Conversely, an acute increase in Hct on average from 51 to 61 vol/dl decreases SNGFR less than Q_A, with R_E increasing more than R_A, allowing an increase of $\overline{\Delta P}$ and filtration fraction (14).

The potential role of Hct in progression of chronic renal disease has not been explored. Recently, the availability of recombinant human erythropoietin (r-HuEpo) and its success in correcting the anemia of end-stage renal disease (15) have stimulated interest in its potential use in patients with less marked renal insufficiency. However, potential adverse consequences of normalizing Hct with r-HuEpo administration include worsening of systemic hypertension, clotting of arteriovenous fistulas, and hyperkalemia in patients on dialysis (16–18). In consequence, we sought to examine the role

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Abbreviations: SN, single nephron; GFR, glomerular filtration rate; Q_A, glomerular plasma flow rate; $\bar{P}_{GC}$, mean glomerular capillary hydraulic pressure; $\overline{\Delta P}$, mean glomerular transcapillary hydraulic pressure gradient; R_A, afferent arteriolar resistance; R_E, efferent arteriolar resistance; Hct, hematocrit; r-HuEpo, recombinant human erythropoietin; $\overline{AP}$, mean arterial pressure; K_f, glomerular capillary ultrafiltration coefficient.

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of Hct on glomerular hemodynamics and progression of glomerular injury in rats with renal ablation.

METHODS

Dietary and pharmacologic means were used to enhance and to prevent the anemia that characterizes severe reduction in nephron mass in the rat. Three groups of male Munich–Wistar rats were fed isocaloric diets differing only in the iron content. An untreated control group (C) (220–250 g) received standard rat chow (35 mg of iron per kg of chow). A second group (A) (120–150 g) was pretreated with an isocaloric diet containing a low (6 mg/kg) iron content, which induces mild anemia in the rat (19); periodic phlebotomy was performed for 8 weeks prior to ablation to further reduce the Hct to 30 vol/dl. A third group (E) (220–250 g) was fed standard rat chow and was treated with r-HuEpo (AMGen, Thousand Oaks, CA), 25 units i.p. twice weekly beginning at the time of renal ablation to maintain Hct at the normal prenephrectomy value of ≈50 vol/dl. Hematocrits were measured every 2 weeks. When the Hct reached 35 vol/dl in the low iron group, and the body weight was comparable to the other groups, the rats were subjected to five-sixths nephrectomy by removal of the right kidney and ligation of two or three branches of the left renal artery (3, 4).

At 4 weeks after renal ablation, six or seven rats from each group underwent micropuncture study. Rats were anesthetized with Inactin (100 mg/kg of body weight, i.p.), placed on a temperature-regulated table, and prepared for micropuncture in standard fashion (3, 4). Euvolemia was maintained by i.v. infusion of isoncotic rat plasma (20). Inulin (4 g/dl in 0.9% NaCl) was also infused at a rate of 1.2 ml/hr. Tubule fluid was collected from surface proximal tubule convolutions for determination of flow rate and inulin concentration (21). Samples of efferent arteriolar blood were obtained for determination of protein concentration (22). Hydraulic pressures were measured in glomerular capillaries, proximal tubules, and efferent arterioles by the servo-null technique (23).

The remaining rats in each group (n = 10–13) were followed for 6–12 weeks, with serial measurements of Hct, systolic blood pressure, and proteinuria. In preliminary experiments rats in group E failed to survive for 12 weeks after renal ablation. Accordingly, subsequent r-HuEpo-treated rats were sacrificed at 6 weeks. Systolic blood pressure was measured every 2 weeks in all rats in the conscious state by the tail cuff method (24). Hct was measured in tail blood following each measurement of systolic blood pressure. Twenty-four-hour urinary total protein excretion was measured at 3-week intervals.

At the time of sacrifice remnant kidneys were fixed by perfusion at the measured arterial pressure with 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After fixation, two 3- to 4-mm-thick midcoronal sections of the remnant kidney were postfixed in buffered formaldehyde solution (4 g/dl) and processed for light microscopy through paraffin embedding. Sections 3 μm in thickness were stained with hematoxylin/eosin and by the periodic acid/Schiff technique. The frequency of focal and segmental sclerosis was determined by examining all glomerular profiles (197 ± 6 profiles per animal) contained in two coronal sections from each animal. For each animal, the number of glomeruli with segmental lesions was expressed as a percentage of the total number of glomeruli counted. Epoxy-resin-embedded fragments of renal cortex were stained with 1% toluidine blue in 1% aqueous borax and examined nonquantitatively by light microscopy for other glomerular changes, such as expansion of the mesangial areas and abnormalities of arteries and arterioles.

Statistical analysis was performed by one-way analysis of variance, and the significance of multiple pairwise comparisons was determined by the method of Bonferroni (25).

RESULTS

Body weight was similar in all groups at the time of renal ablation; thereafter all rats gained weight at comparable rates. Food and water intake was measured at random time points and was comparable among groups. Serial values for Hct are depicted in Fig. 1. Untreated rats exhibited mild anemia, with values for Hct falling from 50 ± 1 vol/dl (mean ± SEM) at the time of renal ablation to 42 ± 1 vol/dl 4 and more weeks thereafter. The low iron diet had the desired effect of reducing Hct to 34 ± 2 vol/dl, at which time five-sixths nephrectomy was performed, leading soon thereafter to a further fall in Hct. R-HuEpo was effective, despite renal ablation, in maintaining Hct at 50 ± 1 vol/dl, the same value measured before nephrectomy.

Untreated rats subjected to five-sixths nephrectomy developed systemic hypertension within 2 weeks of renal ablation. As demonstrated in Fig. 2, systolic blood pressures in group C rats averaged 155 ± 8 mmHg (1 mmHg = 133.3 Pa) by 2 weeks and remained at even higher levels thereafter. Hypertension was even more severe in group E, with values about 20 mmHg higher than those seen in control rats at each time point ($P < 0.05$ vs. group C). Despite equally extensive renal ablation, the development of systemic hypertension was largely prevented in anemic rats (group A), with values for systolic blood pressure maintained at ≈130 mmHg throughout the study. These values are comparable to those seen in intact, two-kidney rats (7).

Table 1 summarizes the mean values for body weight, Hct, whole kidney GFR, mean arterial pressure ($\overline{AP}$), SNGFR, and the pressures, flows, and resistances governing glomerular ultrafiltration for the three groups when studied 4 weeks after ablation. There were no significant differences in body weight among the three groups. In untreated control rats, Hct fell from 50 ± 1 to 42 ± 1 vol/dl following partial nephrectomy. The low iron diet was effective in accentuating anemia in group A, with values falling to 27 ± 1 vol/dl. In contrast, r-HuEpo treatment in group E maintained Hct at 51 ± 1 vol/dl, a value similar to that seen before nephrectomy. Values for $\overline{AP}$ were elevated in group C rats, averaging 143 ± 8 mmHg. $\overline{AP}$ remained in the normal range in anemic rats

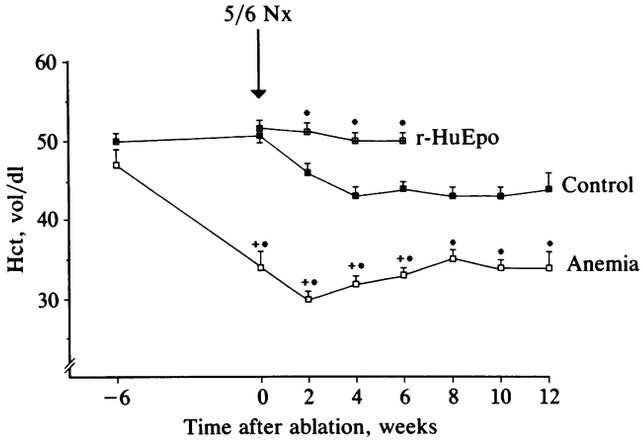

FIG. 1. Hct measured in tail blood in conscious rats followed for 6 weeks (r-HuEpo) and 12 weeks (Control and Anemia) after five-sixths nephrectomy (5/6 Nx). Partial nephrectomy lowered Hct in control rats, whereas r-HuEpo prevented the fall in Hct after five-sixths nephrectomy. Low iron diet in the anemic group accentuated anemia after five-sixths nephrectomy. Values are means ± SEM. *, $P < 0.05$ vs. control; +, $P < 0.05$ vs. r-HuEpo.

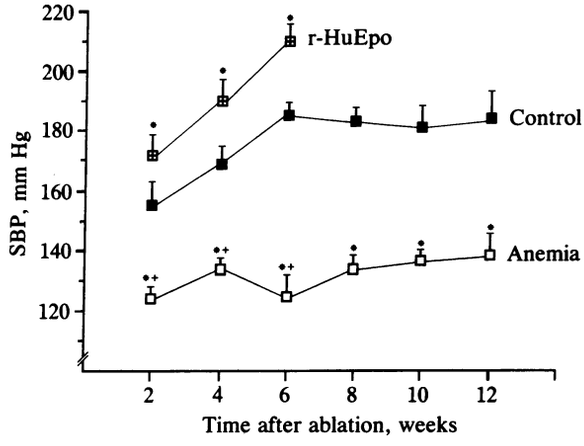

FIG. 2. Systolic blood pressure (SBP) measured by the tail cuff method in conscious rats followed for 6 weeks (r-HuEpo) and 12 weeks (Control and Anemia). Control rats exhibited sustained systemic hypertension. Hypertension was aggravated by normalization of Hct in r-HuEpo-treated rats, whereas anemia markedly ameliorated systemic hypertension. Values are means ± SEM. *, $P < 0.05$ vs. control; +, $P < 0.05$ vs. r-HuEpo.

(123 ± 2, $P < 0.05$ vs. group C), whereas extreme hypertension (166 ± 9, $P < 0.05$ vs. both other groups) occurred in rats with Hct maintained at normal levels with r-HuEpo.

SN hyperfiltration was apparent in all groups, with values considerably higher than those seen in normal rats (7). In the untreated group C rats, SN hyperfiltration resulted from elevations of Q_A, which averaged 257 ± 13 nl/min, and $\overline{\Delta P}$ (48 ± 1 mmHg). Since values for proximal tubule hydraulic pressure were equivalent in all groups, alterations in $\overline{\Delta P}$ reflected differences in values for $\overline{P}_{GC}$. Anemia (group A) resulted in significantly higher values for Q_A as compared with groups C and E. Anemia prevented the development of glomerular capillary hypertension, so that values for $\overline{\Delta P}$ (34 ± 2 mmHg) were maintained at near-normal levels ($P < 0.05$ vs. groups C and E). Values for K_f, the glomerular capillary ultrafiltration coefficient, were preserved at near-normal levels in the anemic group ($P < 0.05$ vs. groups C and E). Values for efferent arteriolar hydraulic pressure, afferent arteriolar protein concentration, and afferent colloid osmotic pressure were comparable in all groups.

Maintenance of Hct at the normal level with r-HuEpo in group E resulted in extremely high values for $\overline{\Delta P}$ (58 ± 2 mmHg), and low values for K_f (36 ± 3 pl/(s·mmHg), both even more extreme than those seen in group C. These contrasting patterns were the result of changes in $\overline{\Delta P}$ and also in the intrarenal resistances. Values for R_A were comparable in the three groups. Anemia lowered R_E, thereby accounting for the reduction in $\overline{\Delta P}$. r-HuEpo numerically increased R_E, which, combined with a more severe degree of systemic hypertension, resulted in extreme glomerular capillary hypertension.

In the untreated group C rats, systemic and glomerular hypertension were associated with increasing levels of proteinuria throughout the 12-week observation period (Fig. 3). The markedly enhanced systemic and glomerular capillary hypertension resulting from r-HuEpo administration in group E was associated with greater severity of proteinuria such that values in group E rats were higher than those in group C rats, as early as 3 weeks postablation ($P < 0.05$). Control of systemic and glomerular hypertension with anemia markedly blunted this proteinuric response ($P < 0.05$ vs. groups C and E).

At the end of the 12-week observation period, group C animals showed prominent and widespread glomerular alterations characterized by focal and segmental collapse of capillaries, hyaline deposition, and adhesion of the glomerular tuft to Bowman's capsule, involving 31.0% ± 4.0% of glomeruli. These areas of collapse often contained vacuolated cells surrounded by basement membrane and matrix material. Epithelial cell abnormalities with increased numbers of lysosomes (reabsorption droplets) and cytoplasmic blebs were often observed. Occasional areas of tubule atrophy, interstitial fibrosis, and mild chronic inflammation, and cast formation in distal tubules and ascending thick segments of the loop of Henle, were observed in association with the glomerular abnormalities. Arteries and arterioles showed rare hypertrophic changes of their media with minimal hyaline deposition. The incidence of lesions in r-HuEpo-treated rats was more than twice that of the untreated group, involving 33.2% ± 6.0% and 12.8% ± 3.0% of glomeruli, respectively, at 6 weeks ($P < 0.05$). Indeed, glomerular injury in rats in group E at 6 weeks was comparable to that seen in untreated rats sacrificed at 12 weeks. In contrast, anemic animals in group A showed much less glomerular injury, with segmental glomerular lesions limited on average to 5.6% ± 3.0% at 12 weeks ($P < 0.05$ vs. group C).

DISCUSSION

The present findings confirm previous observations of a direct relationship between Hct and systemic blood pressure. In this study in rats with partial renal ablation, preventing anemia with r-HuEpo resulted in aggravation of systemic hypertension, whereas enhancing anemia with the low iron diet prevented the development of systemic hypertension. Hct and blood pressure have also been directly correlated in patients with renal disease (12), as well as patients with essential hypertension (26–28). Elevation of Hct raises total peripheral resistance by increasing blood viscosity, as well as by increasing O_2 delivery and reducing peripheral capillary vasodilatation. Increased blood viscosity not only raises peripheral resistance and reduces capillary blood flow but also serves to decrease plasma volume (due to increased hydraulic pressure and extravasation of fluid from the vasculature into the interstitium) (29). Conversely, anemia is associated with peripheral vasodilatation, reduced total peripheral resistance, and lower blood pressure.

Table 1. Summary of renal cortical microcirculation studies

Group	*n*	BW, g	Hct, vol/dl	$\overline{AP}$, mmHg	SNGFR, nl/min	Q_A, nl/min	$\overline{\Delta P}$, mmHg	K_f, pl/(s·mmHg)	R_A, dyne·s·cm^{-5}·10^{10}	R_E, dyne·s·cm^{-5}·10^{10}	R_T, dyne·s·cm^{-5}10^{10}
C. Control	6	279 ± 9	42 ± 1	143 ± 8	80 ± 5	257 ± 13	48 ± 1	61 ± 6	1.5 ± 0.15	1.0 ± 0.05	2.5 ± 0.20
A. Anemia	7	263 ± 1	27 ± 1	123 ± 2	88 ± 4	363 ± 16	34 ± 2	109 ± 7	1.2 ± 0.06	0.6 ± 0.05	1.8 ± 0.10
E. r-HuEpo	7	260 ± 7	50 ± 1	166 ± 9	72 ± 3	226 ± 16	58 ± 2	36 ± 3	1.7 ± 0.19	1.3 ± 0.15	3.1 ± 0.30
P value											
C vs. A		NS	<0.05	<0.05	NS	<0.05	<0.05	<0.05	NS	<0.05	NS
C vs. E		NS	<0.05	<0.05	NS	NS	<0.05	<0.05	NS	NS	NS
A vs. E		NS	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

BW, body weight; RT, total renal resistance; NS, not significant.

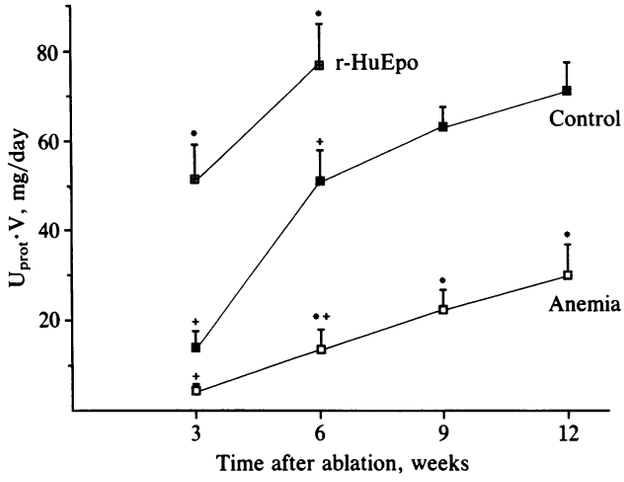

FIG. 3. Urinary protein excretion rates (U_{prot}·V) per day. Untreated control rats developed progressive proteinuria over the 12-week course. Anemia significantly limited proteinuria. Normalization of Hct with r-HuEpo accelerated proteinuria at 3 and 6 weeks after renal ablation. Values are means ± SEM. *, $P < 0.05$ vs. control; +, $P < 0.05$ vs. r-HuEpo.

Variations in systemic Hct also had profound effects on glomerular hemodynamics. In previous animal studies, filtration fraction varied directly with Hct owing to inverse changes in renal plasma flow rate (11, 30–32). The clinical counterpart of these experiments is provided by renal function studies in patients with anemia or polycythemia. Anemia is associated with proportionately greater increases in the renal plasma flow rate than in GFR, so that filtration fraction tends to fall, whereas polycythemia results in disproportionately lesser decreases in GFR than in renal plasma flow, so that filtration fraction tends to rise (33, 34). In the present study, preventing anemia augmented, and enhancement of anemia obviated, the glomerular capillary hypertension that characterizes this model. These contrasting changes in $\overline{\Delta P}$ resulted primarily from directionally opposite changes in R_E.

Much attention has focused on determinants of vascular resistance in the efferent arteriole; in particular, angiotensin II enhances wall tone (35), whereas inhibition of angiotensin II formation with angiotensin I converting enzyme inhibitor therapy relaxes efferent arteriolar tone, thereby decreasing R_E and $\overline{\Delta P}$ (3, 4, 7). Less investigation has centered on physical characteristics of the blood as determinants of vascular resistance and the role of these factors in regulation of R_E and $\overline{\Delta P}$. Of note, however, an important factor in regulation of R_E is blood viscosity, which is determined in large part by Hct (29). Hct in the efferent arteriole always exceeds that of systemic blood, owing to loss of water and solutes during glomerular filtration (36). The impaired autoregulatory capacity of the remnant kidney (37) may render this effect more pronounced in this model; in the isolated perfused dog kidney, blood flow in nonautoregulating kidneys was inversely related to viscosity (38). Our findings suggest that fairly modest alterations in Hct had major effects on R_E, and therefore $\overline{\Delta P}$. The observed changes in R_E may have directly resulted from changes in blood viscosity (which presumably varied directly with Hct) but also from changes in the activity of the renin–angiotensin system, since raising Hct and viscosity has been reported to increase plasma and renal vein renin levels (31, 32).

As in previous studies involving dietary and pharmacologic manipulations of $\overline{\Delta P}$ (1–7), control of glomerular hypertension in this study was associated with marked protection against the progression of renal disease, whereas preventing anemia with r-HuEpo actually accelerated the development of proteinuria and glomerular sclerosis. These findings provide a hemodynamic explanation for the clinical observation that reduction of Hct in polycythemic patients with congenital cyanotic heart disease (39), cor pulmonale (40), and obstructive sleep-apnea syndrome (41) results in a decrease in filtration fraction and remission of proteinuria, which reappears upon return of Hct to polycythemic levels. In addition, the marked polycythemia and hyperviscosity that characterize human cyanotic heart disease have been associated with glomerular capillary congestion and dilatation, massive glomerular enlargement, proteinuria, and progressive glomerular sclerosis (42–44). It seems likely that the markedly increased Hct in these patients results in glomerular capillary hypertension and therefore contributes to hemodynamically mediated glomerular injury.

Severely anemic patients with end-stage renal disease have enjoyed remarkable improvement in systemic symptoms related to hypoxemia with the availability of r-HuEpo. However, restoration of Hct to near-normal levels has resulted in systemic hypertension in some patients, providing a rationale for avoidance of overly vigorous correction of Hct with this valuable therapeutic agent (45). Our findings would also suggest a theoretical basis for avoiding normal levels of Hct in predialysis renal patients, since normalization of Hct may aggravate not only systemic hypertension but also glomerular capillary hypertension, thereby accelerating deterioration in renal function.

In summary, we have shown that vigorous correction of anemia with r-HuEpo in rats with renal ablation is associated with striking acceleration of glomerular injury due to further aggravation of systemic and glomerular hypertension. Control of systemic and glomerular hypertension with chronic anemia retards the development of proteinuria and glomerular injury. Anemia may therefore serve as a protective factor mitigating the risk of progressive injury following loss of renal mass. The accelerated progression associated with normalization of Hct suggests that efforts to fully correct anemia in predialysis patients with advancing renal disease may prove harmful to residual renal function and also increase the risk and/or severity of systemic hypertension.

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