Acute and long-term effects of modified hemoglobin (HBOC-201) in a rat model of hypertension and chronic kidney disease

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BACKGROUND: The goal was to determine whether administration of the oxygen-therapeutic blood substitute HBOC-201 had any deleterious effect on renal function and/or structure in a rat model of chronic kidney disease (CKD).

STUDY DESIGN AND METHODS: Longitudinal studies were conducted in the conscious chronically catheterized male Sprague-Dawley rat with rapidly progressing CKD produced by ablation-infarction of 5/6th of the functional renal mass. Experimental rats received a loading dose (after acute removal of 10% blood volume) and six subsequent daily maintenance doses of HBOC-201 at Weeks 4 to 5 after induction of CKD and were studied again at 6 weeks. Controls received isoosmotic human serum albumin (HSA, 13%) over the same period. Blood pressure (BP) and renal function were measured in acute experiments at Weeks 4, 5, and 6. Proteinuria and 24-hour creatinine clearance was measured longitudinally and renal pathology was assessed at killing.

RESULTS: There were differences in the acute response to the infusions, with rats given HBOC-201 exhibiting an increase in BP and renal vascular resistance, whereas rats given HSA showed a decrease in BP. These changes did not persist, however, because mean BP, glomerular filtration rate, level of proteinuria, and glomerular pathology were all similar at the end of the study (6 weeks after induction of CKD) in rats given HBOC-201 and HSA.

CONCLUSION: In this model of CKD, daily doses of HBOC-201 had no long-term damaging effects on renal function or structure, compared to rats given the osmotic control agent, 13 percent HSA.

Modified hemoglobin (Hb) is being developed as a blood substitute[1] and has great therapeutic potential, although the acute hypertensive effect, thought to be due in part to nitric oxide (NO) scavenging,[2,3] is a cause for concern. Although the pressor effect might be considered beneficial in a hypotensive state such as hemorrhagic shock,[4] the consequent further reduction in tissue perfusion may impair organ function.[5] In particular, additional vasoconstriction in a patient with existing renal disease and hypertension could have acute or long-term damaging effects. Furthermore, there have been a number of reports that some Hb solutions are directly nephrotoxic.[6-9] There are many different Hb formulations in development with varying toxicity profiles,[10] and it is essential to determine the impact of individual modified Hbs in the presence of underlying renal disease.

In this study we investigated the impact of both acute and chronic administration of an ultrapure polymerized bovine Hb-based oxygen carrier (HBOC-201) in a rat model of chronic kidney disease (CKD). HBOC-201 is an isoosmotic solution that contains less than 15 percent

ABBREVIATIONS: A-I = ablation-infarction; BP = blood pressure; BW = body weight; CKD = chronic kidney disease; GFR = glomerular filtration rate; NO = nitric oxide; RF1 = first renal function study; RF2 = second renal function experiment; RPF = renal plasma flow; RVR = renal vascular resistance; $U_{Na}V$ = urinary sodium excretion; U_KV = urinary potassium excretion.

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methemoglobin and less than 0.05 percent EU per mL endotoxin and contains 100 g per L α- and β-Hb chains.[11] We used the 5/6th renal ablation-infarction (A-I) model in the male Sprague-Dawley rat, which produces a rapidly progressing form of CKD in the remnant kidney, with severe systemic hypertension.[12,13] In view of a report that anesthesia blunts the pressor response to infusion of modified Hb in spontaneously hypertensive rats,[14] all studies were conducted in conscious, chronically catheterized rats.

MATERIALS AND METHODS

Male Sprague-Dawley rats (n = 14) were purchased at 12 weeks of age from Harlan SD (Indianapolis, IN; Facility 217), and after approximately 1 week of acclimatization, all rats were subjected to surgical preparation of CKD as follows: functional renal mass was reduced under short-duration barbiturate (1:1 mixture pentobarbital and methohexital sodium (Brevital, Eli Lilly, Indianapolis, IN), 32.5 mg per kg pentobarbital and 25 mg per kg Brevital, ip) anesthesia with full sterile technique. The right kidney was removed and upper and lower thirds of the left kidney infarcted, by ligation of 2 of the 3 branches of the renal artery, with the retroperitoneal approach.[12] All rats were then monitored for 3 weeks and after a 24-hour urine collection (to measure urinary protein excretion) were subjected to chronic catheterization. Under short-duration barbiturate (Brevital; 50 mg/kg ip; 2-5 mg/kg IV, supplemental) general anesthesia and with full sterile technique, vascular (Tygon, Norton Plastics, Akron, OH) catheters were placed in the left femoral artery and vein and exteriorized at the back of the neck. A catheter was placed in the urinary bladder. Details of this technique have been published by us previously.[15,16] Rats were allowed at least 6 days to fully recover from the surgery and/or anesthesia before any functional measurements were made. Before catheterization and afterward, all rats were trained and handled to allow them to become comfortable with the activities in the laboratory. All animal procedures were conducted in accordance with Institutional guidelines and were approved by the West Virginia University Animal Care and Use Committee.

At the end of Week 4 after induction of CKD, rats were randomly assigned to either the HBOC-201 (Biopure Corp., Cambridge, MA) or control, 13 percent human serum albumin (HSA, Baxter Healthcare Corp., Hyland Immuno, Glendale, CA), protocols, as described in Table 1. All rats were studied for baseline renal function measurements (glomerular filtration rate [GFR] and renal plasma flow [RPF]) and arterial blood pressure (BP). A blood sample was also taken for creatinine measurement. Both groups were then hemorrhaged by 10 percent removal of blood volume (over 5 minutes). Group 1 (n = 7) then received IV HBOC-201 at a loading dose of 2 g per kg at the rate of 1.5 mL per 100 g of body weight (BW), given over a 60-minute period. The purpose of the hemorrhage was to prevent an acute volume overload in an already volume expanded model, from confounding the data. After 20 minutes of equilibration, two additional clearance measurements were made to determine the acute effect of HBOC-201 on renal hemodynamics in rats with compromised renal function. Group 2 rats (n = 7) were subjected to an identical protocol except they received HSA in a 13 percent solution instead of HBOC-201, to control for the colloid osmotic effects of a concentrated protein solution, and 50 percent of the red blood cells (RBCs) removed during the hemorrhage were reconstituted in an equal volume of 13 percent HSA and restored to the rat at the end of the HSA infusion. We restored 50 percent of the RBCs in these rats to prevent a severe anemia and to match the fact that due to the short $t_{1/2}$ of the HBOC-201 (approx. 30 hour)[17] Group 1 rats also have a chronic Hb deficit. The volume of 13 percent HSA necessary to reconstitute the RBCs was subtracted from the volume given over the infusion period.

All catheters were then primed and plugged and rats were placed in metabolic cages for 24-hour urine collection and then returned to their home cages. For the next 6 days after the first renal function experiment, rats were returned to the restraining cage and received daily IV infusion of 0.5 g per kg (HBOC-201) or equal volumes of 13 percent HSA. On the 7th day of infusions, a blood sample was taken for creatinine measurement, and clearance studies were conducted before (for baseline) and after HBOC-201 (0.5 g/kg, at 0.4 mL/100 g BW given over a 15-

TABLE 1. Outline of the longitudinal observations made in rats with CKD due to 5/6th renal A-I

Weeks after 5/6 A-I	End Week 3 catheterization surgery	End Week 4	End Week 5	End Week 6
HBOC or HSA administration	None	1 dose during RF experiment	Daily doses	None
24-hr urine collection	Before catheterization	After renal function experiment	After renal function experiment	After renal function experiment
Renal function experiment	None	Baseline and during acute HBOC-201	After 7 days of HBOC-201	After 7 days of HBOC-201 washout

minute period), or equivalent 13 percent HSA, to determine GFR and RPF. Rats were then placed in metabolic cages for determination of 24-hour urine protein excretion and allowed a 7-day washout period in which no measurements were made or HBOC-201 or HSA administered. An additional renal clearance study and 24-hour urine collection were made at Week 6 after induction of CKD, and rats were then killed, and blood and kidney tissue was harvested for chemistry and histology, respectively.

Renal function experiments involved measurement of renal hemodynamics and electrolyte excretion. Rats were placed in a restraining cage to which they had been acclimated, vascular catheters were unplugged, and the arterial line was connected to a pressure transducer, via three-way stopcock, to allow for continual monitoring of arterial BP (MacLab, AD Instruments Pty Ltd, Castle Hill, Australia) and occasional withdrawal of an arterial blood sample. The venous line was connected to a syringe containing 0.9 g-percent NaCl solution with tritiated inulin (1-2 µCi/mL) and PAH (0.33 g% PAH), infused at the rate of 50 µL per 100 g BW per minute. The bladder catheter was also unplugged and the bladder was irrigated with sterile NaCl to check for complete recovery. After equilibration of IV infusion (80 minutes), renal function measurements were begun with 20-minute urine collections each with a midpoint blood sample. Urine volume was measured gravimetrically, and blood and urine inulin, PAH, and sodium concentrations were measured for calculation of GFR, RPF, renal vascular resistance (RVR) and fractional and absolute sodium excretion. Details of analyses and calculations have been given in our earlier publications.[15,16] For the metabolic cage urine samples, total urinary protein concentration was measured by the Bradford assay as reported previously[18] and creatinine was measured with a commercial kit (Kit 555, Sigma, St. Louis, MO). Plasma creatinine was also measured by with this Sigma kit and blood urea nitrogen was measured with Sigma Kit 640.

For histology the kidney remnant was weighed, bisected longitudinally, fixed in 10 percent formalin, and blocked in paraffin wax, and 5-µm-thick sections were cut. Sections were stained with periodic acid Schiff with hematoxylin-eosin counterstain and examined, blind, for the level of glomerular sclerosis and tubulointerstitial injury. Glomerular injury was assessed with a 0 to 4+ scale on 100 cortical glomeruli per section: 0 = no glomerular damage; 1+ = less than 25 percent glomerular sclerosis; 2+ = 25 to 50 percent injury; 3+ = 50 to 75 percent injury; and 4+ = global sclerosis as described previously.[17] The overall injury score gives an index of both total number of sclerosed glomeruli and severity of injury.[19]

Throughout, all data are expressed as means ± standard error. All functional data are analyzed by either unpaired or paired t test, and nonparametric (morphologic) data are analyzed by Kruskal-Wallis and U test. A p value of less than 0.05 is taken to indicate significance. The number of 7 per group was chosen based on our previous experience with this model and the power of the longitudinal design. We use multiple endpoints to assess injury and test the null hypothesis, which protects against Type II error.

RESULTS

This model has already been extensively characterized with regard to functional and structural changes[12,13] and there were therefore no sham animals included in the present study. At the end of Week 3 after 5/6 A-I, all rats had significant and similar proteinuria (131 ± 53 and 137 ± 56 mg/24 hours, Groups 1 and 2, respectively). As shown in Table 2, despite randomization, at Week 4 in the first renal function study (RF1), the baseline GFR was slightly lower in rats that entered the HBOC-201 study (Group 1) versus Group 2 rats (HSA controls). All other baseline values (BP, RVR, filtration fraction, arterial hematocrit [Hct]), however, were similar. This suggests that the small initial difference in GFR was not biologically significant, particularly because the treatment group (plus HBOC) started with a slightly lower GFR than HSA controls. Furthermore, there were no differences in baseline values of plasma electrolytes, urine flow (V), excretion of sodium or potassium ($U_{Na}V$ and U_KV), or the fractional excretion of Na+ (FE_{Na+}) between the two groups (Table 3).

After completion of baseline measurements, rats were acutely hemorrhaged (10% reduction in estimated blood volume over 5 minutes) and then received the loading dose of HBOC-201 or HSA. There was a striking difference between the groups in the BP and RVR response in RF1, with a mild acute pressor and moderate renal vasocon-

TABLE 2. Week 4 after A-I: baseline renal hemodynamics in Groups 1 and 2

Group	BW (g)	BP (mmHg)	GFR (mL/min/ 100 g BW)	RPF (mL/min/ 100 g BW)	Filtration fraction	RVR (mmHg/ (mL/min))	Hct (%)
1. HBOC	334 ± 6	202 ± 6	0.18 ± 0.01	0.71 ± 0.1	0.26 ± 0.01	50 ± 4	41 ± 1
2. HSA	357 ± 10	184 ± 6	0.26 ± 0.03	0.92 ± 0.1	0.28 ± 0.01	38 ± 7	40 ± 1
p Value	NS	NS	< 0.05	NS	NS	NS	NS

TABLE 3. Week 4 after A-I: baseline plasma and urinary electrolyte data in Groups 1 and 2

Group	P Na (meq/L)	P K (meq/L)	V (μL/min)	$U_{Na}V$ (μeq/min)	FE_{Na} (%)	U_KV (μeq/min)
1. HBOC	139 ± 1	4.2 ± 0.1	33 ± 5	0.96 ± 0.28	1.2 ± 0.4	2.8 ± 0.2
2. HSA	138 ± 1	4.2 ± 0.1	42 ± 9	2.69 ± 0.79	2.2 ± 0.6	3.6 + 0.6
p Value	NS	NS	NS	NS	NS	NS

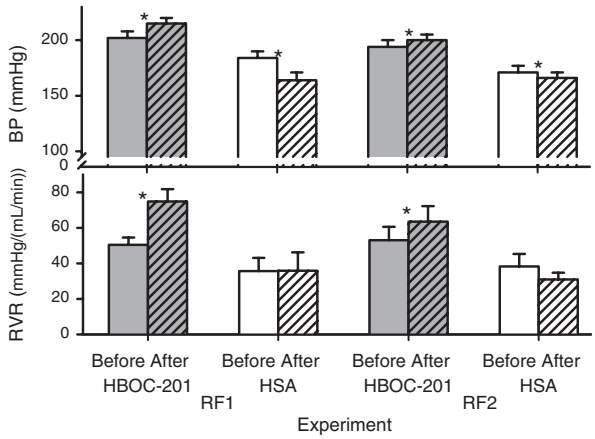

Fig. 1. Mean arterial BP (top) and RVR (bottom) measured at baseline (before intervention; open columns) and after 10 percent hemorrhage and subsequent iv infusion (after intervention; hatched columns) of either HBOC-201 (shaded columns) or HSA (clear columns). RF1 refers to the first renal function experiment at Week 4 after induction of CKD in which a loading dose of infusate (1.5 mL/100 g BW, given over a 60-min period) was given after a 10 percent hemorrhage. RF2 refers to the second renal function experiment (Week 5 of CKD) conducted after the loading dose and 6 consecutive days of daily maintenance infusions (0.4 mL/100 g BW given over a 15-min period). *Significant difference ($p < 0.05$) between the baseline (preinfusion) and postinfusion value.

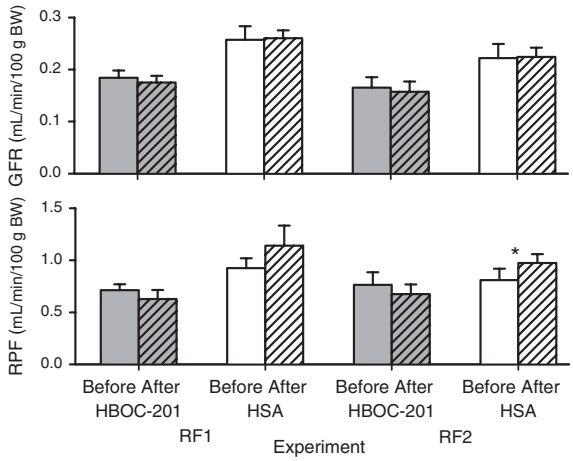

Fig. 2. GFR (top) and RPF (bottom) measured at baseline (before intervention; open columns) and after IV infusion (after intervention; hatched columns) of either HBOC-201 (shaded columns) or HSA (clear columns). All abbreviations are as in Fig. 1. *Significant difference ($p < 0.05$) between the baseline (preinfusion) and postinfusion value.

strictor response to HBOC-201 (Group 1 rats), whereas HSA was moderately antihypertensive and had no effect on RVR (Fig. 1). The BP and RVR changes in each group tended to cancel each other out; hence, there were no net changes in GFR or RPF in either group (Fig. 2).

Rats then received daily maintenance doses (25% of loading dose) and a second renal function experiment (RF2) was conducted with baseline measurements before, as well as measurements after the sixth maintenance dose. A slight acute increase in BP was seen with HBOC-201, whereas a moderate fall was seen with HSA (Fig. 1). RVR increased moderately with HBOC-201 and did not change with HSA (Fig. 1), and there were no effects on GFR with the acute infusion in either group (Fig. 2) although a small decrease in RPF occurred with HBOC-201 and a small increase with HSA (Fig. 2).

An equivalent decrease in Hct occurred (due to hemorrhage and the hemodilution effect of the cell-free infusions) in Groups 1 and 2 in RF1, with similar but smaller declines in RF2 (Fig. 3). Despite the hemorrhage, there was a marked natriuretic response to HBOC-201, and this was even more pronounced with HSA in RF1 due to increased fractional excretion of Na+ (FE_{Na+}) and accompanied by a diuresis. In RF2 only, the HSA produced a significant natriuretic and diuretic response (Fig. 4).

Rats were studied again after a 1-week "washout" period in a third renal function experiment in which only baseline measurements were made. As shown in Fig. 5 (which gives BP and renal hemodynamic data from control measurements of each renal function experiment), despite the acute pressor effect, in the long term HBOC-201 did not raise BP (Fig. 5, top panel). The BP was stable over the 3-week observation period in both groups so that at Week 6 after induction of CKD, BPs were similar. As shown in the bottom panels, other than the lower value for GFR in Group 1 (HBOC-201) rats in RF1, there were no differences between the groups over the 3-week observation period. The 24-hour creatinine clearances give a separate index of GFR and show the same trends as the acutely measured inulin clearances; that is, creatinine clearance is stable over the 3-week period in Group 1 rats

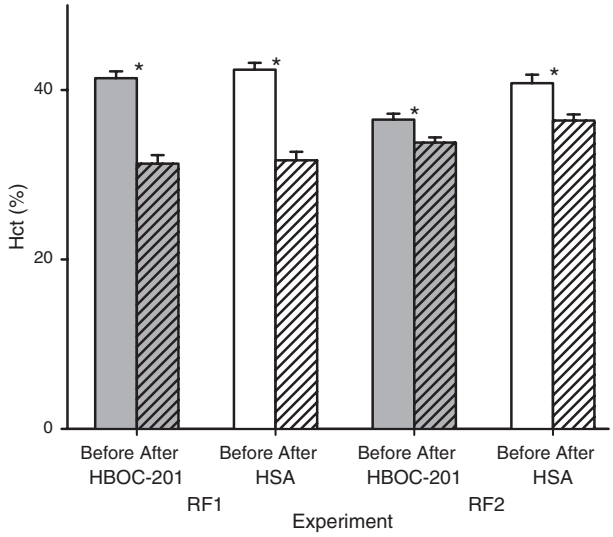

Fig. 3. Mean arterial Hct measured at baseline (before intervention; open columns) and after IV infusion (after intervention; hatched columns) of either HBOC-201 (shaded columns) or HSA (clear columns). All abbreviations are as in Fig. 1. *Significant difference ($p < 0.05$) between the baseline (preinfusion) and postinfusion value.

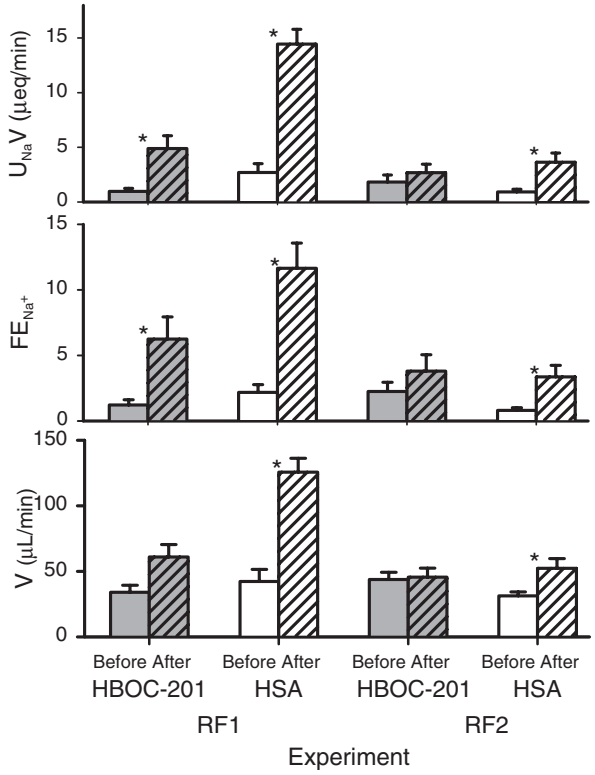

Fig. 4. Urinary sodium excretion ($U_{Na}V$; top panel), fractional excretion of sodium (FE_{Na+} middle panel), and urine flow (V; bottom panel), measured at baseline (before intervention; open columns) and after IV infusion (after intervention; hatched columns) of either HBOC-201 (shaded columns) or HSA (clear columns). All abbreviations are as in Fig. 1. *Significant difference ($p < 0.05$) between the baseline and postinfusion value.

(2.5 ± 0.3, 2.5 ± 0.3, and 2.4 ± 0.4 mL/min) and in Group 2 (3.3 ± 0.4, 3.0 ± 0.4, and 2.8 ± 0.5 mL/min).

The time course of the urine protein excretion is shown in Fig. 6, with a steady increase from Weeks 1 through 3 after 5/6 renal A-I for induction of CKD. In Group 1 rats (given HBOC-201), there was no additional increase in proteinuria, whereas urinary protein excretion increased further in Group 2 rats given HSA but returned by Week 6 to the similar value seen in rats given HBOC-201. According to the pathology, the level of glomerular injury was similar at Week 6 of CKD with the overall glomerular injury score of 72 ± 17 in Group 1 (HBOC-201) versus 105 ± 16 in Group 2 (HSA). The individual level of severity of injury was also similar between the groups, as shown in Fig. 7. There was no difference between the number of tubular casts and/or kidney in rats given HBOC-201 (12 ± 4) versus rats given HAS (21 ±6).

At the end of the study (Week 6 of CKD), the BW was similar in both groups (349 ± 6 and 370 ± 1 g, NS) as was arterial Hct (37 ± 1 and 40 ± 1 vol%, NS), plasma Na+ and K+, and $U_{Na}V$ and U_KV (data not shown).

DISCUSSION

The main finding of this study is that the acute hypertensive and renal vasoconstrictor effect caused by HBOC-201 had no other adverse short-term consequence in rats with chronic hypertension and CKD and did not result in even transient further falls in GFR. Importantly, the HBOC-201-induced hypertension and renal vasoconstriction did not persist during the chronic dosing regime, and at Week 6 after induction of CKD, after the 7-day washout period, BP and renal function were similar in rats treated with HBOC-201 and those receiving the osmotic control, HSA. The rate of development of the CKD was also unaffected by HBOC-201 administration.

Modified Hbs have great potential as a blood supplement in response to acute hemorrhage although the vasoconstrictor effect is a limitation because this produces hypertension due to widespread vasoconstriction and can impair organ perfusion, of particular concern during blood loss. A number of factors have been implicated in this vasoconstriction, including release of thromboxane[7] and autoregulatory vasoconstriction of the microvasculature in response to increased oxygen delivery and low viscosity.[5] In addition, NO scavenging effects have been widely reported and this is the focus of the present discussion, given the evidence that a state of NO deficiency already occurs in CKD (see below). A number of workers have reported that L-arginine supplementation (which

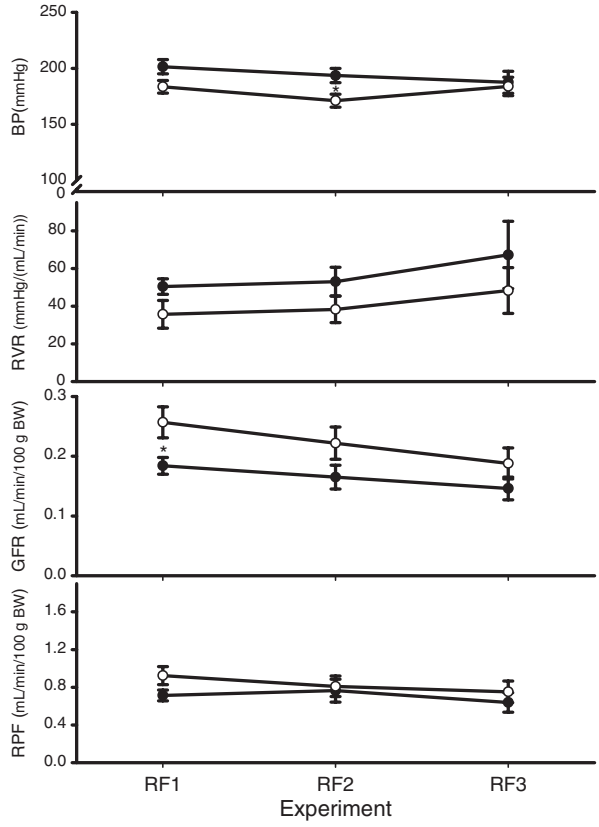

Fig. 5. Mean arterial BP, RVR, GFR, and RPF measured in the baseline state over a 3-week period (renal function experiments, RF1-RF3) at Weeks 4 through 6 after induction of CKD. The solid symbols denote data obtained in rats given HBOC-201 and the open symbols denote data from rats given HSA. *Significant difference ($p < 0.05$) between rats given HBOC-201 and those given HSA.

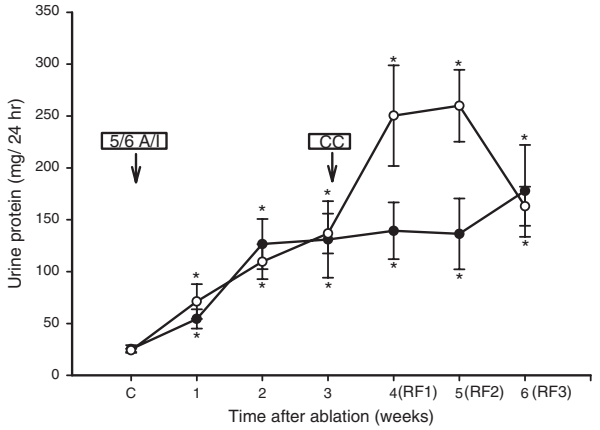

Fig. 6. The 24-hr urinary total protein excretion in control and at weekly intervals for 6 weeks after induction of CKD in rats given HBOC-201 (●) and rats given HSA (○). CKD was induced by A-I of 5/6th of functional renal mass (5/6 A/I) in control (C) rats. Rats were chronically catheterized (CC) at Week 3 of CKD. *Significant difference ($p < 0.05$) between the baseline (preinfusion) and postinfusion value.

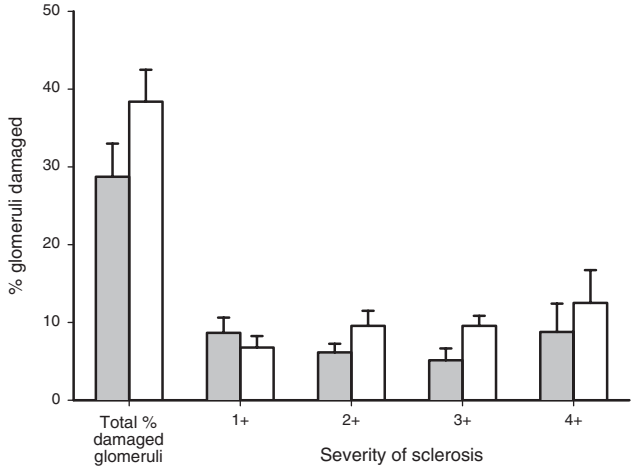

Fig. 7. The extent of glomerular injury at 6 weeks after induction of CKD in rats given HBOC-201 (solid columns) and rats given HSA (open columns). Injury is defined at the total number of glomeruli exhibiting glomerular sclerosis and the extent of injury and/or glomerulus with 1+ = less than 25 percent glomerular sclerosis; 2+ = 25 to 50 percent injury; 3+ = 50 to 75 percent injury; and 4+ = global sclerosis.

boosts endogenous NO production) is a helpful adjunct during resuscitation from hemorrhage[20,21] and that concomitant NOS inhibition reduces survival.[22] Alternatively, raising the BP may be helpful in maintaining organ perfusion, at least when hypotension is present (as in hemorrhage). Indeed, there have also been reports that NO scavenging or NOS inhibition is beneficial during recovery from hemorrhage.[23,24] Lieberthal and colleagues[23] found that acute systemic NOS inhibition produced a blunted hypertensive and renal vasoconstrictor response in rats with hemorrhagic hypotension versus normals and that GFR was actually elevated by NOS inhibition in hypotensive rats while being reduced in normotensive rats. As suggested by these workers, this is likely a matter of balancing adequate perfusion pressure with adequate arteriolar vasodilation and tissue perfusion, because while low-dose systemic NOS inhibition was protective, high-dose NOS inhibition caused severe hypertension and increased organ damage within a few hours after hemorrhage.[25]

Additionally, some Hb solutions have been reported to cause renal dysfunction in man and animals with normal kidneys, particularly the early formulations. There have been reports of renal vasoconstriction due to NO scavenging and thromboxane release as well as tubular damage and even acute renal failure with permanent and complete loss of kidney function.[6-9] HBOC-201 is an ultrapure glutaraldeyde-polymerized bovine Hb without sig-

nificant renal toxicity when administered to rats with normal renal function. With top-load infusions of HBOC-201 that increased blood volume acutely by 10 to 25 percent there were no acute or chronic changes in BP, serum creatinine, inulin clearance, or renal ATP content although massive expansions of 50 to 75 percent caused dose dependent, reversible declines in GFR.[8,26] At very high (75%) expansion a focal, nonischemic tubular injury was observed at 24 hours after infusion and appeared to be resolving at 48 hours.[8] During isovolemic hemodilution in normal dogs, no acute changes were seen in kidney structure at the light or electron microscope level.[27] In an endotoxin-induced model of septic shock in the rat, where high levels of endogenous NO are pathogenic, HBOC-201 was equally effective with NO synthesis inhibition in restoring BP and also restored renal perfusion to baseline, whereas NO synthesis inhibition markedly increased RVR and lowered RPF.[28] There have been no investigations into the impact of HBOC-201 in states of CKD, where chronic NO deficiency and endothelial dysfunction occur.[26,29,30,31] In the 5/6 renal A-I animal model of CKD, there is systemic and intrarenal NO deficiency that contributes to the progression of the disease.[13,32-34] Based on the NO scavenging effects of Hb solutions, it is possible that preexisting states of NO deficiency may be particularly vulnerable to damage if further NO loss occurs, particularly during recovery from hemorrhage.

For these reasons we conducted the current study, with the rapidly progressing rat model of CKD produced by 5/6 renal A-I, to determine whether seven daily administrations of HBOC-201 had any adverse impact on BP and/or renal function and/or structure. This model has been extensively characterized previously and exhibits a predictable, time-dependent proteinuria and glomerular sclerosis.[12,13] For this study we chose to examine Weeks 4 to 6 after 5/6 renal A-I, a time with substantial damage and reduced function, but before end stage.

Our findings were reassuring in that the renal hemodynamics, and most importantly the GFR, were not impaired by chronic HBOC-201 administration after a 10 percent blood loss and in fact GFR was maintained acutely, despite transient increase in RVR. The injury indices of proteinuria and structural damage (assessed by histology) were not exacerbated by chronic HBOC-201 administration and, in fact, the injury measures were often nonsignificantly better at killing in those rats receiving HBOC-201 compared to HAS-treated rats. Further, the level of hypertension was similar at the end of the study between groups receiving HBOC-201 and HSA.

Overall, this study demonstrates that acute administration of HBOC-201 to rats with renal disease and with moderate acute hemorrhage causes an acute peripheral and renal vasoconstriction. Despite this, chronic administration of HBOC-201 to rats with progressive renal disease has no long-term deleterious effects on the natural course of the CKD and does not have a chronic effect to exacerbate the hypertension.

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REFERENCES

1. Chang TM. Present status of modified hemoglobin as blood substitutes and oral therapy for end stage renal failure using artificial cells containing genetically engineered cells. Ann N Y Acad Sci 2001;944:362-72.
2. Doherty DH, Doyle MP, Curry SR, et al. Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin. Nat Biotechnol 1998;16:672-6.
3. Sakai H, Hara H, Yuasa M, et al. Molecular dimensions of Hb-based O (2) carriers determine constriction of resistance arteries and hypertension. Am J Physiol Heart Circ Physiol 2000;279:H908-15.
4. Przybelski RJ, Daily EK, Birnbaum MI. The pressor effect of hemoglobin-good or bad?. In: Winslow RM, Vandegriff KD, Intaglietta M, editors. Advances in blood substitutes: industrial opportunities and medical challenges. Boston: Birkauser; 1997.
5. Tsai AG, Friensenecker B, Intaglietta M. Microcirculatory consequences of blood substitution with aa-hemoglobin. In: Winslow RM, Vandergriff KD, Intaglietta M, editors. Blood substitutes: physiological basis of efficacy. Boston: Birkhauser; 1995. pp. 155-74.
6. Blantz RC, Evan AP, Gabbai FB. Red cell substitutes in the kidney. In: Winslow RM, Vandergriff KD, Intaglietta M, editors. Blood substitutes: physiological basis of efficacy. Boston: Birkhauser; 1995. pp. 132-42.
7. Lieberthal W, LaRaia J, Valeri CR. Role of thromboxane in mediating the intrarenal vasoconstriction induced by unmodified stroma free hemoglobin in the isolated perfused rat kidney. Biomat Artif Cells Immobil Biotech 1992;20:663-7.
8. Somers M, Piqueras AI, Strange K, et al. Interactions of ultrapure bovine hemoglobin with renal epithelial cells in vivo and in vitro. Am J Physiol 1997;273:F38-52.
9. Winslow RM. The toxicity of hemoglobin. In: Hemoglobin-based red cell substitutes. Baltimore: Johns Hopkins University Press; 1992. pp. 136-63.
10. Chang TM. Modified hemoglobin-based blood substitutes: crosslinked, recombinant and encapsulated hemoglobin. Vox Sang 1998;74:233-41.
11. Gawryl M, Clark T, Rausch C. Dose related renal effects of intravenous administration of polymerized bovine hemoglobin solution to rats. In: Sekiguchi S, editor. Red cell substitutes. Tokyo: Kindai-Shuppan; 1991. pp. 28-40.
12. Erdely A, Wagner L, Muller V, et al. Protection of Wistar Furth rats from chronic renal disease is associated with

maintained renal nitric oxide synthase. J Am Soc Nephrol 2003;14:2526-33.
13. Szabo AJ, Wagner L, Erdely A, et al. Renal neuronal nitric oxide synthase protein expression as a marker of renal injury. Kidney Int 2003;64:1765-71.
14. Ning J, Wong LT, Christoff B, et al. Haemodynamic response following a 10% topload infusion of HemolinkTM in conscious, anaesthetized and treated spontaneously hypertensive rats. Transfus Med 2000;10:13-22.
15. Baylis C, Collins RC. Angiotensin II inhibition on blood pressure and renal hemodynamics in pregnant rats. Am J Physiol 1986;250:F308-314.
16. Baylis C, Harton P, Engels K. Endothelial derived relaxing factor controls renal hemodynamics in the normal rat kidney. J Am Soc Nephrol 1990;1:875-81.
17. Lee R, Neya K, Svizzero TA, et al. Limitations of the efficacy of hemoglobin-based oxygen-carrying solutions. J Appl Physiol 1995;79:236-42.
18. Baylis C. Age-dependent glomerular damage in the rat. Dissociation between glomerular injury and both glomerular hypertension and hypertrophy: male gender as a primary risk factor. J Clin Invest, 1994;94:1823-9.
19. Raij L, Azar S, Keane W. Mesangial immune injury, hypertension, and progressive glomerular damage in Dahl rats. Kidney Int 1984;26:137-43.
20. Benyo Z, Szabo C, Csaki C, et al. Effect of L-arginine on adrenal and renal blood flows during hemorrhage in cats. Kidney Int Suppl 1998;67:S221-3.
21. Loeb AL, McIntosh LJ, Raj NR, et al. Resuscitation after hemorrhage using recombinant human hemoglobin (rHb1.1) in rats: effects on nitric oxide and prostanoid systems. Crit Care Med 1998;26:1071-80.
22. Mellander S, Bjornberg J, Ekelund U, et al. Cardiovascular regulation by endogenous nitric oxide is essential for survival after acute hemorrhage. Acta Physiol Scand 1997;160:57-65.
23. Lieberthal W, Thompson A, Valeri CR. Effects of nitric oxide inhibition on systemic and renal hemodynamics in the hemorrhaged rat. Kidney Blood Press Res 1996;19:340-6.
24. Menezes J, Hierholzer C, Watkins SC, et al. A novel nitric oxide scavenger decreases liver injury and improves survival after hemorrhagic shock. Am J Physiol 1999; 277:G144-51.
25. Yao YM, Bahrami S, Leichtfried G, Redl H, Schlag G. Significance of NO in hemorrhage-induced hemodynamic alterations, organ injury, and mortality in rats. Am J Physiol 1996;270:H1616-23.
26. Lee R, Atsumi M, Jacobs EE Jr, et al. Ultrapure, stroma-free, polymerized bovine hemoglobin solution: evaluation of renal toxicity. J Surg Res 1989;47:407-11.
27. Lipfert B, Standl T, Dullmann J, et al. Histology and ultrastructure of liver and kidney following blood exchange with ultrapurified, polymerised bovine hemoglobin in comparison with hydroxyethyl starch. Lab Invest 1999; 79:573-82.
28. Haneka MT, Loschmann PA, Osswald H. Polymerized hemoglobin restores cardiovascular and kidney function in endotoxin-induced shock in the rat. J Clin Invest 1997;99:47-54.
29. Aiello S, Noris M, Todeschini M, et al. Renal and systemic nitric oxide synthesis in rats with renal mass reduction. Kidney Int 1997;52:171-81.
30. Schmidt RJ, Baylis C. Total nitric oxide production is low in patients with chronic renal disease. Kidney Int 2000;58: 1261-6.
31. Xiao S, Schmidt RJ, Baylis C. Plasma from ESRD patients inhibits nitric oxide synthase activity in cultured human and bovine endothelial cells. Acta Physiol Scand 2000; 168:175-9.
32. Erdely A, Greenfeld Z, Wagner L, et al. Sexual dimorphism in the aging kidney: Effects on injury and nitric oxide system. Kidney Int 2003;63:1021-6.
33. Vaziri ND, Ni Z, Wang XQ, et al. Downregulation of nitric oxide synthase in chronic renal insufficiency: role of excess PTH. Am J Physiol 1998;274:F642-9.
34. Wagner L, Riggleman A, Erdely A, et al. Reduced nitric oxide synthase activity in rats with chronic renal disease due to glomerulonephritis. Kidney Int 2002;62:532-6.