Recombinant Human Hemoglobin Does Not Affect Renal Function in Humans: Analysis of Safety and Pharmacokinetics

Maureen K. Viele, M.D.,* Richard B. Weiskopf, M.D.,† Dennis Fisher, M.D.‡

Background: Recombinant human hemoglobin (OptroD; rHb1.1) is a genetically engineered protein produced in *Escherichia coli*. The two a-globin polypeptides are genetically joined, resulting in a stable tetramer that does not dissociate into dimers or monomers. Historically, infusion of humans of acellular human hemoglobin preparations has resulted in renal toxicity. This study was performed to evaluate the safety and pharmacokinetics of rHb1.1 when infused in humans.

Methods: After giving informed consent, 48 healthy male volunteers were randomly assigned to receive either 0.015–0.32 g/kg 5% rHb1.1 (n = 34) or an equivalent amount of 5% human serum albumin (HSA; n = 14) infused intravenously over 0.8–1.9 h. Serum creatinine, creatinine clearance, urine N-acetyl-D-glucosaminidase, and serum rHb1.1 concentrations were measured before and at timed intervals after infusion.

Results: Postinfusion urine N-acetyl-D-glucosaminidase activity did not exceed preinfusion values at any interval in either group. Serum creatinine did not differ from preinfusion values at 1 day, 2–5 days, or 7 days after infusion for either group. Creatinine clearance increased significantly for the HSA group 12 h after infusion (138 ± 16 ml/min, mean ± SE) and in the rHb1.1 group 1 day after infusion (112 ± 5 ml/min; P < 0.05). Values for creatinine clearance did not differ from preinfusion values for either group at any other postinfusion interval; serum creatinine and creatinine clearance did not differ between groups at any time. The amount of hemoglobin excreted in the urine did not exceed approximately 0.04% of the administered rHb1.1 dose in any volunteer. Plasma clearance of rHb1.1 decreased and half-life increased as a function of increasing plasma concentration (e.g., the half-life was 2.8 h at a plasma concentration of 0.5 mg/ml and 12 h at 5 mg/ml). The incidence of gastrointestinal symptoms, fever, and chills was greater after infusion of rHb1.1 than after HSA (P < 0.05).

Conclusions: No evidence for rHb1.1-mediated nephrotoxicity was observed in volunteers given doses of rHb1.1 as large as 0.32 g/kg. Because the clearance of rHb1.1 varies inversely with its concentration, additional studies with larger doses are necessary to determine the half-life expected in clinical use. Administration of rHb1.1 to conscious humans is associated with some side effects, such as gastrointestinal upset, fever, chills, headache, and backache. (Key words: Hemoglobin, recombinant; safety; renal. Hemoglobin, recombinant; side effects. Pharmacokinetics: hemoglobin. NONMEM: population techniques).

IN an effort to produce a fluid with substantial oxygen-carrying capacity and intravascular retention, but without the potential for toxicity or transmission of blood-borne viral diseases, hemoglobins from horses, cows, pigs, and humans have been chemically modified and purified to varying degrees. Infusions of early products resulted in transient renal damage.1,2 Because these products contained varying amounts of red cell stroma, it was thought that stromal elements produced the renal toxicity. Because complete elimination of red cell stroma was difficult, it was not possible to determine whether hemoglobin itself produces renal toxicity. Indeed, the current teaching is that hemoglobin is nephrotoxic.

Recently recombinant DNA techniques enabled production of human hemoglobin by *Escherichia coli*3 and *Saccharomyces cerevisiae*.6 These techniques yield a pure solution of hemoglobin without remnants of red cell stroma. Synthetic genes encoding the human a- and b-globin polypeptides expressed from a single operon resulted in a fully assembled tetrameric molecule incor-
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Table 1. Numbers of Volunteers Receiving Each Dose of Recombinant Human Hemoglobin or Human Serum Albumin

<table>
<thead>
<tr>
<th>Dosage (g/kg)</th>
<th>Number Given Rhb1.1</th>
<th>Number Given HSA</th>
<th>Infusion Rate (mg·kg⁻¹·hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.015</td>
<td>4</td>
<td>2</td>
<td>18.75</td>
</tr>
<tr>
<td>0.075</td>
<td>8</td>
<td>3</td>
<td>93.75</td>
</tr>
<tr>
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<td>4</td>
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<td>0.15</td>
<td>8</td>
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<td>187.5</td>
</tr>
<tr>
<td>0.18</td>
<td>2</td>
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<tr>
<td>0.22</td>
<td>2</td>
<td>1</td>
<td>187.5</td>
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<tr>
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<td>2</td>
<td>1</td>
<td>187.5</td>
</tr>
<tr>
<td>0.29</td>
<td>2</td>
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<tr>
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<td>1</td>
<td>187.5</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

rHb1.1 = recombinant human hemoglobin. HSA = human serum albumin.

porating heme moieties, with functional characteristics similar to those of human hemoglobin Α₂. Genetic modifications provided fusion of the aα chains to prevent molecular disassociation, and an amino acid substitution (as in the mutant hemoglobin Presbyterian: β108 Asn → Lys) to decrease affinity for oxygen (half-saturation pressure [P₅₀] = 33 mmHg).⁸

To evaluate the safety and possible toxicity of recombinant human hemoglobin (rHb1.1) in humans, we infused rHb1.1 intravenously to determine its renal effects and its pharmacokinetic profile.

Methods

With approval of the review boards of each institution (University of California, San Francisco, California, and Pharmaco, Austin, Texas), informed consent was obtained from 48 healthy male volunteers, ages 18–55 yr. Volunteers had uncomplicated medical histories and normal results of physical examinations, electrocardiograms, and laboratory evaluations (urine drug screen, viral infection screen, complete blood count, serum chemistries, and urinalysis).

Volunteers were randomly assigned to receive either 5% (5 g/dl) recombinant hemoglobin solution (OptroD[rHb 1.1], Somatogen Inc., Boulder, CO; protein oncotic pressure of 12 mmHg and osmolality of 298 mOsm), or an equal volume of 5% human serum albumin (HSA; Baxter Healthcare, Glendale, CA). Volun-
teers, uninformed of their group assignment, were assigned to one of nine groups based on the amount of rHb1.1 and HSA to be infused (table 1). For safety reasons, investigators were not blinded and rHb1.1 (and HSA) was administered in a dose-escalating format: Each subsequent group received a larger dose of either substance than did the preceding group. The smallest dose selected was an arbitrarily small dose in case toxicity occurred. At the largest doses, we believe that we administered more recombinant protein than had ever been given to a human (at that time). Hemodynamic effects and toxicity of each dose were evaluated before proceeding with the next larger dose. Some volunteers in the groups receiving the larger doses of rHb1.1 (≥0.15 mg/kg) were given terbutaline (0.25 mg given subcutaneously every 2 h up to six times), nifedipine (10 mg given sublingually), naloxone (0.2 mg given intravenously), nitroglycerin (0.4 mg given sublingually), or glucagon (1 mg given intravenously) in an effort to ameliorate symptoms observed in the groups given smaller doses.

Both rHb1.1 and albumin solutions were infused intravenously by a constant-rate pump for a period of 0.8–1.9 h, and duration varied with dose (table 1). Immediately before, during, and at various times after infusion, blood was sampled from each volunteer to determine creatinine and serum hemoglobin concentrations. Recombinant hemoglobin concentrations were measured using an rHb1.1-specific high-pressure liquid chromatography assay. The assay is sensitive to 25 μg rHb1.1/ml plasma (2.5 μg/ml of a 1:10 dilution of plasma), with a coefficient of variation of 18% at that concentration. Urine was collected for urinalysis and to measure creatinine and N-acetyl-beta-glucosaminidase (NAG; Boehringer Mannheim Biochemicals, Indianapolis, IN) concentrations. Urine was also analyzed for the presence of hemoglobin by the "dipstick" method. Standardization, relating rHb1.1 concentrations with "dipstick" results (detection limit for hemoglobin 0.6 μg/ml), was used to convert these results into hemoglobin concentrations. The amount of hemoglobin excreted in the urine of each volunteer was estimated by multiplying the volume of urine collected for each time period by the estimated rHb1.1 concentration. Blood was sampled at times of gastrointestinal symptoms to measure serum amylase and lipase activity by standard clinical methods.

Blood pressure, oral temperature, electrocardiograph, heart rate, respiratory rate, and oxyhemoglobin saturation (by pulse oximetry [Sₒ₂]) were monitored continuously during infusion and for 1 h after infusion. Blood


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pressure, temperature, heart rate, and respiratory rate were monitored at frequent intervals for 24 h after infusion. Volunteers were assessed clinically for 8 weeks after the infusion for evidence of adverse reactions.

**Statistical Analyses**

Data are reported as means ± SE unless otherwise specified. Blood pressure and heart rate are reported for only the first four dose groups (0.015–0.15 g/kg) because of pre- and postinfusion treatment regimens with various drugs (see above). Because of the small number of volunteers in each of the largest dose groups, data from groups given 0.18 and 0.22 g/kg rHb1.1 were combined, as were the data from groups given 0.25–0.32 g/kg rHb1.1. Data for hemodynamic variables, serum creatinine concentration, creatinine clearance, and urine NAG activity before, during, and after infusion of rHb1.1 and HSA were compared by analysis of variance with repeated measures and the Newman-Keuls method of multiple comparisons. The incidence of symptoms between rHb1.1 and HSA groups was compared by Fisher's exact test. In all cases, P < 0.05 was accepted as significant.

**Pharmacokinetic Analysis**

The pharmacokinetics of rHb1.1 were determined using a population approach; that is, values for all volunteers were analyzed simultaneously to yield estimates for "typical values" of the pharmacokinetic parameters and interindividual variability. The analysis was performed using the NONMEM program, Version V, Level 1.04.

Parameters for the one-compartment model were volume of the central compartment (V) and clearance (Cl, equal to V/\( k_0 \)), where \( k_0 \) is the elimination rate constant); each parameter included one or both of two components—one constant and one proportional to weight. Parameters for the two-compartment model were volume of the central compartment (\( V_1 \)), volume of the second compartment (\( V_2 \)), clearance (Cl, elimination clearance equal to \( V_1 \cdot k_0 \)), and distribution clearance (Cl\(_{distribution}\) equal to \( V_1 \cdot k_{12} \)), where \( k_{12} \) is the rate constant for drug movement from the central to the peripheral compartment). Volume of distribution at steady state (Vss) was equal to \( V_1 \) plus \( V_2 \). For one- and two-compartment models, half-lives were determined using standard equations. \(^{10}\)

\[ \ln(Cl_i) = \ln(Cl) + \eta_i \]  

where Cl is the estimate for clearance for the \( i \)th person, Cl is the typical value for the population, and \( \eta_i \) is a random variable with a mean of 0.0. All models permitted interindividual variability in Cl and V (or \( V_1 \)). Analyses were performed using NONMEM's "centered" option of the first-order conditional estimate method; this option assures that the \( \eta_i \) values are centered around 0.0, thereby eliminating a problem with NONMEM reported previously by Kataira et al. \(^{11}\) Residual errors between the observed and predicted values were assumed to have two components, one proportional to the predicted value and the other constant; this error model is consistent with the coefficient of variation of the assay; that is, the fractional coefficient of variation increases with smaller concentration.

When initial analyses suggested an influence of rHb1.1 dose on its pharmacokinetics, additional models were tested in which each of the pharmacokinetic terms was permitted to vary as a function of either the dose or the predicted plasma concentration. Several variants of these models were tested. For those models in which Cl became saturated as a function of plasma concentration of rHb1.1, we tested two variants. In the first variant, Cl decreased as plasma concentration increased:

\[ Cl_p = Cl_0 \cdot [1 - ClFactor \cdot \log(1 + Cp)] \]  

where Cp is the predicted (rather than observed) plasma concentration, Cl\(_p\) is clearance at a plasma concentration Cp, Cl\(_0\) is clearance as Cp approaches 0, and Cl\(_{factor}\) is determined in the analysis. In the second variant, Cl decreased as plasma concentration increased (as in equation 2); then as Cp decreased, Cl was maintained at the value attained at peak Cp.

For those models in which Cl became saturated as a function of dose of rHb1.1, we also tested two variants. In the first variant, Cl varied as a function of total dose of rHb1.1 given to that person:

\[ Cl_{Dose} = Cl_0 \cdot [1 - ClFactor \cdot \log(Dose)] \]  

where Cl\(_{Dose}\) is clearance for a person given that dose
of rHb1.1, $C_l_0$ is clearance for an infinitesimally small dose, $C_l_{\text{factor}}$ is determined in the analysis, and Dose is the total dose of rHb1.1 for that person. In the other model, $C_l$ varied as a function of the dose of rHb1.1 administered until that specific time; that is, $C_l$ decreased during the infusion (as the cumulative dose of rHb 1.1 increased), then at the end of the infusion remained constant for that person.

Because each of these new models requires additional terms (called “$\theta$” in NONMEM parlance), they were added to the model only if they significantly improved both the pattern of residual differences between observed and predicted values and the objective function (equivalent to the residual sum of squares in least-squares analysis). For a model with one additional $\theta$, a decrease in the objective function of 3.8 units was necessary for $P < 0.05$. In addition, for each model the differences between individual parameter estimates were determined using the NONMEM post hoc step, as were “typical” values, from NONMEM’s population fit. These differences were plotted against covariates (weight, height, age, dose); trends were sought by plotting a smoother (Supersmooth) through these data and examining for a systematic deviation of this smoother from the horizontal line at $y = 0$.

**Results**

Of 48 volunteers, 47 completed the infusion. A reaction developed in one volunteer assigned to receive 0.15 g/kg rHb1.1 after he received 0.05 g/kg (see Adverse Reactions). The infusion was stopped and the volunteer was treated with epinephrine and diphenhydramine. Cardiovascular and pharmacokinetic data from this volunteer were excluded from analysis, but results from all other tests are included. Of 48 volunteers, 47 completed all postinfusion testing. One volunteer was assessed for 14 rather than 56 days.

**Renal Effects**

In volunteers given rHb1.1, mean urine NAG activity at 12, 24, and 48-72 h did not differ from preinfusion values (fig. 1; normal value = 6.1 U/l). In volunteers given 5% HSA, urine NAG activity decreased 12 h after infusion but did not differ from preinfusion values 24 h or 48-72 h after infusion. Consequently, 12 h after infusion, urine NAG activity was less in the HSA group than in the rHb1.1 group ($P = 0.04$). The NAG activity was unchanged at other times. One volunteer given 0.25 g/kg rHb1.1 had a urine NAG activity (6.3 U/l) that minimally exceeded the reference range 12 h after rHb1.1 infusion, but not 24 or 48 h after infusion (4.7 and 4.3 U/l, respectively). He had been given terbutaline and ibuprofen, the former in an effort to obviate esophageal/gastrointestinal symptoms, the latter to treat headache. At the time of the small increase in urinary NAG activity, this volunteer’s serum bicarbonate level was normal and there was no glucose in his urine.

**Serum Creatinine Concentrations**

Serum creatinine concentrations did not differ significantly from preinfusion values at any postinfusion interval for either group (table 2). Twelve hours and 2-3 days after rHb1.1 infusion, the creatinine clearance concentration did not differ from the preinfusion value; however, 1 day after rHb1.1 infusion, creatinine clearance exceeded ($P < 0.05$) the preinfusion value. After infusion of HSA, creatinine clearance increased ($P < 0.05$) at 12 h but did not differ from the preinfusion value at other times. Values for serum creatinine and creatinine clearance did not differ between rHb1.1 and HSA groups at any time.

After infusion, 19 of 34 volunteers receiving rHb1.1 (56%) and 5 of 14 volunteers receiving HSA (36%) had positive dipstick readings for urine hemoglobin ($P = 0.3$ between groups). The fraction of the dose of rHb1.1 excreted correlated with the dose administered (fig. 2). No volunteer given rHb1.1 excreted more than approxi-
Table 2. Serum Creatinine and Creatinine Clearance Before and After Infusion of Human Serum Albumin or Recombinant Human Hemoglobin in Volunteers

<table>
<thead>
<tr>
<th></th>
<th>Before Infusion</th>
<th>12h</th>
<th>24h</th>
<th>2–3 days</th>
<th>7 days</th>
</tr>
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<tbody>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td></td>
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<tr>
<td>HSA (N = 14)</td>
<td>1.1 ± 0.04</td>
<td>1.0 ± 0.05</td>
<td>1.0 ± 0.04</td>
<td>1.1 ± 0.04</td>
<td>1.1 ± 0.02</td>
</tr>
<tr>
<td>rHb1.1 (N = 34)</td>
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<td>1.1 ± 0.03</td>
<td>1.1 ± 0.02</td>
<td>1.1 ± 0.04</td>
<td>1.1 ± 0.03</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
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<td></td>
</tr>
<tr>
<td>HSA (N = 14)</td>
<td>85 ± 7</td>
<td>138 ± 16*</td>
<td>109 ± 8</td>
<td>85 ± 10</td>
<td>Not determined</td>
</tr>
<tr>
<td>rHb1.1 (N = 34)</td>
<td>93 ± 6</td>
<td>110 ± 6</td>
<td>112 ± 5*</td>
<td>103 ± 4</td>
<td>Not determined</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
* P < 0.05 vs. preinfusion.

There were not statistically significant differences between groups at any time.
HSA = human serum albumin; rHb1.1 = recombinant human hemoglobin.

approximately 9 mg hemoglobin or more than approximately 0.04% of the infused dose in the urine (range, 0.00–0.04%).

Cardiovascular Effects

Infusion of HSA did not alter heart rate or mean arterial blood pressure (fig. 3). Infusion of rHb1.1 produced non-dose-related increases in mean arterial pressure (MAP) 1 and 2 h after infusion (9 ± 2 mmHg and 10 ± 2 mmHg). Heart rate decreased during and for 2 h after infusion (fig. 3; P < 0.05). Heart rate increased in one group 8 h after infusion (0.015 g/kg) and one group 12 h after infusion (0.11 g/kg). There were few statistically significant differences in MAP between rHb1.1 and HSA groups and none 2 h after infusion. Six to 24 h after infusion, values for MAP did not differ from those measured before infusion. There was no correlation between plasma rHb1.1 concentration and increased MAP.

Pharmacokinetics

Peak concentrations of rHb1.1 varied with dose (fig. 4). Initial one-compartment analyses suggested an influence of rHb1.1 dose on CI (model 1, fig. 5). As a result, additional models were tested in which CI varied with dose or predicted plasma concentration. The model (model 2) in which CI varied with the total dose of rHb1.1 fit the data markedly better, the objective function decreased by 55 units (P < 0.01). The model in which CI varied with the dose of rHb1.1 administered before that time further improved the fit. The model (model 3) in which CI varied as a function of the predicted concentration of rHb1.1 further improved the fit (fig. 6); the objective function decreased by 61 units compared with model 1 and by 106 units compared with model 2 (P < 0.01). The model in which CI decreased as Cp increased, then remained constant as Cp decreased, failed to improve the fit. With model 3, plots of post hoc individual pharmacokinetic parameters against the covariates failed to demonstrate additional influences of the covariates (such as age, weight, or height) on the pharmacokinetic parameters (fig. 7); that is, there was no longer a systematic relation between the post hoc values and CI (fig. 7). The model in which pharmacokinetic parameters contained a weight-normalized component, without a constant component, fit as well as the model with both components. Addition of a second compartment failed to improve the model further.

Thus the optimal model had one compartment. Typi-
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Fig. 3. Changes (compared with preinfusion values) in (A) heart rate and (B) mean arterial blood pressure during and after infusion of 5% human serum albumin (HSA; ○ all doses combined) or 5% recombinant human hemoglobin (rHb1.1; • 0.015 g/kg; ■ 0.075 g/kg; ◻ 0.11 g/kg; ▲ 0.15 g/kg). The rate of infusion varied from 0.8–1.9 h (see text). *P < 0.05 versus HSA at the same time point. #P < 0.05 versus preinfusion values for the same group. Data are means ± SE.

Fig. 4. Values for plasma concentration of rHb1.1 are plotted against time. Symbols indicate dose ranges.

Fig. 5. Values for clearance (CI) of rHb1.1 determined from NONMEM's post hoc step are plotted against the dose of rHb1.1. Values for CI were obtained from a one-compartment model in which weight-normalized pharmacokinetic parameters were equal for all volunteers and CI did not vary with plasma concentration or dose of rHb1.1. The thin line, determined using a smoother (Supersmooother), suggests that the assumption of the same CI for all volunteers is incorrect.

Fig. 6. The quality of fit of the one-compartment model in which weight-normalized clearance (CI) varies as a function of predicted plasma concentration of rHb1.1 is displayed. The x axis is (log) time after the start of the rHb1.1 infusion. The y axis is the ratio of the observed values to the population-predicted values. If observed values were identical to predicted values (i.e., if the pharmacokinetic variation in all volunteers could be explained entirely by the plasma concentration of rHb1.1), each line would lie horizontally at 100%.
predicted concentration in units of milligrams per milliliter. Interindividual variability (expressed as one standard deviation of the typical value) was 11% for CI and 13% for V. Because CI varies continuously and inversely with Cp, the half-life also varies directly as a function of Cp (table 3).

**Adverse Reactions**

Adverse reactions occurred in 8 of 14 volunteers given HSA and in 30 of 34 volunteers given rHb1.1 ($P = 0.045$ between groups; table 4). With HSA, one half of the reactions were either headaches or gastrointestinal symptoms. With rHb1.1, the most prevalent adverse reactions were those affecting the gastrointestinal tract (68%), including nausea, vomiting, diarrhea, dysphagia, and midepigastric or abdominal pain.

Twenty-three volunteers given rHb1.1 reported gastrointestinal symptoms. The incidence of these symptoms was greater with rHb1.1 doses of $\geq 0.15$ g/kg than with smaller doses ($P < 0.05$, Fisher’s exact test). Of these 23, six volunteers (at doses of 0.15 g/kg - 0.52 g/kg) had increases in lipase above the reference range: Peak lipase values were $3.041 \pm 1.313$ IU/l (reference range, 25 - 300 IU/l). Four of these six also had increases of amylase above the reference range: Peak amylase values for these four were $286 \pm 84$ IU/l (reference range, 30 - 110 IU/l). All increases were detected 2–4 h after infusion. In five of six volunteers, amylase, lipase, or both returned to normal within 18 – 24 h; in the sixth, they were normal 48 h after infusion. No volunteers receiving HSA had increases in pancreatic enzymes.

There were no significant changes in oral temperature in volunteers given HSA (fig. 8). Temperatures of volunteers given rHb1.1 did not increase during infusion but did increase 6–12 h after infusion ($P < 0.05$). Some volunteers were given oral ibuprofen (400 mg to 800 mg) to treat postinfusion increases in body temperature. Body temperature returned to normal within 24 h after infusion.

Some volunteers given rHb1.1 reported a “flu-like” syndrome consisting of fever, chills, headache, and myalgias (table 4). Symptoms typically began 4–8 h after infusion and were ameliorated within 12–24 h by ibuprofen.

Urticaria, pruritus, or both were seen in 8 of 34 (24%) volunteers receiving rHb1.1. These resolved either spontaneously or with diphenhydramine (15 mg to 50 mg given intravenously). One volunteer given 0.15 g/kg rHb1.1 experienced a reaction (bronchospasm, sneezing, and arterial oxyhemoglobin desaturation) during the infusion. The infusion was terminated and the volunteer was treated with epinephrine (0.3 mg given subcutaneously), oxygen, and diphenhydramine (50 mg given intravenously) with rapid improvement of his symptoms.

**Discussion**

Development of acellular hemoglobin solutions has been hindered by previous reports of renal damage in

<table>
<thead>
<tr>
<th>Plasma Concentration (mg/ml)</th>
<th>Clearance (l \cdot kg(^{-1}) \cdot hr(^{-1}))</th>
<th>Half-life (h)</th>
</tr>
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<tbody>
<tr>
<td>0.1</td>
<td>15.4</td>
<td>2.4</td>
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<tr>
<td>0.3</td>
<td>14.2</td>
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<td>11.1</td>
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<td>12.0</td>
</tr>
<tr>
<td>6</td>
<td>1.9</td>
<td>18.9</td>
</tr>
</tbody>
</table>

rHb1.1 = recombinant human hemoglobin.

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Table 4. Adverse Reactions Observed with Recombinant Hemoglobin or Human Serum Albumin

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Recombinant Hemoglobin (g/kg)</th>
<th>Human Serum Albumin (All doses combined)</th>
<th>P Value (HSA vs. Hb1.1)</th>
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</thead>
<tbody>
<tr>
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<td>0.015–0.11</td>
<td>0.15–0.22</td>
<td>0.25–0.32</td>
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<tr>
<td>N</td>
<td>16</td>
<td>12†</td>
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<tr>
<td>Fever</td>
<td>10</td>
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<tr>
<td>Chills</td>
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</tr>
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<td>Myalgia</td>
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<td>Headache</td>
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</tr>
<tr>
<td>Allergic</td>
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<td>3</td>
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<tr>
<td>Gastrointestinal symptoms†</td>
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<td>6</td>
</tr>
<tr>
<td>Chest pain or cardiac signs</td>
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</tr>
<tr>
<td>Other‡</td>
<td>7</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* Includes one volunteer in whom the infusion was not completed (see Results).
† Nausea, vomiting, abdominal or epigastric pain and cramps, dysphagia, diarrhea, heartburn.
‡ Insomnia, somnolence, restlessness, dizziness, pericardial paraesthesia, anxiety, weakness, jaw, shoulder, or knee pain.
ND = not determined.

animals and humans after infusion of hemoglobin solutions. 1–4 Our important finding is that recombinant human hemoglobin in intravenous doses as large as 0.32 g/kg does not produce detectable renal toxicity even when sensitive markers of renal function are assessed. N-acetyl-beta-glucosaminidase is a lysosomal enzyme contained in renal tubular cells, the appearance of which in urine is a sensitive, consistent, and early marker of renal tubule cell damage. 5 The sole urine sample with abnormal NAG activity (a value only slightly exceeding the upper limit of normal) occurred in a volunteer given both terbutaline and ibuprofen. Although long-term administration of ibuprofen can produce both renal insufficiency 15 and increased urine NAG activity, 16 there are no reports of the effect of a single dose of ibuprofen on urine NAG activity. The proximal renal tubule is the source of urinary NAG. In the absence of other evidence of proximal tubular damage (e.g., glucosuria, increased serum bicarbonate concentration), as was the case for this volunteer, the clinical significance of a minimally increased value of urinary NAG activity is unknown. No individual or mean value was above the reference range for serum creatinine or below normal for creatinine clearance. Our results suggest that renal damage detected after previous infusions of hemoglobin solutions of uncertain purity probably was due to contamination of the solutions by other elements. For example, Savitsky et al. 17 found decreased creatinine clearance and oliguria after administration to humans of 16 g (approximately 0.2 g/kg) hemoglobin solution labeled “stroma-free” but that contained 1% of the original red cell stroma. Infusion of a

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Fig. 8. Oral temperature before, during, and after infusion of 5% human serum albumin (HSA; all doses combined) or 5% recombinant human hemoglobin (Hb1.1; ○ 0.015 g/kg; ● 0.075 g/kg; ● 0.11 g/kg; ▲ 0.15 g/kg; ▼ 0.18–0.22 g/kg; ● 0.25–0.32 g/kg). Rate of infusion varied from 0.8–1.9 h (see text). *P < 0.05 versus HSA at same time point. #P < 0.05 versus preinfusion for the same group. Data are means ± SE.

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filtered hemoglobin solution (prepared from lysates of human erythrocytes) in rats decreased glomerular filtration rate and increased urinary NAG activity. Binding of the filtered hemoglobin to dextran, followed by further washing and dialysis of the product prevented its renal excretion. Infusion of this dextran-bound hemoglobin did not affect the glomerular filtration rate in rats, but it did decrease urine production and excretion of sodium. Urinary NAG activity increased, but to a smaller extent than after infusion of the unbound filtered hemoglobin.

The recombinant human hemoglobin infused in this study differs structurally from human hemoglobin A₉ in that the two α chains are joined by a glycine residue, a result of a modification of the gene. Linking of the two α chains prevents dissociation of the four amino acid chains into dimers, preserving the molecule's tetrameric structure and preventing renal excretion. Although the amount of rHb1.1 excreted in the urine appeared to vary directly with dose administered, the amount excreted did not exceed 0.04% of the total dose. However, the magnitude of the amount excreted should not be taken as exact because of the inherent difficulties in attempting to evaluate a semiquantitative analysis. The genetically α-α cross-linked hemoglobin evaluated in this study appears to have an approximately 100-fold smaller renal excretion than does an α-α crosslinked hemoglobin (prepared from outdated human erythrocytes) in rats. α-α Cross-linkage of normal human hemoglobin is known to occur in some dimers and produces visible hemoglobinuria. The method of elimination of rHb1.1 has not been studied in humans, but in rats it appears to be eliminated in a manner similar to that of native hemoglobin (hepatic clearance). The absence of renal toxicity of rHb1.1 infusion, perhaps, is to be expected from the absence of red cell stroma and the small amount of rHb1.1 appearing in the urine after infusion.

The pharmacokinetic analysis suggests that clearance of rHb1.1 varied inversely as a function of the predicted plasma concentration. This implies that elimination of rHb1.1 is a saturable process. The analysis also suggested that this saturable process was reversible; that is, as Cp decreased, clearance recovered. The mechanism for this saturation remains to be determined. Although studies in rats suggest that rHb1.1 is eliminated in a manner similar to that of native hemoglobin, there are no other reports in humans regarding the influence of rHb1.1 dose or concentration on its elimination. However, a study of pharmacokinetic characteristics of smaller doses of a diaminopropylamine cross-linked hemoglobin in patients undergoing hemodialysis suggests that a fourfold increase in dose (from 0.025 to 0.1 g/kg) is associated with a threefold decrease in clearance. The largest dose in the present study, 0.52 g/kg, is smaller than would be given to many patients (one unit of blood contains approximately 55 g of hemoglobin, equivalent to approximately 0.8 g/kg), resulting in a peak rHb1.1 plasma concentration of approximately 6 mg/ml. Because our model could not predict the effect of larger concentrations of rHb1.1, we cannot speculate about the expected clearance and half-life of clinical doses of rHb1.1, producing larger plasma concentrations.

We observed that a one-compartment model was sufficient to describe the pharmacokinetic characteristics of rHb1.1. Our “typical” value for the volume of distribution of rHb1.1 was 53 ml/kg, a value similar to that of plasma. This suggests that the distribution of rHb1.1 is limited to the vascular space, presumably because of its molecular size.

Infusion of recombinant human hemoglobin was not without hemodynamic effects. Arterial blood pressure increased and heart rate decreased. Intravenous infusion of acellular hemoglobin has previously been reported to increase blood pressure. Hemoglobin scavenges nitric oxide (NO), a potent naturally occurring vasodilator, thought to play an important role in maintenance of normal blood pressure. Both hemoglobin A and recombinant human hemoglobin inhibit the NO-mediated vasorelaxation of nitroglycerin and nitroprusside.

The increased blood pressure was not dose-related or large. Most increases were less than 10 mmHg, and the largest mean increase of MAP was 16 mmHg. Although we could not analyze the hemodynamic effects of the largest doses of recombinant hemoglobin (because of the limitation imposed by administering other drugs with potential cardiovascular activity), for the smaller doses there was no correlation between plasma hemoglobin concentration and increased blood pressure. The largest increase in MAP occurred 1-2 h after infusion. We did not measure the state of vascular dilatation or constriction either directly or indirectly, but it seems likely that the mild increases in blood pressure were probably related to scavenging of NO by infused hemoglobin. The reason for the lack of a dose-response relation between amount infused or blood concentration
of rHb1.1 and blood pressure increase is uncertain. Perhaps the smallest dose we infused is larger than that required for maximal scavenging of NO or exceeds the maximal pharmacologic effect achieved by NO scavenging. If maximal increases of MAP are achieved with relatively small concentrations of rHb1.1, the mild increase of MAP should not deter future development of hemoglobin solutions. Alternatively, larger increases in arterial blood pressure after partial exchange transfusion in rats with 8% diaspirin cross-linked hemoglobin and larger and dose-related increases after infusion of 0.025–0.10 g/kg diaspirin cross-linked hemoglobin in patients undergoing hemodialysis have been reported. This blood pressure increase could, in part, result because the diaspirin cross-linked hemoglobin preparation is hyperoncotic and hyperosmotic. In contrast, rHb1.1 is iso-osmotic and hypotonic, and these physical properties should not increase blood pressure.

Nitric oxide scavenging by intravenously infused acellular hemoglobin may also explain the frequent occurrence of gastrointestinal symptoms reported in this and other studies. In this study, the incidence of gastrointestinal symptoms could have been affected by the administration of other drugs before infusion of the larger doses of rHb1.1. Nitric oxide plays an important role in the control of smooth muscle function in the gastrointestinal tract. Nitric oxide is thought to reduce both lower esophageal sphincter tone and esophageal peristalsis in response to swallowing or electrical stimulation. These functions of the esophagus are inhibited when acellular hemoglobin is introduced into test systems. The in vivo effect of rHb1.1 on normal human esophageal function is not known; however, gastrointestinal symptoms that we report are consistent with NO scavenging by rHb1.1.

The cause of the amylase and lipase elevations in six volunteers may also relate to inhibition of the NO pathway. Nitric oxide relaxes the sphincter of Oddi and affects its phasic activity. Inhibition of NO by hemoglobin completely abolishes relaxation of isolated rabbit sphincter of Oddi in response to nicotinic agonists. Abnormalities in sphincter of Oddi function are postulated as causing idiopathic pancreatitis in humans by obstructing the pancreatic duct transiently. Although there is no evidence in humans, inhibition of NO-mediated sphincter relaxation by rHb1.1 may cause the pancreatic enzyme elevations. Other potential causes, such as pancreatic hypoxia or direct pancreatic injury, are possible, but there is no evidence to support these causes.

Fever and chills experienced by volunteers receiving rHb1.1 are consistent with symptoms of a pyrogenic response. Although the rHb1.1 product had unmeasurable levels of endotoxin (lipus amoebocyte lysate assay), it is possible that Escherichia coli elements were present in sufficient quantity to produce these symptoms but could not be detected by the assay. The late increase of body temperature is the likely cause of the increased heart rate at 8 and 12 h after rHb1.1 infusion.

In summary, recombinant human hemoglobin infused intravenously in humans is not nephrotoxic at doses 0.32 g/kg or less. Plasma clearance varies with plasma concentration, suggesting that the elimination process is saturable. The mechanism of this saturation remains to be determined, although it appears to be immediately reversible. Administration of recombinant human hemoglobin is also associated with findings and symptoms consistent with hemoglobin scavenging of NO oxide: increased blood pressure, decreased heart rate, and gastrointestinal symptoms.

References


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