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INCREASED VASCULAR RESISTANCE WITH HEMOGLOBIN-BASED OXYGEN CARRIERS

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ABSTRACT

Purpose: To compare the effects of resuscitation with hemoglobin-based oxygen-carriers and conventional crystalloid and colloid resuscitation fluids on hemodynamics, oxygen transport, and oxygen consumption in an animal model of the field medical use of these fluids in the treatment of hemorrhagic shock.

Protocol: Twenty-eight immature swine were surgically prepared, allowed to recover five days, water deprived for 48 hours, hemorrhaged of 25 ml/kg over one hour, resuscitated promptly with 1) Ringer's lactate, 75 ml/kg, 2) 7% albumin in Ringer's acetate, 25 ml/kg, 3) 9% unmodified hemoglobin in Ringer's acetate, 25 ml/kg, or 4) 9% αα-crosslinked hemoglobin in Ringer's acetate, 25 ml/kg, observed with three hours of hemodynamic and oxygen transport measurements, and, finally, blood which had been removed previously was returned.

Results: Systemic and pulmonary vascular resistance were increased in hemoglobin-treated animals to more than twice the levels seen in crystalloid- or colloid-treated controls. Oxygen consumption and the rate of correction of lactic acidosis were not increased in hemoglobin-treated animals. The return of blood which had previously been removed induced a small additional increase

in vascular resistance and a slight reduction in cardiac output.
Conclusions: Increased vascular resistance limits the oxygen transport benefit of cell-free-hemoglobin-based oxygen carriers. Cell-free-hemoglobin-induced increases in vascular resistance may place animals' hearts on an unfavorable portion of the Frank-Starling curve as well as complicate the use of red cells in further medical treatment by reducing the animals' tolerance to increases in blood viscosity.

INTRODUCTION

Cell-free hemoglobin causes vasoconstriction in isolated vascular rings with intact endothelium (1) and in perfused coronary arteries (2). Except in the virtual absence of red blood cells (3), hemoglobin solutions have produced hypertension in animals or have not supported an increase in cardiac output with blood volume expansion. Half of all the humans administered hemoglobin in published trials demonstrated hypertension (4), and a recent human trial of a modified hemoglobin was stopped when patient volunteers developed symptoms suggestive of acute pulmonary hypertension (5). All of these findings imply that cell-free hemoglobin can be expected to increase vascular resistance in many clinical situations.

We have studied hemoglobin-based oxygen carriers in swine, modeling the conditions of resuscitation from hemorrhagic shock in a field medical environment. Specifically, we have produced solutions of modified and unmodified hemoglobin of defined function and high purity, and we have used these solutions in an animal model that consistently demonstrates increased vascular resistance after administration of hemoglobin solutions. We have tried to understand the implications of the observed increases in systemic and pulmonary vascular resistance on the future development of hemoglobin-based oxygen carriers.

MATERIALS AND METHODS

Primary reports of the experiments described here have been published elsewhere (6). The production, oxygen-binding properties, and formulation of the hemoglobin solutions used in these studies have also been reported separately (7,8). The immature swine model was developed at our institution and extensive descriptions of the surgical preparation (9), normal physiology (10), and response to dehydration and hemorrhage (11) have been published.

The renal vascular resistance was calculated from measurements of aortic blood pressure and renal blood flow, which were monitored continuously along with pulmonary artery pressure during the four-hour hemorrhage and resuscitation procedure. Examples of changes in blood pressure after the beginning of the administration of hemoglobin were taken directly from the primary strip-chart records. Cardiac output was measured intermittently by thermodilution. Consequently, the calculated values for vascular resistance are also discontinuous.

The viscosity of hemoglobin cross-linked between Lys-99αα residues with bis(3,5-dibromosalicyl)fumarate (ααHb) mixed with whole pig blood was obtained with a cone-plate viscometer in a previous study (3). Those data are provided here in greater detail.

Data on the intravascular and whole-body persistence of unmodified Hb and ααHb were calculated from exponential curves fitted to the plasma hemoglobin concentrations.

RESULTS

Resuscitation of hemorrhagic shock with hemoglobin solutions raised mean systemic blood pressure from 62.3±3 (Mean±SEM) to 140±4 Torr (40% higher than prehemorrhage levels). The increase in mean systemic blood pressure in the control groups of swine resuscitated with Ringer's lactate and albumin solutions was only half as great, rising to 101±6 Torr (close to normal

prehemorrhage levels). In the hemoglobin-treated animals, the blood pressure achieved this rise after only 2 minutes when approximately 10 ml/kg of hemoglobin solution had been administered (Fig. 1). The resultant hypertension persisted for the full three hours of observation following resuscitation.

Mean pulmonary blood pressure increased from 15±3 to 40±4 Torr in the groups resuscitated with hemoglobin solutions. The final pressures were 19 Torr lower in the groups resuscitated with Ringer's lactate and albumin solutions. Pulmonary hypertension developed with occasionally startling rapidity (Fig. 2), and the final pressures exhibited greater animal-to-animal variation than was seen in the systemic pressures.

Cardiac output did not increase significantly after resuscitation with hemoglobin solution, changing from 0.16±0.02 to 0.20±0.02 l/min/kg. Resuscitation with conventional crystalloid and colloid solutions doubled the cardiac output, increasing it from 0.15±0.01 to 0.35±0.02 l/min/kg. Post-resuscitation cardiac output values remained constant in the albumin and both hemoglobin groups, but declined to 0.26±0.03 l/min/kg in the Ringer's lactate group one hour after resuscitation.

Systemic and pulmonary vascular resistances calculated at all time points after resuscitation were uniformly more than twice as large in the hemoglobin-treated groups compared with the groups treated with Ringer's lactate or albumin solution. Systemic and pulmonary vascular resistance declined approximately 20% after resuscitation with the conventional solutions.

Whole blood viscosity decreased with hemodilution from 3.8 cP at a hematocrit of 30% to 3.2 cP at a hematocrit of 17% with 3 g/dl of $\alpha\alpha$Hb.

Renal vascular resistance was also increased at all time points after hemoglobin administration (Fig 3), but was not associated with significant changes in renal function.

The return of 25 ml/kg of blood which had previously been removed at three hours after resuscitation increased intravascular volume and hematocrit. This simultaneous increase in vascular volume and viscosity was well tolerated by all animals.

The initial or redistribution phase rates of change in plasma concentration of unmodified Hb and αα-Hb were similar with half-concentration times of about 4.5 hours. During the next day, unmodified hemoglobin continued to be eliminated at the same rate, but the whole body half-clearance time of αα-Hb was 13 hours.

DISCUSSION

"Hemoglobin is a vasoconstrictor," according to Amberson (12), but the clinical implications of this observation have not been explored. Massive hemolysis in humans which occurs with hemolytic transfusion reactions, hemolytic anemias, *Clostridial* sepsis, and falciparial malaria is sufficiently desperate that reliable studies of the vascular resistance changes associated with these conditions are not available. A standard textbook of transfusion medicine says that cell-free hemoglobin is safe, and mentions its development as a blood substitute as proof (13). However, measurements of vascular resistance in human trials of hemoglobin-based oxygen carriers have never been published. Previous animal studies of the effects of cell-free hemoglobin have been interpreted to view hypertension, or the failure to increase cardiac output in the face of decreased blood viscosity, as efficacy (14). The implications of increased vascular resistance on the usefulness of cell-free hemoglobin solutions have thus not been addressed in critical experiments.

We conducted an animal study searching for any toxicity of cell-free hemoglobin that might occur under the anticipated conditions of use in resuscitation. We measured aortic blood pressure, pulmonary artery blood pressure, cardiac output, and renal artery blood flow, because we believed that these hemodynamic measures might be important in understanding renal tubular and hepatic central-lobular necrosis reported previously (15). Here, we review the data of that study (6) with specific attention to potential complications of increased vascular resistance.

Swine resuscitated with αα-Hb exhibited the rapid onset of marked

systemic hypertension. The blood pressure rose within seconds of the beginning of hemoglobin infusion and achieved hypertensive pressures after the delivery of less than 0.5 grams of hemoglobin per kilogram of body mass. The blood pressure often achieved its maximum before the completion of hemoglobin infusion and persisted for at least the three hours of hemodynamic observation without diminution. In the one animal that was measured 24 hours later, hypertension persisted.

The hypertension was consistently associated with a doubling of systemic vascular resistance. In the hemoglobin-treated animals, cardiac output changed very little with resuscitation or during the period of hemodynamic observation. Changes in blood pressure may thus be viewed as reflecting changes in vascular resistance. Renal vascular resistance, a component of the systemic vascular resistance, but derived from continuously collected data, was also increased. The addition of more hemoglobin solution after the achievement of the hypertensive plateau did not further raise pressure or reduce renal flow. At the end of the experiment, when the blood which had previously been removed was returned, increases in pressure and reductions in flow were only minimal. The results of these two volume challenges suggest that the effects of hemoglobin on blood pressure and resistance are independent of effects on vascular compliance. Alternatively, the failure to respond to increases in volume raises the possibility that the increased cardiac work places the animal's heart near the apex of the Frank-Starling curve.

In our study, one animal with preexisting ventricular dysfunction died with hypertension and low cardiac output after receiving hemoglobin. In the absence of methods to treat the increased vascular resistance, human subjects enrolled in clinical trials should be carefully screened for cardiovascular disease.

Pulmonary hypertension also occurred consistently with the administration of hemoglobin solutions. The pulmonary hypertension varied from animal to animal. It achieved its highest pressure during hemoglobin administration then decreased to a lower but still elevated level during the next 30 minutes. Pulmonary vascular resistance was also elevated, although the

exact degree of elevation is less certain, because resting pulmonary artery wedge pressures were not consistently obtainable in these awake animals.

In our study one animal died with acute pulmonary edema after receiving hemoglobin. The animal had pulmonary artery blood pressure greater than 80/30 Torr. Because several animals demonstrated pressures only slightly lower, and because humans have suffered apparently similar clinical episodes, this reaction needs to be understood and a treatment developed before large scale human trials of hemoglobin are undertaken.

Despite these incidents, the hypertension and increased vascular resistance were usually well tolerated. Hemoglobin administration did not appear to interfere with vascular compliance or limit further treatment with whole blood. The majority of these young, healthy animals thus tolerated the increased vascular resistance, but the reduced cardiac output may have offset the increased oxygen-carrying-capacity of hemoglobin-based resuscitation fluid so that hemoglobin appeared to confer no benefit in this resuscitation model.

The safety and efficacy of cell-free hemoglobin remains in doubt. Over 200 people have been given varying amounts of the solutions with only one reported death, but many individuals have experienced untoward reactions. Even an ideal oxygen-carrying solution may have limited benefits in the resuscitation from acute hemorrhagic shock of young, previously healthy individuals who respond well to volume expansion. Clearly, more research on the basic physiology and toxicity of hemoglobin-based oxygen carriers is needed.

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The experimental studies the authors described in this report were reviewed and approved by the Institutional Review Committee/Animal Care

and Use Committee at Letterman Army Institute of Research. The manuscript was peer reviewed for compliance before submission for publication. In conducting the research described herein, the authors adhered to the "Guide for Care and Use of Laboratory Animals" (NIH Publ. 85-23).

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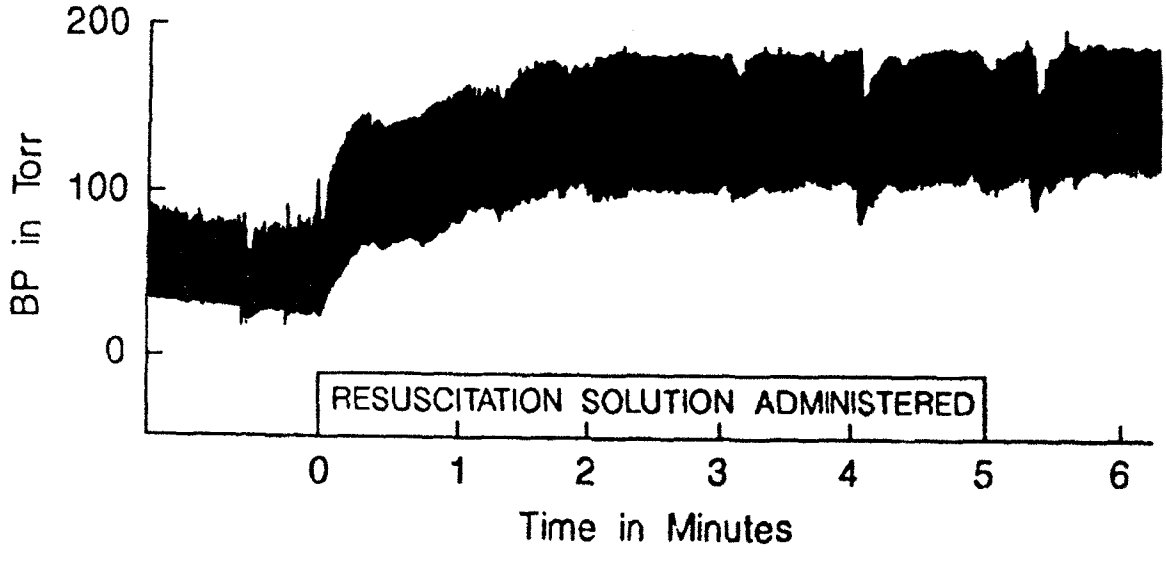
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RESUSCITATION SOLUTION ADMINISTERED
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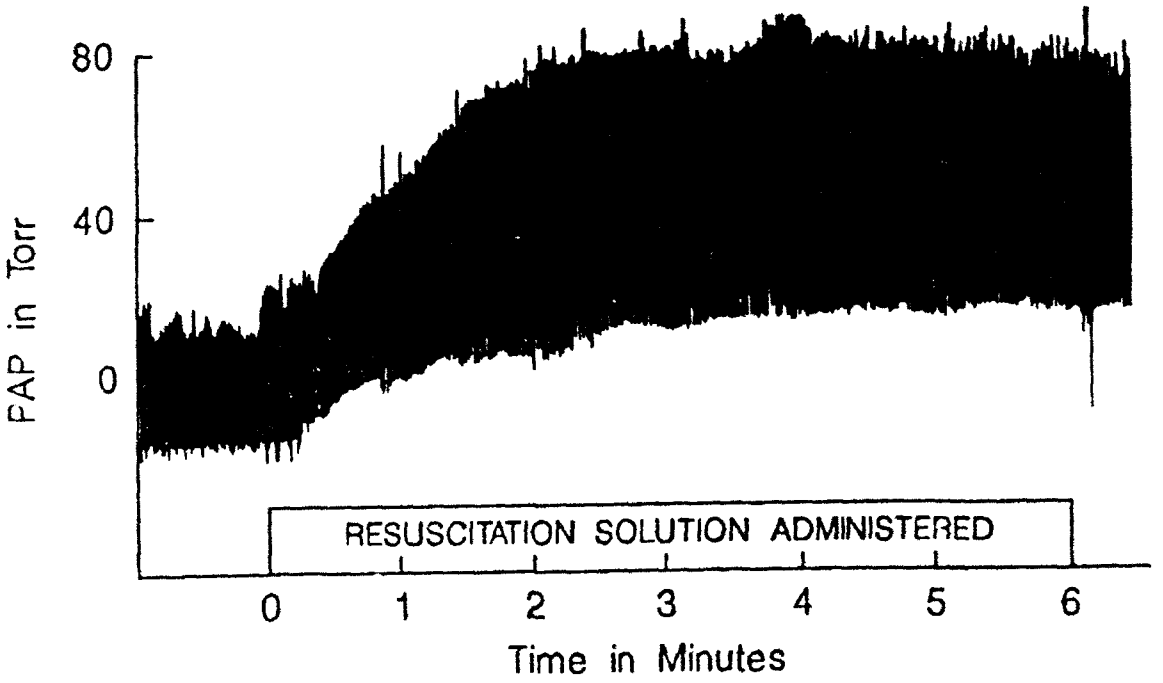
Time in Minutes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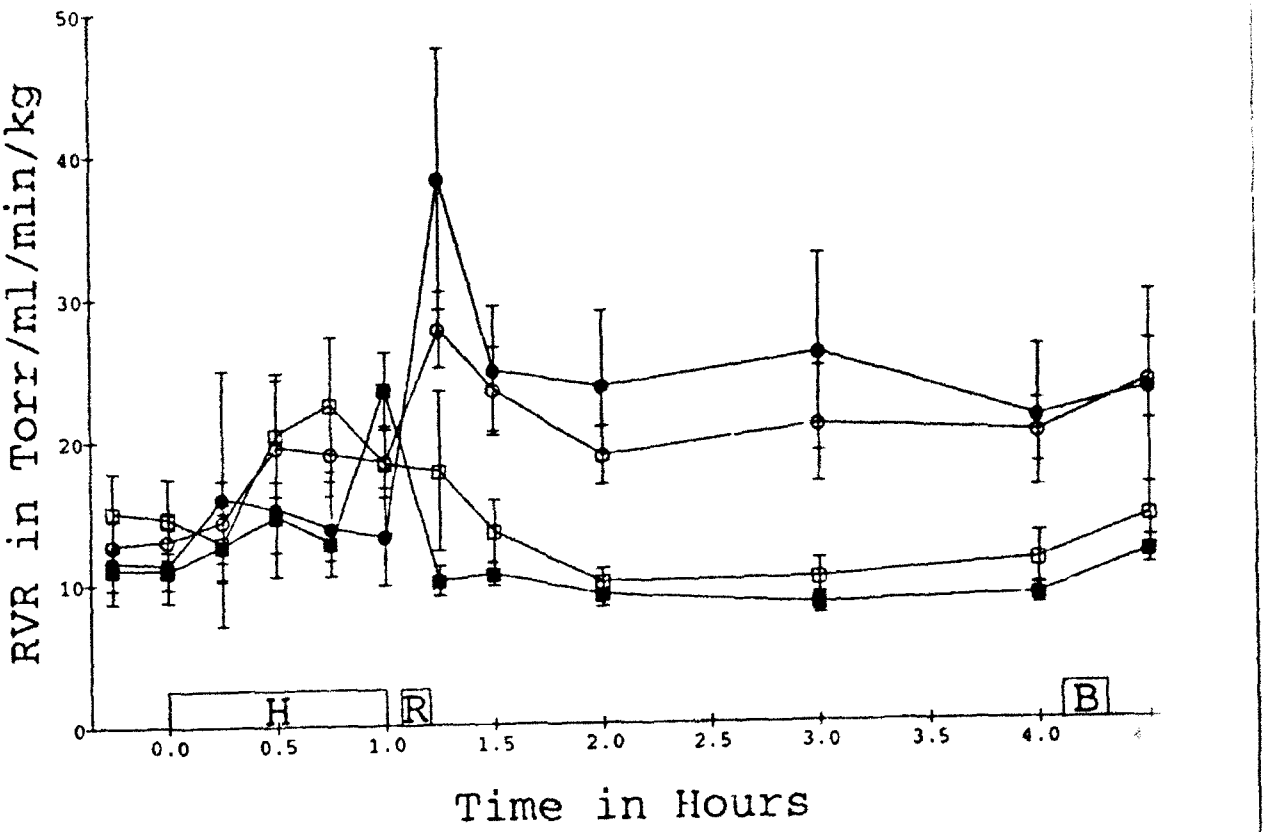
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2.0
2.5
3.0
3.5
4.0
Time in Hours

FIGURE 1. Aortic blood pressure during resuscitation with ααHb, 9.9 g/dl, in Ringer's acetate. The tracing was photographed from the original strip-chart recording of the period just prior to, during, and immediately after administration of 25 ml/kg ααHb solution through the jugular vein catheter. Note the very rapid onset of the hypertensive response during the first 30 seconds with the administration of 60 ml of solution containing 0.3 g/kg of ααHb. The full response was attained when only 10 ml/kg had been administered, about 1 g/kg of Hb. Blood pressure rose at an equivalent rate for both uncross-linked HbA_0 and ααHb, lending no support to the argument that the amount of Hb dimer is a determinant of the hypertensive response.

FIGURE 2. Response of pulmonary artery pressure to resuscitation with 9.9 g/dl ααHb in Ringer's acetate. The tracing was photographed from the original strip-chart as in Fig 1, but from a different animal. The animal that died with acute pulmonary edema achieved similar pulmonary artery pressures of 80/30 Torr.

FIGURE 3. Renal vascular resistance (RVR) at discreet time points before, during and just after hemorrhage (H); after resuscitation (R); and after return of shed blood (B). Animals were resuscitated with equivolume amounts of 9.9 g/dl ααHb (●), 9.9 g/dl unmodified hemoglobin (○), or 7 g/dl human serum albumin (■) in Ringer's acetate or with Ringer's lactate at three times the volume of shed blood (□). Values are Mean±SEM.