The Efficacy of Polymerized Pyridoxylated Hemoglobin Solution as an O_2 Carrier

STEVEN A. GOULD, M.D., LAKSHMAN R. SEHGAL, PH.D., ARTHUR L. ROSEN, PH.D., HANSA L. SEHGAL, B.S.M.T., and GERALD S. MOSS, M.D.

A polymerized pyridoxylated hemoglobin solution (Poly SFH-P) has been prepared with a normal [Hb] of 14 g/dL, a normal COP of 20 to 25 torr, a P_{50} of 16 to 20 torr, and a plasma $T_{½}$ of 40 to 46 hours. Animals underwent a total exchange transfusion with Poly SFH-P to assess its ability to support hemodynamics and oxygen transport in the absence of red cells. All animals survived the exchange transfusion. Mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), and oxygen consumption ($\dot{V}O_2$) remained at baseline values at zero hematocrit after the exchange. The final plasma [Hb] at Hct less than 1% was 9.7 ± 0.4 g/dL. These results are significantly better than previous data with unmodified tetrameric hemoglobin solution (SFH). Poly SFH-P supports life in the absence of red cells. In contrast to SFH, Poly SFH-P achieves a nearly normal [Hb], a longer $T_{½}$, and maintains baseline hemodynamics and oxygen consumption at zero hematocrit. These results document that Poly SFH-P is an effective oxygen carrier that offers greater potential than previous products as a clinically useful red cell substitute.

From the Department of Surgery, Michael Reese Hospital and Medical Center, and The University of Chicago, Pritzker School of Medicine, Chicago, Illinois

WE HAVE SHOWN PREVIOUSLY that stroma-free hemoglobin solution (SFH) with a hemoglobin concentration ([Hb]) of 7 g/dL supports life in primates in the absence of red blood cells.[1,2] Although animals survive such a total exchange transfusion, there is a significant decrease from baseline values in oxygen consumption ($\dot{V}O_2$), mean arterial pressure (MAP), and cardiac output (CO) and an increase in heart rate (HR) at zero hematocrit. It is possible that these changes are due to the low [Hb] of SFH because the final plasma [Hb] in these animals is only 4 g/dL.[3]

The SFH can be prepared with a [Hb] equal to 14 g/dL; however such a solution has an unacceptable colloid osmotic pressure (COP more than 60 torr). We have developed a polymerized pyridoxylated hemoglobin solution (Poly SFH-P) with a normal COP and a normal [Hb] of 14 g/dL.[4] The purpose of this report is to assess the ability of Poly SFH-P to support hemodynamics and oxygen transport in the absence of red blood cells.[5]

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Address reprint requests to Steven A. Gould, M.D., Department of Surgery, Michael Reese Hospital and Medical Center, Lake Shore Drive at 31st Street, Chicago, IL 60616.

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Materials and Methods

Animal Preparation

Six adult baboons were anesthetized, paralyzed, intubated, and mechanically ventilated on room air. The respiratory rate and tidal volume were adjusted to maintain a $PaCO_2$ between 35 and 45 torr before the start of the study, and were not changed during the study. The animals were surgically prepared with arterial and central venous catheters for infusion, blood sampling, and monitoring. A thermal dilution balloon-tipped catheter was floated into the pulmonary artery. A Foley catheter was inserted into the urinary bladder. Standard hemodynamic monitoring was performed for EKG, arterial pressures, pulmonary capillary wedge pressures, and central venous pressures. Cardiac output was determined by the thermal dilution method.

Hemoglobin Solution

The hemoglobin solution was prepared by a method we have previously described.[4] Briefly, outdated washed red cells are lysed with hypotonic phosphate buffer and the stroma removed by filtration. The stroma-free hemoglobin is then pyridoxylated to raise the P_{50} to 22 to 24 torr. The pyridoxylated hemoglobin (SFH-P) is sub-

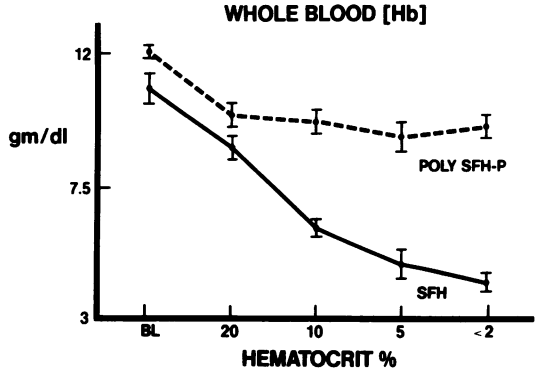

FIG. 1. Whole blood [Hb] *versus* hematocrit. Data presented as mean ± SEM.

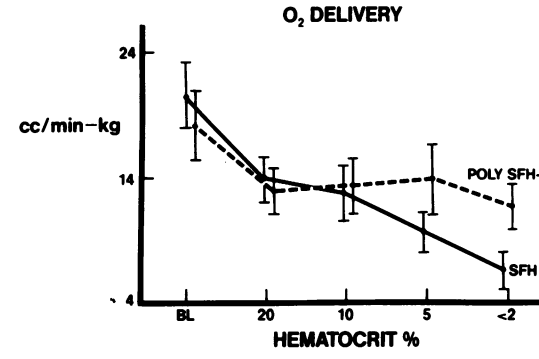

FIG. 3. Total O_2 delivery *versus* hematocrit. Data presented as mean ± SEM.

sequently polymerized with glutaraldehyde. The Poly SFH-P is reconstituted in a balanced electrolyte solution and sterilized. The Poly SFH-P thus prepared has a [Hb] of 14.3 ± 0.2 g/dL and a P_{50} of 16.7 ± 0.4 torr.

Exchange Transfusion

The study was conducted under ketamine anesthesia. After stabilization of the animals, a set of baseline measurements was obtained. An isovolemic exchange transfusion with the Poly SFH-P was then performed. Whole blood was removed in 50-cc aliquots and was replaced with approximately equal volumes of the infusate. Additional volume adjustments were made as required to maintain the pulmonary capillary wedge pressure at baseline values. The exchange was stopped at hematocrits of 20%, 10%, 5%, and less than 2% to obtain additional sets of measurements. The animals were killed at the end of the study.

Measurements

Cardiac outputs were determined by the thermal dilution technique and indexed for body surface area. MAP and HR were monitored using standard electrodes. $\dot{V}O_2$ was calculated as the product of the cardiac output and the total arteriovenous oxygen content difference ($AVDO_2$). Total oxygen delivery was calculated as the product of the cardiac output and the total arterial oxygen content. Oxygen delivery and consumption were indexed for body weight. The oxygen extraction ratio was calculated as

$$\frac{\text{Total } O_2 \text{ Consumption}}{\text{Total } O_2 \text{ Delivery}} \times 100$$

and expressed as a percentage.

Arterial and mixed venous blood gases were measured by standard electrodes [IL-813]. Whole blood and plasma hemoglobin levels were determined on the IL-282 Co-oximeter and corrected for methemoglobin.[6] Oxygen carried by whole blood hemoglobin was measured by the IL-282 Co-oximeter. The physically dissolved oxygen in the aqueous phase was calculated from the PO_2 and the Bunsen solubility coefficient.[7] Hematocrits were determined by the microhematocrit method.

Statistics

Statistical significance between groups at baseline and after total exchange was ascertained by an unpaired Stu-

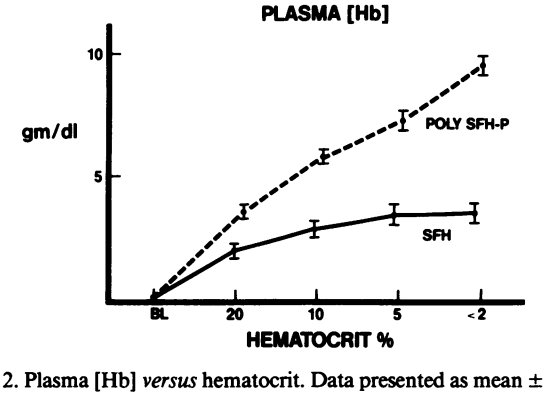

FIG. 2. Plasma [Hb] *versus* hematocrit. Data presented as mean ± SEM.

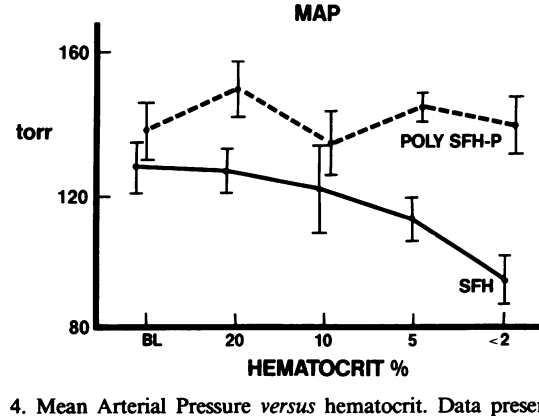

FIG. 4. Mean Arterial Pressure *versus* hematocrit. Data presented as mean ± SEM.

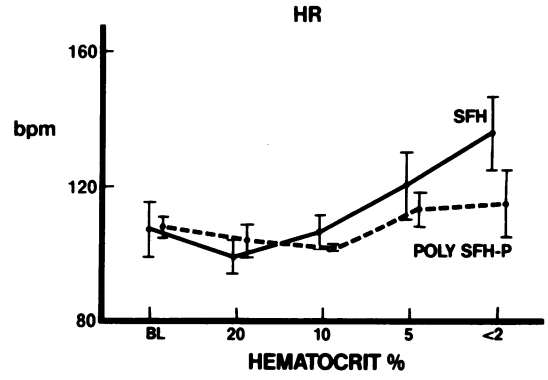

FIG. 5. Heart rate *versus* hematocrit. Data presented as mean ± SEM.

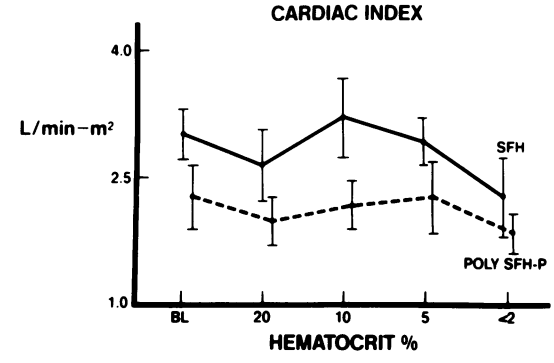

FIG. 7. Cardiac index *versus* hematocrit. Data presented as mean ± SEM.

dent's t test. Differences within each group between baseline and total exchange was determined by a paired t test. All results are expressed as mean ± standard error (SE). The data were compared to those previously reported with our SFH.[1-3] The [Hb] of the unpolymerized SFH was 6.6 ± 0.2 g/dL and the P_{50} was 18.3 ± 2.4 torr.

Results

All animals receiving Poly SFH-P survived the total-exchange transfusion, as did the previous animals receiving SFH. The final hematocrit was 0.8 ± 0.4% (mean ± SE). Figures 1 and 2 show the whole-blood and plasma [Hb] values during the exhange. At the end of the exchange, the group receiving poly SFH-P had significantly higher ($p < 0.001$) whole-blood and plasma [Hb] values than did the SFH group. The final plasma [Hb] at Hct less than 2% for this group is 9.7 ± 0.4 g/dL. Figure 3 shows total oxygen delivery, which fell ($p < 0.01$) in both groups. There were no differences between groups during the study.

Figures 4 to 7 show the MAP, HR, $\dot{V}O_2$, and cardiac index during the study. The animals receiving Poly SFH-P showed no change from their baseline values in any of these variables throughout the exchange. In contrast animals receiving SFH showed a significant decrease at Hct less than 2% from baseline values in MAP ($p < 0.025$), $\dot{V}O_2$ ($p < 0.025$), and cardiac index ($p < 0.05$), and a significant rise in HR ($p < 0.05$).

The mixed venous PO_2 ($P\bar{v}O_2$) data are shown in Figure 8, and the oxygen extraction ratio is shown in Figure 9. There was a significant decrease in $P\bar{v}O_2$ and rise in extraction ratio ($p < 0.025$) at the end of the exchange in both groups. Both were better maintained in the Poly SFH-P group, although the differences were not significant. Finally there were no significant changes in arterial pH, pCO_2, or base excess between the start and end of the exchange transfusion.

Discussion

The data in this study describe the ability of polymerized pyridoxylated hemoglobin solution to support hemodynamics and oxygen transport in a primate model. The stimulus to prepare the current Poly SFH-P was a desire to obtain a product with a normal [Hb], P_{50}, and COP.[4,8] Initial reports with SFH were with the stripped or unmodified tetrameric form of the hemoglobin mol-

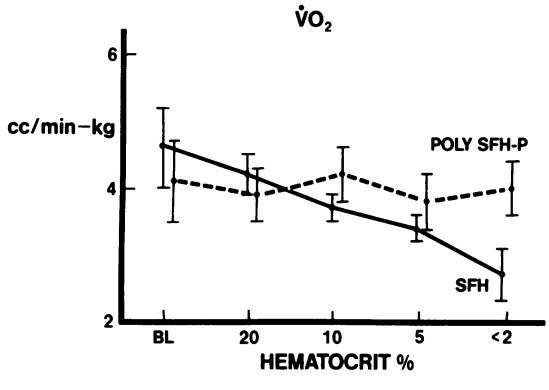

FIG. 6. Total O_2 consumption *versus* hematocrit. Data presented as mean ± SEM.

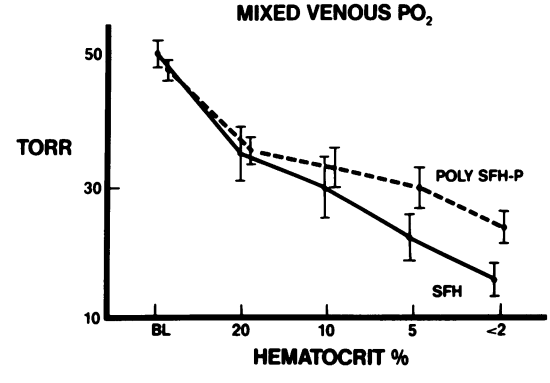

FIG. 8. Mixed venous PO_2 *versus* hematocrit. Data presented as mean ± SEM.

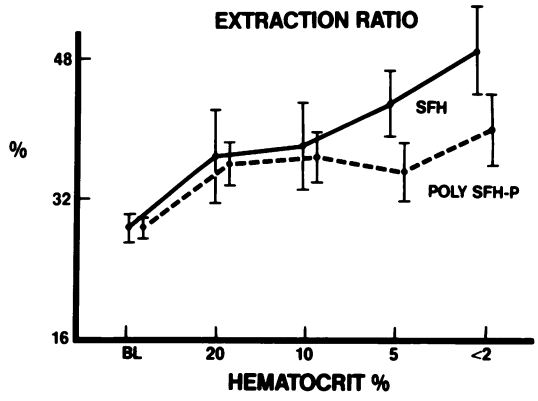

FIG. 9. O_2 Extraction ratio *versus* hematocrit. Data presented as mean ± SEM.

ecule (Table 1).[1-3] The product has a [Hb] of 6 to 8 g/dL and a P_{50} of 12 to 14 torr. This low [Hb] is required to maintain an iso-oncotic solution. The low P_{50} is due to the loss of 2,3-DPG from the solution. The plasma half-life ($T_{½}$) of this product is only 2 to 4 hours. Despite these limitations, this unmodified SFH will support life in baboons at zero hematocrit, documenting effective oxyxgen transport in the absence of red cells.[1] However a significant decrease in oxygen consumption, mean arterial pressure, and cardiac output, and an increase in HR occur at zero hematocrit.[5] It is possible that these changes reflect the final plasma [Hb] of only 4 g/dL, which is due to the low [Hb] and short $T_{½}$ of the infused SFH.

Poly SFH-P represents an improvement over previous red cell substitutes. Poly SFH-P has a normal oxygen-carrying capacity and an improved intravascular persistence.[4,5,8] After a 900-mL partial exchange, the plasma half-life ($T_{½}$) of the SFH was 5.9 ± 0.7 hours. In contrast, the $T_{½}$ of the poly SFH-P was 46.2 ± 4.3 hours.[4] The process begins with the preparation of SFH.[1] The next step is to use pyridoxyl phosphate to increase the P_{50} of the unmodified tetramer from 12 to 14 torr to 20 to 24 torr.[9] This pyridoxylated SFH (SFH-P) is then prepared with a [Hb] equal to 14 g/dL. Polymerization of this hyperoncotic product reduces the total number of molecules and increases the average molecular weight. There is no loss of hemoglobin mass. Because the COP is proportional to the number of particles in solution, the COP is lowered to an iso-oncotic range, as the polymerization proceeds. Poly SFH-P is therefore the first product to achieve the goal of both a normal [Hb] and COP. The P_{50} of this product is 16 to 20 torr. Concurrently there is a significant increase in the $T_{½}$ to 40 to 48 hours due to the larger size of the hemoglobin molecules (Table 2).

TABLE 1. *Properties of SFH*

Characteristic	Range of Values
[Hb]	6–8 g/dL
P_{50}	12–14 torr
COP	20–25 torr
$T_{1/2}$	2–4 hours

TABLE 2. *Properties of Poly SFH-P*

Characteristic	Range of Values
[Hb]	12–14 g/dL
P_{50}	16–20 torr
COP	20–25 torr
$T_{1/2}$	40–48 hours

The principal requirements of any oxygen carrier are effective oxygen loading (delivery) and unloading (consumption), and support of hemodynamics. Many reports have raised concerns about the ability of a low P_{50} hemoglobin solution to effectively unload oxygen in the clinical setting in the presence of red cells with their higher P_{50} of 26 torr.[2,10,11] Although the P_{50} of Poly SFH-P is 16 to 20 torr, this value is still considerably lower than the normal red cell P_{50} of 26 torr. We have addressed this specific issue in a previous study. Using quantitative assessments, we have shown that Poly SFH-P significantly contributes to both total oxygen delivery and consumption in the presence of red cells in a clinically relevant range of hematocrits.[12] Poly SFH-P is thus an effective oxygen carrier and, in comparison with SFH, makes greater contributions to both oxygen loading and unloading.

The objective of this study was to describe the ability of Poly SFH-P to support hemodynamics and oxygen transport in the absence of circulating red cells. The animals acutely survive exchange transfusion with Poly SFH-P to hematocrits less than 2%. This survival at an otherwise lethal hematocrit level documents effective oxygen transport by Poly SFH-P in the virtual absence of red cells. In addition the data illustrates that at zero hematocrit, Poly SFH-P results in a final plasma hemoglobin concentration of nearly 10 g/dL. We consider this an important result. In the absence of red cells, animals can maintain a nearly normal circulating hemoglobin concentration with the infused Poly SFH-P. At zero hematocrit, the values for $\hat{V}O_2$, MAP, HR, and CI remain at baseline values. There is no physiologic difference in any of these variables between the animals at baseline hematocrit and zero plasma hemoglobin, or a hematocrit of zero and a plasma hemoglobin of 10 g/dL. The modest decrease in total oxygen delivery between baseline hematocrit and a hematocrit of 20% is due to the decrease in the level of whole blood [Hb]. This is most likely secondary to the fluid equilibration that occurs as part of the exchange transfusion. From a hematocrit of 20 to the end of the exchange, the whole-blood hemoglobin and total oxygen delivery do not decrease. As seen in Figure 6, this results in the ability to

maintain oxygen consumption at baseline values throughout the entire study. Consistent with this was the maintenance of baseline pH and base excess throughout the exchange.

Figures 8 and 9 show that $P\bar{v}O_2$ and oxygen extraction ratio show some changes from baseline during the course of the study. $P\bar{v}O_2$ and extraction ratio are both influenced by many variables, including cardiac output, $\mathring{V}O_2$, [Hb], $P\bar{v}O_2$, and P_{50}.[3] Because there is a modest decrease in P_{50} and [Hb] from baseline during the course of the exchange transfusion, we believe that they represent the basis of the changes that are seen in $P\bar{v}O_2$ and extraction ratio. However both parameters are better maintained with Poly SFH-P than with the tetrameric SFH.

This study verifies that Poly SFH-P permits animals to survive total-exchange transfusion. Poly SFH-P achieves a nearly normal total [Hb], and maintains normal hemodynamics and oxygen consumption throughout a total-exchange transfusion. Because the $T_{½}$ is increased to 40 to 48 hours, our hypothesis that Poly SFH-P would support animals more effectively at zero hematocrit appears to be confirmed. Poly SFH-P is an effective oxygen carrier both in the presence and absence of red cells. We believe that with these improvements and these results, that Poly SFH-P is an improved oxygen carrier and offers greater potential than previous products as a clinically useful red cell substitute.

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