Anesthesiology 2006; 105:1153–63

A Multicenter Clinical Study of the Safety and Activity of Maleimide-Polyethylene Glycol–modified Hemoglobin (Hemospan®) in Patients Undergoing Major Orthopedic Surgery

Christina Olofsson, M.D., Ph.D., Torbjörn Ahl, M.D., Ph.D.,† Torsten Johansson, M.D., Ph.D.,‡ Sune Larsson, MD, Ph.D.,§ Per Nellgård, M.D., Ph.D.,‖ Sari Ponzer, M.D., Ph.D.,# Bengt Fagrell, M.D, Ph.D.,** Robert Przybelski, M.D.,†† Peter Keipert, Ph.D.,‡‡ Nancy Winslow, B.S.,§§ Robert M. Winslow, M.D.‖‖*

Background: **Hemospan® (Sangart Inc., San Diego, CA), a polyethylene glycol–modified hemoglobin with unique oxygen transport properties, has successfully completed a phase I trial in healthy volunteers. Because adverse events are expected to increase with age, the authors conducted a phase II safety study of Hemospan in elderly patients undergoing elective hip arthroplasty during spinal anesthesia.**

Methods: **Ninety male and female patients, American Society of Anesthesiologists physical status I–III, aged 50–89 yr, in six Swedish academic hospitals were randomly assigned to receive either 250 or 500 ml Hemospan or Ringer's acetate (30 patients/group) before induction of spinal anesthesia. Safety assessment included vital signs and Holter monitoring from infusion to 24 h, evaluation of laboratory values, and fluid balance. The hypothesis to be tested was that the incidence of adverse events would be no more frequent in patients who received Hemospan compared with standard of care (Ringer's acetate).**

Results: **Three serious adverse events were noted, none of which was deemed related to study treatment. Liver enzymes, amylase, and lipase increased transiently in patients in all three groups. There were no significant differences in electrocardiogram or Holter parameters, but there was a suggestion of more bradycardic events in the treated groups. Hypotension was less frequent in the treated patients compared with controls.**

Conclusions: **In comparison with Ringer's acetate, Hemospan mildly elevates hepatic enzymes and lipase and is associated with less hypotension and more bradycardic events. The absence of a high frequency of serious adverse events suggests that further clinical trials should be undertaken.**

A SUBSTITUTE for erythrocytes for transfusion ("blood substitute") is a long-sought goal.[1] In spite of the worthiness of the goal, however, no product is currently approved for clinical use despite a large amount of research and development by both industry and academia. Many different approaches have been used to produce a safe and effective cell-free oxygen carrier, many of which use hemoglobin in some form. A model compound based on intramolecularly cross-linked human hemoglobin, $\alpha\alpha$-hemoglobin, was developed by the US Army and was found to be intensely vasoactive, resulting in increased systemic and pulmonary resistance and hypertension.[2] Therefore, although administration of $\alpha\alpha$-hemoglobin increased hemoglobin concentration, it did not increase delivery of oxygen to tissue.[3] A commercial version of the same product, HemAssist® (Baxter, Deerefield, IL), failed clinical testing[4,5] and was subsequently abandoned. Other hemoglobin-based products also have been studied in clinical trials, but their properties have been less well documented in the literature.

The physiologic basis for the vasoactivity of hemoglobin is complex. Hemoglobin mutants with reduced rates of nitric oxide binding seem to be less vasoactive.[6] However, nitric oxide binding is not the complete explanation: Different modifications of hemoglobin can result in differing degrees of vasoactivity but with similar rates of nitric oxide binding.[7] One explanation of this apparent contradiction is that excessive delivery of oxygen to vessel walls, particularly arterioles, may also be vasoconstrictive; thus, by designing larger, less diffusive molecules with higher oxygen affinity, vasoconstriction might be reduced.[8] Increased molecular size has been proposed to also reduce the tendency to extravasate and could therefore reduce interference with the diffusion and action of nitric oxide. [9]

We have incorporated many of the results of this more recent research into the design of a new hemoglobin-based product, maleimide-polyethylene glycol–modified human hemoglobin.[10] Formulated as a 4.2-g/dl solution in Ringer's acetate (RA), the product is called Hemospan® (Sangart, Inc., San Diego, CA). Ligand binding studies with Hemospan have shown that the hemoglobin molecule is constrained to the R-state (high oxygen affinity) conformation with a low P50 (approximately 5 mmHg) and cooperativity (Hill parameter approximately 1.2).[11] Nevertheless, Hemospan can support life in rats with no detectable erythrocytes.[12] Microcirculatory studies in the hamster window model have shown direct

* Associate Professor, Anesthesiology, Karolinska University Hospital, Solna, Stockholm, Sweden. † Assistant Professor, Orthopedic Surgery, Danderyd Hospital, Stockholm, Sweden. ‡ Assistant Professor, Orthopedic Surgery, Linköping University Hospital, Linköping, Sweden. § Professor, Orthopedic Surgery, Uppsala University Hospital, Uppsala, Sweden. ‖ Assistant Professor, Anesthesiology, Sahlgrenska University Hospital, Göteborg, Sweden. # Associate Professor, Orthopedic Surgery, Department of Clinical Science and Education, Karolinska Institute at Södersjukhuset, Stockholm, Sweden. ** Professor Emeritus, Medical Consultant, Karolinska University Hospital, Solna, Stockholm, Sweden. †† Associate Professor, Medical Consultant, University of Wisconsin, Madison, Wisconsin. ‡‡ Vice President, §§ Clinical Trials Administrator, Clinical Research, Sangart, Inc., San Diego, California. ‖‖ Chairman, CEO and President, Sangart, Inc., San Diego, California. Adjunct Professor of Bioengineering, University of California, San Diego, California.

Received from Karolinska Institute at Södersjukhuset and Karolinska University Hospital, Solna, Stockholm, Sweden. Submitted for publication April 12, 2006. Accepted for publication July 19, 2006. All aspects of the study were supported solely by Sangart, Inc., San Diego, California. Dr. Winslow, Nancy Winslow, and Dr. Keipert are Sangart employees. Drs. Fagrell and Przybelski are consultants to Sangart, Inc. Presented as an abstract at the 35th Critical Care Congress, Society of Critical Care Medicine, San Francisco, California, January 21–25, 2006.

Address correspondence to Dr. Winslow: Sangart, Inc., 6175 Lusk Boulevard, San Diego, California 92121. rwinslow@sangart.com.

improvement in tissue capillary oxygen transfer at extreme hemodilution.[13] Studies in swine did not demonstrate increased pulmonary or systemic vascular resistance,[14] and animals resuscitated with Hemospan after uncontrolled hemorrhage had significantly improved 24-h survival compared with conventional resuscitation with crystalloid or pentastarch.[15] A phase I study in healthy volunteers did not show the hypertension or gastrointestinal side effects that have characterized previous hemoglobin-based solutions.[16]

Based on these clinical and preclinical studies, it was thought that a larger safety study of Hemospan administration to patients undergoing major orthopedic surgery procedures with spinal anesthesia would be justified. These patients frequently become hypotensive because of sympatholysis[17]; therefore, maintenance of hemodynamic stability might be of benefit because Hemospan can maintain blood pressure[15] and tissue perfusion.[13] A double-blind, randomized infusion of Hemospan or RA in elective hip replacement patients before induction of spinal anesthesia was undertaken in six Swedish academic hospitals. Participation was limited to patients aged older than 50 yr, because cardiac events increase with increasing age in this patient group.[18]

Materials and Methods

The clinical study was performed according to the principles stated in the Declaration of Helsinki and Good Clinical Practices as described by the International Conference on Harmonisation. The protocol and consent forms were approved by the centralized institutional review (ethics) committee of the Karolinska University Hospital, Stockholm, and the study was approved by the Swedish Medical Products Agency. The design of the case report forms, handling of data, statistical analysis, and report writing were by Quintiles AB, Uppsala, Sweden. The study was performed at six Swedish academic hospitals, and the Principal Investigator (C.O.) was a member of the faculty and staff of the Karolinska Institute and Hospital. A Co-Principal Investigator at each site was responsible for the conduct of the study at that site.

Study Population

Within 2 weeks of scheduled surgery, potential candidates for the study were identified by an investigator, who conducted an initial interview, performed screening history and physical examination, requested various laboratory tests, and obtained informed consent if all criteria were met. The study included adult males or surgically sterile or postmenopausal females, American Society of Anesthesiologists physical status I–III, aged 50 yr or older, undergoing spinal anesthesia for primary hip arthroplasty, although some acute hip fractures were included as well as a few revision arthroplasties (table 1).

Table 1. Number of Patients in Each Study Group by Type of Surgery

	A (250 ml Hemospan)	B (500 ml Hemospan)	C (RA)
All patients	28	31	31
Primary arthroplasty	22	24	28
Revision arthroplasty	5	5	2
Other procedures*	1	2	1

* Group A: 405 (internal fixation of fracture). Group B: 403 (internal fixation of fracture), 625 (nail extraction). Group C: 615 (nail extraction).

RA = Ringer's acetate.

Patients were not included if they had any acute or chronic condition that would limit their ability to complete the study or jeopardize their safety in the judgment of the investigator. Specifically, patients were excluded from the study if they had clinical manifestations of uncontrolled metabolic, cardiovascular, or psychiatric disorders, if the screening blood pressure was greater than 180 mmHg (systolic) or 105 mmHg (diastolic), or if they had a history of myocardial infarction or stroke within the preceding 6 months. Patients were also excluded if they had a known drug or alcohol dependency, hemoglobinopathy, or coagulopathy; if they were on a chronic anticoagulation regimen (other than daily aspirin); or if they had participated in another clinical trial during the preceding 30 days. The prospective patients received both written and verbal information on the study and were given an opportunity to ask questions. Patients were informed that they were free to withdraw from the study at any time without any prejudice to further medical care.

The goal for the study was to enroll a total of 90 patients, 30 in each arm. This number was chosen arbitrarily, because adverse events (AEs) in our previous clinical trials were too few to use as a rational power calculation. Ninety-eight patients were screened for the study. Of these, 94 met the criteria for inclusion and were randomized. Four patients withdrew from the study before dosing. One was randomized before informed consent was obtained, and this patient withdrew from the study. Surgery for a second patient was cancelled as the indication for surgery was no longer considered to be valid. In the third instance, the bottle of test article was broken when the plastic hanger containing it failed. A fourth patient withdrew before dosing when it was discovered that he did not fulfill the age criterion for inclusion. One patient died 36 h after dosing from massive food aspiration related to incarcerated intestinal hernia. Eighty-nine patients completed the protocol.

Randomization and Blinding

The treatment assigned to each patient number was determined according to a computer-generated, sequentially numbered randomization code list produced by

Quintiles AB. The treatment groups were as follows: A, 250 ml Hemospan; B, 500 ml Hemospan; C, RA. The randomization was done in permuted blocks and stratified by center. One site-specific randomization list was delivered to each study site pharmacy. Upon notification/confirmation of patient randomization, the pharmacy staff prepared the randomized treatment.

Every effort was made to ensure that the patient and staff could not see the solution for infusion and remained blinded throughout the study. The Hemospan/control solution was shrouded when brought to the patient by an unblinded nurse, who personally infused the product through a shrouded infusion line in a way that precluded the patient, the attending anesthesiologist, and other staff from observing the identity of the infusion. The removal of the Hemospan/control solution was also performed by the unblinded nurse, who was not permitted to participate in any therapeutic decisions or safety or efficacy evaluations.

Patients received premedication only on request before arrival in the operating room. All received midazolam or propofol intravenously for sedation before induction of the spinal block and during surgery. Control or study solution was infused before induction of the spinal block. The subarachnoid injection was performed in the L2–L3 interspace, with the patient in the lateral decubital position. The block dose was 12–15 mg bupivacaine solution, 0.5% (Marcaine® spinal; AstraZeneca, Södertälje, Sweden). A block level of T12 was sought before the start of surgery. The height of the sensory block was tested with cold stimulus (ethanol drop) after 10 min. Hypotension was treated with ephedrine (Efedrin®; Merck NM, Stockholm, Sweden).

Study Medication and Infusion

Hemospan was manufactured and supplied by Sangart, Inc., in 300-ml glass bottles filled to deliver 250 ml of solution. RA was obtained from Fresenius Kabi (Bad Homburg, Germany) and supplied by the allocated pharmacy at each study site. Approximately 24 h before administration, bottles of Hemospan were removed from the freezer (−20°C) and thawed. Infusions of either Hemospan or control were *via* established intravenous lines by a calibrated volumetric infusion pump, at an infusion rate of 15 ml/min. Spinal anesthesia was induced not more than 30 min from the end of infusion, and the infusion did not influence normal care of the patient. A total of 1 l of fluid was infused in all patients; *i.e.*, 250 ml Hemospan + 750 ml RA in group A, 500 ml Hemospan + 500 ml RA in group B, and 1,000 ml RA in group C. Patients receiving either test or control solution also received any additional treatments or drugs deemed necessary. All medical procedures and treatments were according to the site's standard care.

Study Protocol and Measurements

The definitions of serious and severe adverse events were as supplied in guidance documents from the European Agency for the Evaluation of Medicinal Products and the International Conference on Harmonisation.## For each AE, the observer was required to check whether the event was serious, the maximum intensity (mild, moderate, severe), the relation to study drug (definite, probable, possible, unlikely, no connection, impossible to judge), action taken (none, concomitant medication given, investigational drug withdrawn), and outcome (recovered, persistent, still present, death, lost to follow-up).

A 12-lead electrocardiogram and body weight were recorded not more than 12 h before the start of infusion, and preinfusion measurements including vital signs (blood pressure, pulse, respiratory rate, and pulse oximetry) and laboratory tests were made within 1 h before the start of infusion.

Holter electrocardiogram monitoring began not less than 1 h before the infusion and continued for the next 24 h. Vital signs (blood pressure, pulse, respiratory rate, and pulse oximetry) were recorded every 15 min during infusion and then every 15 min during anesthesia. Blood pressure was recorded every 5 min from start of anesthesia to the end of surgery. After surgery, vital signs were measured hourly up to 6 h and then 12 h after the start of infusion. A hypotensive episode was defined as a systolic blood pressure less than 90 mmHg or 75% of the preinfusion value. Each registration of a systolic blood pressure fulfilling the definition was counted as one hypotensive episode. All Holter recordings were evaluated at a central site by a blinded cardiologist who had no other connection to the study.

Laboratory status, including clinical chemistry, hematology, coagulation testing, and urinalysis, was performed before anesthesia induction and 6 h after the start of infusion. Blood chemistry measurements were made according to local hospital methods in the clinical laboratories. No corrections were made for possible interference of Hemospan in the plasma. Urine volume, oral and intravenous fluid type and amount, and blood loss and transfusions were recorded in each 24-h period of the study. Preinfusion, postinfusion, 6-h postinfusion, and daily plasma samples were frozen for later measurement of plasma hemoglobin. Analysis of plasma hemoglobin concentration was measured as the cyanomet derivative, and the percent methemoglobin was measured by the method of Evelyn and Malloy.[19]

Statistics

Data were compiled and recorded in a central database at Quintiles, using Good Laboratory Practices, including

ICH Topic E2A, Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, CPMP/ICH/377, 1995. Available at: www.emea.eu.int./pdfs/human/ich/037795en.pdf. Accessed August 7, 2006.

Table 2. Demographic Characteristics of the Primary Arthroplasty Patients

	A (250 ml Hemospan)	B (500 ml Hemospan)	C (RA)
n	22	24	28
Age ± SEM, yr	68 ± 1.9	65 ± 2.0	68.8 ± 1.8
Age range, yr	53–84	53–84	52–89
Weight ± SEM, kg	71.8 ± 2.2	78.1 ± 3.3	77.4 ± 2.8
Female/male	14/8	4/20	15/13
ASA I	7	12	6
ASA II	19	14	19
ASA III	2	5	6
Surgery time ± SEM, min	113 ± 9	136 ± 16	121 ± 10

ASA = American Society of Anesthesiologists [physical status]; RA = Ringer's acetate.

double data entry and primary data source verification. Statistical analysis and graphical presentation were performed using Microsoft Excel® (Redmond, WA), JMP® software (SAS, Cary, NC), or Sigmaplot® (Systat Software, Inc., Richmond, CA) as appropriate. Comparisons of group mean data were analyzed with one-way analysis of variance using the Tukey-Kramer test of the t distribution. This method is appropriate where group sizes are not equal and where no *a priori* differences between group variation are assumed. Differences between means were considered significant if P was less than 0.050.

Results

Dosage Groups and Demographics

Ninety patients were enrolled in the study (table 1), and 1 patient died 36 h after surgery, before completion of the protocol. Therefore, a total of 89 patients completed the study. The bulk of the surgical procedures were elective, primary hip replacements in patients with osteoarthritis. Two sites included fracture patients, and some enrolled revision arthroplasty patients. The numbers of revision arthroplasties in groups A, B, and C were 5, 5 and 2, respectively, and 1 patient underwent internal fixation of a fracture in each of groups A and B. One nail extraction patient was included in each of groups B and C. Therefore, a significantly larger proportion of the patients in the treated groups underwent the more complicated procedures. The primary arthroplasty patients are presented in table 2.

Adverse Events

The AEs are listed in table 3. The listing in table 3 is as determined by the blinded investigators, and the relation to the study drug was determined by them at the time of the event. The total number of AEs was similar in all three treatment groups. There was a suggestion that bradyarrhythmias may be more frequent in groups A and B compared with C, but the number of total incidents was too small for detailed comparison.

Table 3. Most Common Adverse Events

MedDRA System Organ Class Term	A	B	C
Cardiac disorders	7	7	4
Bradyarrhythmias	6	6	3
Ventricular arrhythmias	1	1	1
Gastrointestinal disorders	24	24	17
General disorders and administration site conditions	3	7	8
Infections and infestations	2	2	3
Injury, poisoning, and procedural complications	3	4	2
Investigations	3	3	0
Metabolism and nutrition disorders	0	1	0
Musculoskeletal and connective tissue disorders	3	0	1
Nervous system disorders	0	2	4
Psychiatric disorders	0	0	1
Respiratory, thoracic, and mediastinal disorders	0	0	3
Skin and subcutaneous tissue disorders	2	2	1
Surgical and medical procedures	0	0	1
Vascular disorders	12	11	14
Hypotension	10	7	13
Hypertension	2	4	1
Total	59	63	59

MedDRA = Medical Dictionary for Regulatory Activities.

Four patients had AEs regarded as severe. In one patient (group B), there were four such separate AEs: hypertension, hypotension, dysuria, and aspiration. A second patient (group C) experienced an epileptic seizure in the postoperative period. This patient had a history of epilepsy but had not reported this in the screening evaluation. A third patient (group C) experienced hypotension when cement was applied to the new prosthesis. Ephedrine was given, and the hypotension resolved. A fourth patient (group B) was a 74-yr-old man who experienced 30 multifocal premature ventricular beats during the 24-h monitoring period. Approximately 20 h after the start of surgery, 17 beats of ventricular tachycardia were recorded. No medications were given, and the event resolved.

Serious Adverse Events

There were three serious adverse events (SAEs), none of which was considered to be related to study drug. The first (patient 506) was in an 84-yr-old man scheduled to undergo revision arthroplasty with known coronary artery disease, angina, hypertension, mild renal insufficiency, and history of total hip replacement in 1989, who was enrolled into study group B. The patient reported weakness and worsening abdominal and back pain after surgery, and he was thought to have an acute surgical abdomen but died before further studies could be performed. At autopsy, large amounts of food were found in the larynx, trachea, and bronchi, and a loop of colon was incarcerated in an inguinal hernia. Evidence of an old myocardial infarction was observed but was not considered to be acute or subacute. The cause of death was listed as "massive aspiration."

Table 4. Intraoperative Estimated Blood Loss and Transfusion (Mean ± SEM)

	A (250 ml Hemospan)	B (500 ml Hemospan)	C (RA)
Primary hip arthroplasty, n	22	24	28
Preoperative Hb, g/l	130 ± 2	134 ± 2	127 ± 6
Postoperative Hb, g/l	105 ± 2	104 ± 3	118 ± 6
Estimated blood loss, ml	670 ± 75	910 ± 91	812 ± 98
Transfusion, units	0.30 ± 0.12	0.35 ± 0.22	0.22 ± 0.16
Revision hip arthroplasty, n	5	5	2
Preoperative Hb, g/l	127 ± 6	144 ± 10	138 ± 5
Postoperative Hb, g/l	118 ± 2	113 ± 4	79 ± 6
Estimated blood loss, ml	650 ± 206	3,100 ± 1,397	625 ± 375
Transfusion, units	0.60 ± 0.40	2.50 ± 1.04	1.00 ± 1.00

Hb = hemoglobin; RA = Ringer's acetate.

The second SAE (patient 405) was in an 89-yr-old woman admitted for internal fixation after a trochanteric hip fracture, who was assigned to group A. On the evening of the day of surgery, the patient became hypertensive and was given intravenous clonidine with a subsequent precipitous decrease in systolic blood pressure from more than 200 mmHg to 85 mmHg. She was transiently oliguric, and Holter monitoring showed ST-segment depression during the blood pressure decrease. Troponin I and creatinine kinase–myocardial isoenzyme were elevated on the morning of postoperative day 1. The patient was stable and remained hospitalized, awaiting placement for rehabilitation, but was found dead on postoperative day 9. An autopsy found evidence of an extended posterior–lateral myocardial infarction, signs of an older infarction, and signs of circulatory failure with stasis in organs and lung edema.

The third SAE (patient 313) was in a 77-yr-old woman with a history of angina and hypertension who was admitted for primary arthroplasty and randomized to group B. Electrocardiograms and Holter monitoring did not show significant abnormalities, but creatinine kinase–myocardial isoenzyme and troponin I levels were elevated on postoperative day 2, and a diagnosis of non–ST-elevating myocardial infarction was presumed. The patient was transferred to a cardiology medical ward for 24 h but then recovered uneventfully.

Blood Loss and Transfusion

Blood loss seemed to be greater in group B compared with either group A or C (table 4). However, the greater loss in group B was because of a small number of complex surgical procedures, and the differences in blood loss are not statistically significant. For example, one patient in group B was estimated to have lost 8.4 l of blood in a prolonged and complicated revision procedure. This patient also received 5 units of blood intraoperatively and 2 more units postoperatively. This single patient accounts for most of the trend to a larger amount of blood transfused in group B compared with either groups A or C in both the intraoperative and postoperative periods, but only the cumulative total is significantly greater in B compared with A ($P < 0.020$). Revision arthroplasty procedures tend to be longer, involve more blood loss, and may require more blood transfusion than primary arthroplasties.[20] The amounts of blood loss and blood transfusion in primary arthroplasty procedures were not different among the three study groups.

Hemoglobin concentrations before and 6 h after surgery are also presented in table 4. The decrease in hemoglobin seems to be somewhat greater in primary arthroplasty patients in groups A and B compared with C, but in the revisions, the greatest decrease was in group C. Because fluid administration was not controlled in this study, no conclusions can be drawn with respect to the hemodilution effect of Hemospan.

Fluid Balance

No restrictions were placed on fluid or blood administration in the protocol, and oral fluids were regulated according to the standard procedures of the recovery room. There were no significant differences among the groups in the quantities of oral fluids taken during any of the observation periods.

There were some differences in the use of colloids among the study sites: one used dextran routinely for its anticoagulant effects, and four sites used Voluven (hydroxyethyl starch 130/0.4; Fresenius Kabi AG) intraoperatively as a plasma volume expander. When the dosing volumes and compositions are considered (fig. 1), group A received less crystalloid than groups B and C, group B received more colloid than either groups A or C, and group A received less total intravenous fluid during surgery compared with group C. For the total duration of the study, the only significant difference in administered fluids was that group C received less colloid than either group A or B ($P < 0.005$), as expected. The volumes of Hemospan were included as colloid in these data.

The only significant difference noted in urine output was a greater volume in group C compared with A ($P < 0.0001$) and B ($P < 0.020$) during the 12 h from start of anesthesia (fig. 2). Urine volume during the intraoperative period (0–12 h) was in the order of groups C > B > A. The differences between groups A and B *versus* C were both significant ($P < 0.020$). However, in the remaining periods of the study, outputs were not different among the groups. There were no statistically significant differences in the body weight change over the 7- to 10-day study period among the three study groups.

Vital Signs

Mean arterial blood pressure increased in all groups during the infusion and preparation periods (fig. 3); however, there were no significant differences in mean arterial pressure between either dose group compared with group C, or between the two dose groups (groups

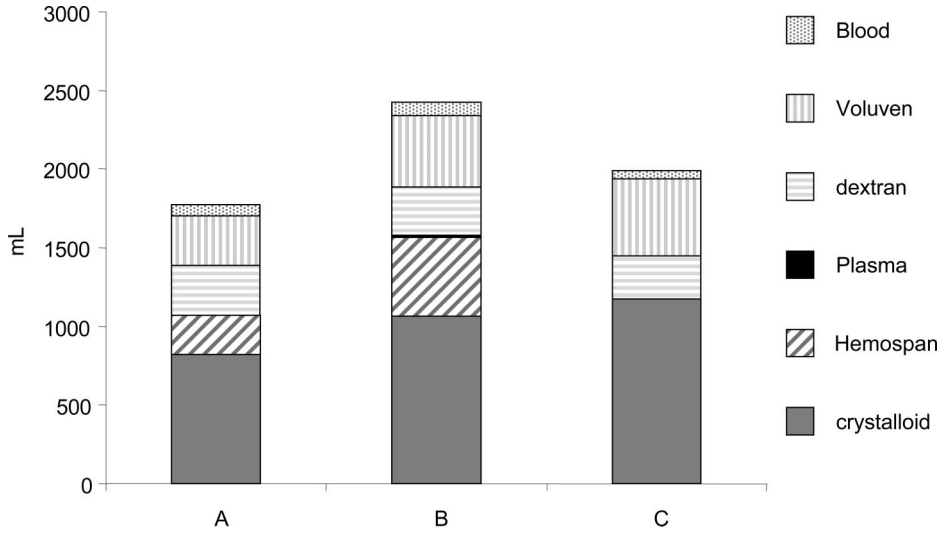

Fig. 1. Intravenous fluids during the surgical period. Group A, 250 ml Hemospan; group B, 500 ml Hemospan; group C, control (Ringer's acetate). The various solutions are identified in the key. The total height of each *bar* corresponds to the total volume of fluid infused starting with the experimental solutions up to the end of surgery.

A and B) during the preanesthesia infusion. After induction of spinal anesthesia, mean arterial pressure decreased somewhat in all three groups. However, mean arterial pressure was significantly higher in group B compared with group C during the postanesthesia and surgical periods. The differences between pressures between groups A and B were not statistically significant. Heart rate (fig. 4) decreased in groups A and B compared with C after infusion, and remained in this order throughout the 6 h of intensive monitoring. Thereafter, this difference disappeared. There were no differences between groups in body temperature, respiration rate, or pulse oximetry at any time in the study.

Hypotensive Episodes and Pharmacologic Intervention

Hypotensive episodes were defined as systolic blood pressure less than 90 mmHg or less than 75% of the baseline (predose) value. Analysis of hypotensive episodes for the primary hip arthroplasty patients by treatment group is presented in table 5. The percentage of patients with any hypotensive episodes was 54% in treatment group A, 39% in treatment group B, and 89% in treatment group C. The extended Mantel-Haenszel test of the effect of treatment on incidence of hypotensive episodes indicated a significant association ($P = 0.0003$). Therefore, the number and incidence of hypotensive

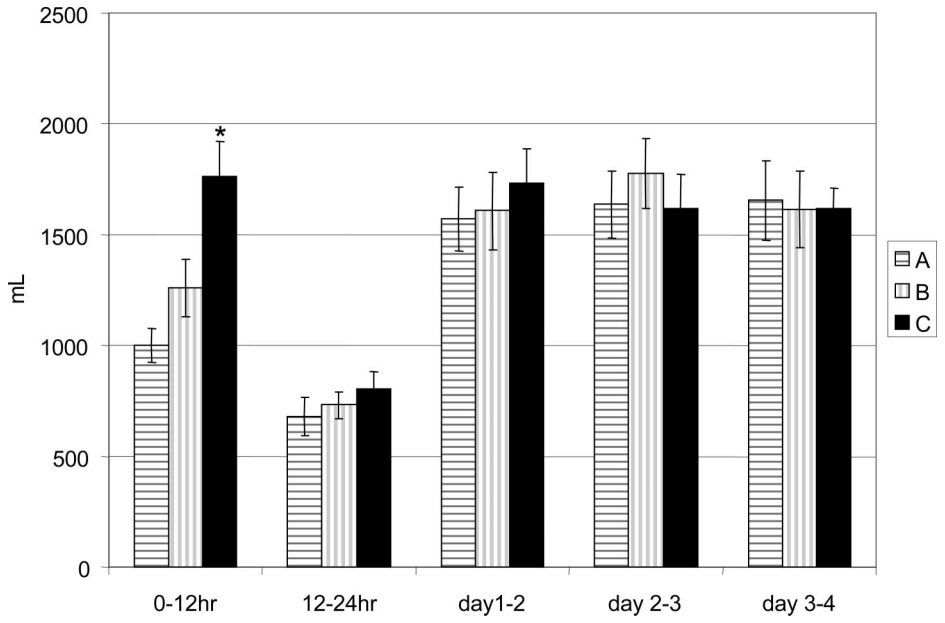

Fig. 2. Urine output. Volumes for each of the three experimental groups are shown. The only significant between-group differences are group C *versus* A in the 0–12 h period ($P < 0.0001$) and group C *versus* B ($P < 0.02$). The differences between groups A and B are not significant at any time period.

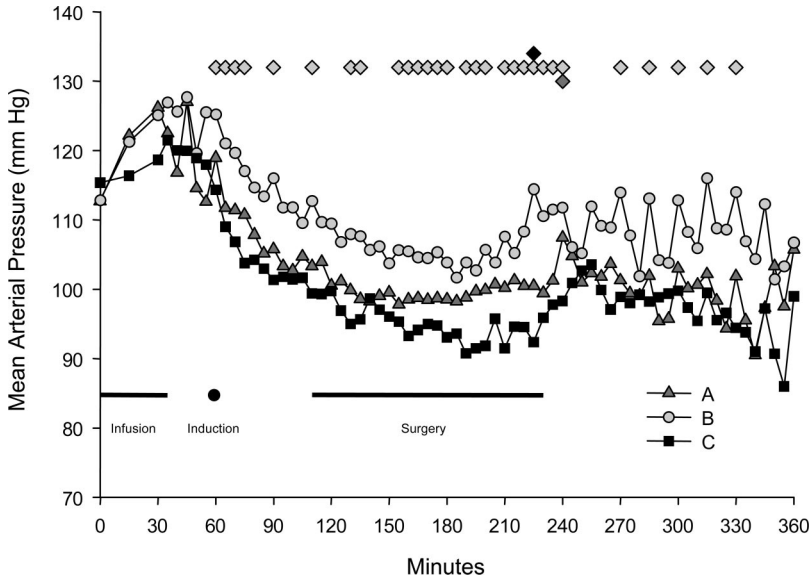

Fig. 3. Mean arterial blood pressure. The infusion periods, induction of anesthesia, and duration of surgery are indicated, and the groups are identified in the key. The symbols at the top of the figure indicate statistical significance ($P < 0.05$) comparing groups A and C (*triangle*), B and C (*circle*), and A and B (*square*).

episodes were both lower for patients treated with Hemospan compared with patients in the control group.

Treatment intervention of hypotensive episodes was not subjected to protocol control. Therefore, some instances of moderate hypotension were treated by infusion of fluids, most often RA. However, in cases of more severe or prolonged hypotension, in which the episode was recorded as an AE, the hypotension was often treated with ephedrine, usually 5 mg intravenously. Fifteen patients were treated with ephedrine for hypotension (two patients were treated with ephedrine for nausea and are not tabulated as pharmacologic interventions). Of this group, five were in group A, three were in group B, and seven were in group C. This corresponds to 21%, 13%, and 25% of the respective groups.

Chemistry and Enzymes

No differences were noted between groups in sodium, potassium, chloride, calcium, glucose, albumin, urea, or creatinine. In general, there were no statistical differences between groups in maximum levels of any of the analytes, with the possible exception of lipase (fig. 5). The difference between mean maximum levels in group B (1.808 μkat/l) *versus* C (0.867 μkat/l) was significant

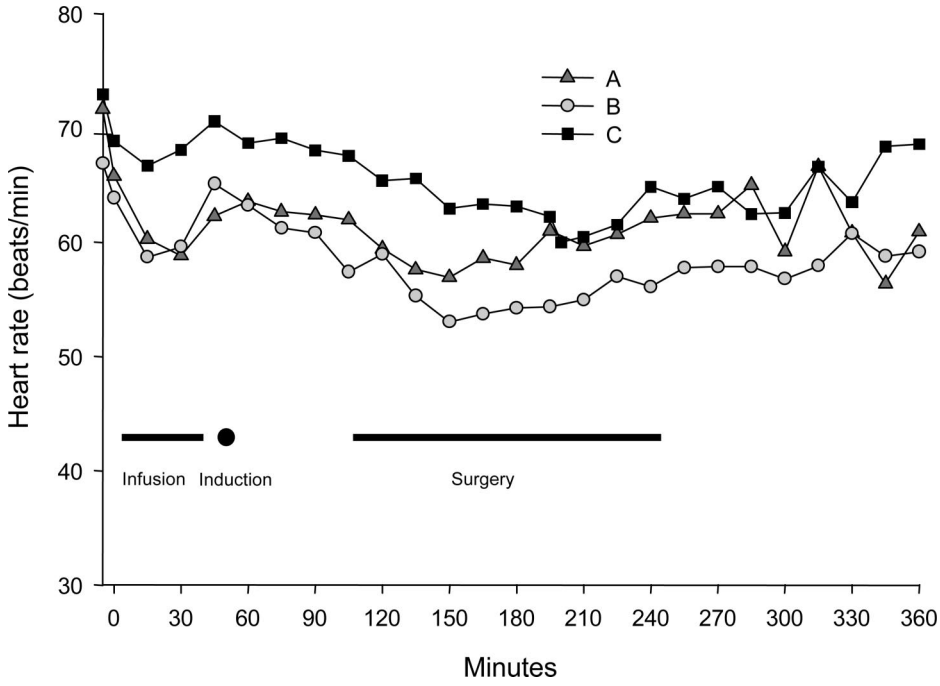

Fig. 4. Mean heart rate. Events (infusion, anesthesia induction, and surgery) and experimental groups are defined as in figure 1. Beginning in infusion and throughout the surgery period, heart rate is significantly lower in the treatment groups, with a slightly greater reduction in group B compared with groups A and C.

Table 5. Hypotensive Episodes* in Primary Arthroplasty Patients

	A (250 ml Hemospan)	B (500 ml Hemospan)	C (RA)
No. of patients	22	24	28
No. with hypotension	13	9	25
% with hypotension	54	39‡	89†
No. of episodes in hypotensive patients, mean ± SEM	10.5 ± 2.1	9.7 ± 3.1	8.8 ± 1.9
No. of patients treated with ephedrine	5	3	7
% treated with ephedrine	21	13	25

* Systolic blood pressure less than 90 mmHg or 75% of baseline. † C *vs.* A or B, $P = 0.0003$. ‡ A *vs.* B, not significant.
RA = Ringer's acetate.

by Student *t* test, but not by Dunnett comparison with controls or by Tukey-Kramer method of the significance in the differences. The greatest elevations in lipase were at 6 h in two group B patients (10.6 and 6.5 μkat/l), one of which was still elevated on the morning of postoperative day 1 but had returned to normal in the other. Elevations in four additional patients were much less and occurred only at a single time point. Because these elevations involved patients from all sites except site 5, they do not seem to be related to any practice that is site specific. Elevated lipase values were also observed in two patients in group C (5.3 and 2.8 μkat/l), one of which was also slightly elevated at the screening evaluation. Amylase followed a similar pattern. In group A, amylase was elevated in one patient on day 1 and in another at preinfusion. In group B, amylase in one patient (who also had elevated lipase on day 1) was elevated on day 1 but was normal on day 2. Amylase in another group B patient was elevated at 6 and 24 h after infusion, time points at which lipase was also mildly elevated.

Aspartate aminotransferase was elevated to abnormal values at 6 h postoperatively in groups A and B but was still within the normal range for group C. Thereafter, aspartate aminotransferase was slightly elevated in all groups. Alanine aminotransferase was within the normal range at all times, but there was a small peak at day 1 in group B because of a single patient (SAE patient 506).

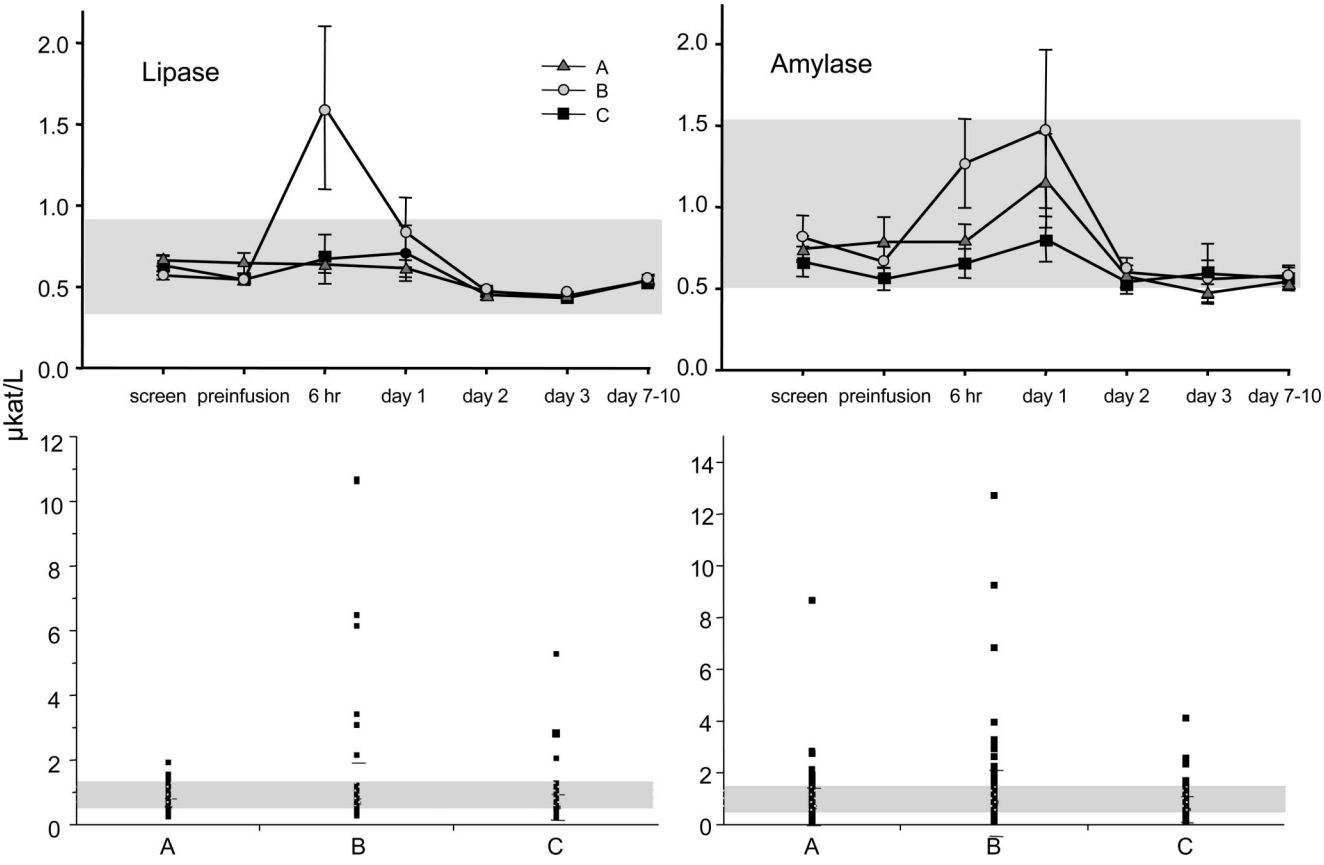

Fig. 5. Serum lipase and amylase. The *shaded areas* indicate the normal range. Lipase, noted to be elevated in two patients in group A, six patients in group B, and five patients in group C, was within normal limits at all subsequent sampling times. There seems to be a trend to rising amylase, but mean values remained within the normal range. As shown in the *lower panels*, individual measurements were significantly elevated but in all cases returned to the normal range.

Table 6. Incidence of Electrocardiogram Abnormalities by Continuous Holter Monitoring during 24 h from Start of Infusion of Test Article in Primary Arthroplasty Patients

	A (250 ml Hemospan)	B (500 ml Hemospan)	C (RA)
n	22	24	28
Maximum frequency, mean ± SEM	122 ± 5	112 ± 3	117 ± 3
Mean frequency, mean ± SEM	64 ± 2	62 ± 2	74 ± 4
Minimum frequency, mean ± SEM	40 ± 2	39 ± 1	48 ± 2
Any premature ventricular contractions	18 (75%)	17 (74%)	23 (82%)
Any ventricular tachycardia	1 (4%)	2 (9%)	1 (4%)
Any periods of couplets	1 (4%)	4 (17%)	2 (7%)
Any pauses > 2 s	3 (12%)	4 (17%)	2 (7%)
Any AV block II	1 (4%)	3 (13%)	3 (11%)
Any AV block III	1 (4%)	2 (9%)	3 (11%)
Any supraventricular beats	24 (100%)	23 (100%)	26 (93%)
Any ST elevation	4 (17%)	1 (4%)	5 (18%)
Any ST depression	1 (4%)	1 (4%)	2 (7%)

AV = atrioventricular; RA = Ringer's acetate.

There was a trend to increased values in all patients at the follow-up visit. Alkaline phosphatase and glutamine transferase similarly remained within normal limits, with a trend upward at follow-up in all treatment groups. Lactate dehydrogenase was slightly elevated in groups A and B after dosing but remained at the upper limit of normal for group C. This elevation could have been due to laboratory interference.[16]

Creatinine kinase and creatinine kinase–myocardial isoenzyme were elevated in most patients in all three treatment groups but returned to normal by the follow-up. Generally, troponin did not increase, except in the patients who had established diagnosis of myocardial infarction (SAE patients 405 and 313).

Electrocardiogram and Holter Monitoring in Primary Arthroplasty Patients

The results from the 24-h Holter monitoring in the primary arthroplasty patients (table 6) showed that the mean heart rate was slightly less in the treatment groups compared with the controls. Premature ventricular contractions were recorded in 75%, 74%, and 82% of the patients in groups A, B, and C, respectively. Ventricular tachycardia and couplets were rare and evenly distributed among the groups. There were no recordings of R on T during the Holter monitoring.

Pauses (> 2 s) were recorded for three patients in group A, four patients in group B, and two patients in group C. Atrioventricular block II and III occurred rarely and equally in all groups. Supraventricular beats were recorded in nearly all patients in the study and were equally distributed across treatments.

ST-segment elevation was recorded in four patients in group A, one patient in group B, and five patients in group C. ST-segment depression was recorded in one patient in group A, one patient in group B, and two patients in group C.

Hematology and Coagulation

Except for day 1, where the leukocyte count was higher in group B compared with groups A and C, the leukocyte counts did not differ. Platelets decreased in all groups to the same extent, presumably due to hemodilution, and were significantly elevated at follow-up in response to surgical blood loss. Reticulocytes increased in all groups, demonstrating appropriate erythropoietic response to the blood loss. No abnormalities were noted in international normalized ratio, fibrinogen, or D-dimer. The activated partial thromboplastin time was prolonged in patients who received Hemospan, but this is attributed to the use of one activator in the automated measuring instrument and is therefore a laboratory artifact (unpublished data provided by Bengt Fagrell, M.D., Ph.D., Karolinska University Hospital, Solna, Stockholm, Sweden, 2004).

Urine

All of the patients had indwelling urinary catheters during the surgical procedures; therefore, evaluation of urinary hemoglobin was of limited value. However, in no case did the urine collected in the postdosing period appear red, although occasional measurements of urine hemoglobin using the dipstick method were positive at 6 h after dosing, decreasing significantly by day 3 and normal at follow-up in groups A and B. There were no apparent differences between either of the treated groups and the control patients. Isolated elevations of β-microglobulin and urine *N*-acetyl-α-D-glucosaminidase were noted but bore no relation to the dosing group. There were no significant between-group differences in albumin, glucose, or acetone. Creatinine clearance, calculated by the method of Jelliffe,[21] was not different between groups after dosing.

Plasma Hemoglobin

Plasma hemoglobin and methemoglobin levels are reported in figure 6. The highest level achieved in group A

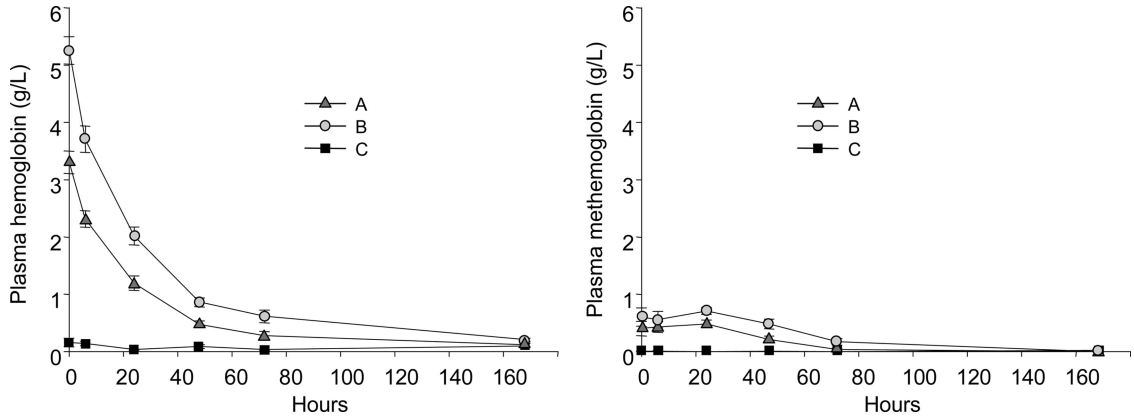

Fig. 6. Plasma hemoglobin and methemoglobin. Values are shown for group A, *triangles*; B, *circles*; and C, *squares*. The disappearance of plasma hemoglobin fits a single exponential expression with a half-life of approximately 20 h. Plasma methemoglobin reaches a maximum of approximately 1 g/l at 20 h and declines thereafter.

(3.31 g/l) is 68% of the highest level achieved in group B (5.32 g/l) after correction for the difference in body weight between the two groups (46.8 and 68.4 mg/kg, respectively) rather than the expected 50%. Therefore, using only these two dose levels, the plasma hemoglobin does not seem to be linear with administered dose. Disappearance of Hemospan from the plasma is approximately exponential in both groups A and B and exhibits a 20-h half-life. The actual half-life of Hemospan in the circulation may be somewhat longer than 20 h, as expected from animal studies,[10] but this can only be resolved by a careful pharmacokinetic study with more frequent samples taken.

Discussion

The primary goal of this study was to evaluate the safety of two dosages (250 and 500 ml) of Hemospan when administered before induction of spinal anesthesia in a group of elderly patients undergoing elective primary hip replacement (arthroplasty). The choice of elderly patients was based on the presumption that the number of AEs would be higher than in younger patients.[17] The secondary goal of the study was to evaluate the potential of Hemospan to increase hemodynamic stability during the surgical procedure, as measured by the incidence of hypotension (defined as systolic blood pressure less than 90 mmHg or 75% of baseline value) and the use of vasopressors. The usefulness of this secondary endpoint is tempered primarily by two considerations. First, hypotension resulting from spinal anesthesia is an expected consequence of the anesthetic agent and may not necessarily be associated with ischemia. Second, the efficacy comparison in this study is between Hemospan, a potent colloid, and a crystalloid (RA). Because the study was designed primarily to evaluate safety of Hemospan, it was thought that the most useful comparison of adverse effects would be with standard of care, rather than a nonconventional therapy (colloid) given before spinal anesthesia.

The study groups are not strictly comparable. First, the number of revision arthroplasties was greater in the treated groups (nine in groups A and B combined) compared with only two in group C. These revision procedures required longer surgery time, involved greater blood loss, and required more transfusions. Second, group A (250 ml) was predominantly female (21 of 28 patients), whereas group B (500 ml) was predominantly male (20 of 31 patients). The mean body weight was greater in group B. For these reasons, some of the analyses (blood loss and transfusion, Holter monitor data, incidence of hypotension) are more meaningful when comparison is restricted to the primary arthroplasty cases, which represent the majority of patients enrolled in this study.

There were three SAEs, none of which were attributed to Hemospan by the clinical team caring for the patients. Common side effects noted in previous clinical trials with other hemoglobin-based products, including chest and abdominal pain and excessive hypertension, were not observed. Mean arterial blood pressure increased in all patients during the infusion period, but the increase in the Hemospan patients was indistinguishable from that in the control patients, suggesting that the increase may have been due primarily to volume expansion.

Other common findings in previous clinical trials of hemoglobin-based oxygen carriers included liver, muscle, and pancreatic enzyme elevations. A phase I study of HemoLink (polymerized human hemoglobin; Hemosol, Inc., Ontario, Canada) noted amylase elevations in 2 of 33 patients and lipase elevations in 12 of 33.[22] That study also noted a dose-dependent increase in lactate dehydrogenase. In a study of 12 high–blood use surgical patients, Schubert *et al.*[23] found significant elevations of amylase, lipase, and creatinine kinase on postoperative day 2 (measurements could not be made on postoperative day 1 because of interference) after administration of up to

1,500 mg/kg HemAssist. A large number of patients in that study (58%) also had jaundice, and 2 of 4 nonurologic surgery patients had gross hemoglobinuria. Although not statistically significant, our data suggest a trend toward elevation of amylase and lipase in patients dosed with Hemospan compared with controls. No elevations in creatinine were noted, and calculated creatinine clearance remained normal in all patients throughout the study period. It is possible that if Hemospan were administered in doses similar to those used in the HemAssist studies, it might also increase pancreatic enzymes; however, the fact that only relatively low doses are physiologically beneficial in animals[24] may result in smaller doses being used in clinical practice.

The number of patients who had abnormalities found in the Holter recordings was relatively small. The most commonly reported abnormalities related to bradyarrhythmias (bradycardia, pauses, heart block). Bradycardia may be a real consequence of Hemospan administration.

The secondary goal of the study was to evaluate possible efficacy. The endpoints chosen were the incidence of hypotensive episodes and the use of vasopressors to treat hypotension, both of which were reduced. Hypotension, when accompanied by significant blood loss, would be a key element in any decision to transfuse in the perioperative period. Blood loss in our patients was approximately as is reported in the literature for similar patients,[25] but there was no correlation between the incidence of hypotension, blood loss, or blood transfusion. It is not clear whether prevention of hypotension would also reduce or eliminate allogeneic blood transfusions. Further clinical trials in a larger number of patients would be required to pursue this question.

The current data provide an indication that Hemospan, administered at either 250 or 500 ml, is well tolerated by patients undergoing elective primary hip arthroplasty, and that the incidence of all AEs is not different in the patients treated with Hemospan compared with those who receive standard of care (RA). Further studies involving more patients would be needed to determine whether Hemospan causes transient increases in liver and pancreatic enzymes and bradycardia. Such studies seem to be justified.

References

1. Winslow R: Historical background, Blood Substitutes. Edited by Winslow R. London, Elsevier, 2006, pp 5–16
2. Hess J, Macdonald V, Brinkley W: Systemic and pulmonary hypertension after resuscitation with cell-free hemoglobin. J Appl Physiol 1993; 74:1769–78
3. Winslow RM: aa-Crosslinked hemoglobin: Was failure predicted by preclinical testing? Vox Sang 2000; 79:1–20
4. Sloan EP, Koenigsberg M, Gens D, Cipolle M, Runge J, Mallory MN, Rodman G Jr: Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock: A randomized controlled efficacy trial. JAMA 1999; 282:1857–64
5. Saxena R, Wijnhoud AD, Carton H, Hacke W, Kaste M, Przybelski RJ, Stern KN, Koudstaal PJ: Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. Stroke 1999; 30:993–6
6. Doherty DH, Doyle MP, Curry SR, Vali RJ, Fattor TJ, Olson JS, Lemon DD: Rate of reaction with nitric oxide determines the hypertensive effect of cell-free hemoglobin. Nat Biotechnol 1998; 16:672–6
7. Rohlfs RJ, Bruner E, Chiu A, Gonzales A, Gonzales ML, Magde D, Magde MDJ, Vandegriff KD, Winslow RM: Arterial blood pressure responses to cell-free hemoglobin solutions and the reaction with nitric oxide. J Biol Chem 1998; 273:12128–34
8. McCarthy MR, Vandegriff KD, Winslow RM: The role of facilitated diffusion in oxygen transport by cell-free hemoglobins: Implications for the design of hemoglobin-based oxygen carriers. Biophys Chem 2001; 92:103–17
9. Matheson B, Kwansa HE, Bucci E, Rebel A, Koehler RC: Vascular response to infusions of a nonextravasating hemoglobin polymer. J Appl Physiol 2002; 93:1479–86
10. Vandegriff K, Malavalli A, Wooldridge J, Lohman J, Winslow R: MP4, a new nonvasoactive PEG-Hb conjugate. Transfusion 2003; 43:509–16
11. Vandegriff K, Bellelli A, Samaja M, Malavalli A, Brunori M, Winslow R: Kinetics of, NO, and O_2 binding to MP4, a polyethylene glycol conjugated hemoglobin. Biochem J 2004; 382:183–9
12. Winslow RM, Lohman J, Malavalli A, Vandegriff KD: Comparison of PEG-modified albumin and hemoglobin in extreme hemodilution in the rat. J Appl Physiol 2004; 97:1527–34
13. Tsai A, Vandegriff K, Intaglietta M, Winslow R: Targeted O_2 delivery by cell-free hemoglobin: A new basis for O2 therapeutics. Am J Physiol 2003; 285:H1411–9
14. Drobin D, Kjellstrom B, Malm E, Malavalli A, Lohman J, Vandegriff K, Young M, Winslow R: Hemodynamic response and oxygen transport in pigs resuscitated with maleimide-polyethylene glycol-modified hemoglobin (MP4). J Appl Physiol 2004; 96:1843–53
15. Young M, Riddez L, Kjellstrom B, Bursell J, Winslow F, Lohman J, Winslow R: MalPEG-hemoglobin (MP4) improves hemodynamics, acid-base status, and survival after uncontrolled hemorrhage in anesthetized swine. Crit Care Med 2005; 33:1794–804
16. Bjorkholm M, Fagrell B, Przybelski R, Winslow N, Young M, Winslow R: A phase I single blind clinical trial of a new oxygen transport agent (MP4), human hemoglobin modified with maleimide-activated polyethylene glycol. Haematologica 2005; 90:505–15
17. Zarin J, Grosvenor D, Schurman D, Goodman S: Efficacy of intraoperative blood collection and reinfusion in revision total hip arthroplasty. J Bone Joint Surg Am 2003; 85-A:2147–51
18. Mantilla C, Horlocker T, Schroeder D, Berry D, Brown D: Frequency of myocardial infarction, pulmonary embolism, deep venous thrombosis, and death following primary hip or knee arthroplasty. ANESTHESIOLOGY 2002; 96:1140–6
19. Evelyn K, Malloy H: Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. JBC 1938; 126:655–62
20. Toy P, Kaplan E, McVay PA, Lee S, Strauss R, Stehling L: Blood loss and replacement in total hip arthroplasty: A multicenter study. Transfusion 1992; 32:63–7
21. Jelliffe RW: Creatinine clearance: Bedside estimate (letter). Ann Intern Med 1973; 79:604–5
22. Carmichael FJ, Ali AC, Campbell JA, Langlois SF, Biro GP, Willan AR, Pierce CH, Greenburg AG: A phase I study of oxidized raffinose cross-linked human hemoglobin. Crit Care Med 2000; 28:2283–92
23. Schubert A, O'Hara J, Przybelski R, Tetzlaff J, Marks K, Mascha E, Novick A: Effect of diaspirin crosslinked hemoglobin (DCLHb HemAssist) during high blood loss surgery on selected indices of organ function. Artif Cells Blood Substit Immobil Biotechnol 2003; 30:259–83
24. Winslow RM, Gonzales A, Gonzales M, Magde M, McCarthy M, Rohlfs RJ, Vandegriff KD: Vascular resistance and the efficacy of red cell substitutes in a rat hemorrhage model. J Appl Physiol 1998; 85:993–1003
25. Olofsson C, Nygards EB, Bjersten AB, Hessling A: Low-dose bupivacaine with sufentanil prevents hypotension after spinal anesthesia for hip repair in elderly patients. Acta Anaesthesiol Scand 2004; 48:1240–4