The Effect of Hemopure® on Coagulation in Clinically Relevant Concentrations

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Hemopure® is a new colloidal blood substitute that may influence coagulation. We designed this study to examine the influence of this product on *in vitro* coagulation of whole blood by using the thrombelastograph® (TEG®). Blood samples from 20 volunteers were obtained. Hemopure® was added to blood samples to obtain 0.5, 1, and 2 g/dL mixtures of Hemopure® in blood. Control consisted of an undiluted sample and, for comparison, two samples diluted with volumes of lactated Ringer's solution (LR) equivalent to the two higher Hemopure® dilutions. TEG® with Hemopure® at a concentration of 2 g/dL showed significantly shorter reaction and clot formation k times and an increased α angle compared with control. LR dilution with equivalent volume to 2 g/dL Hemopure® solution also resulted in significantly shorter reaction and k times, as well as an increased α angle. Coagulation in samples with Hemopure® at concentrations of 0.5 and 1 mg/dL did not vary significantly from control. Maximum amplitude did not vary significantly from control in any samples. The effect of Hemopure® on TEG® measures of coagulation is not significantly different from that of LR at clinically relevant concentrations.

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Hemopure® (hemoglobin glutamer-250 [bovine]), or hemoglobin-based oxygen carrier (HBOC)-201, is a cell-free glutaraldehyde-polymerized bovine hemoglobin that carries oxygen in plasma. It is approved in South Africa for the treatment of adult surgical patients who are acutely anemic and for the purpose of eliminating, reducing, or delaying the need for red blood cell transfusion in these patients (1–3).

Hemopure® contains 13 g/dL polymerized hemoglobin of bovine origin suspended in a modified lactated Ringer's solution (LR) (Table 1). The molecular weight of the product is 250 kd. Other colloidal substances of this order of molecular size significantly interfere with coagulation (4,5). Although there is no clinical evidence that Hemopure® does interfere with coagulation, the effect on coagulation of similar-sized colloid solutions suggests that this possibility should be investigated. Several studies have examined the effect of Hemopure® on measures of coagulation, but these studies have all evaluated the interference of the color change in plasma induced by the HBOC of the measurement techniques of coagulation, rather than the effects that molecule may have on the coagulation process (6–9).

We therefore decided to investigate the effect of this new oxygen-carrying colloid on coagulation. We examined the effects of Hemopure® on coagulation in clinically relevant concentrations as prescribed by the manufacturer, namely, 0.5–2 g/dL. We used the Thrombelastograph® (TEG®) as a method of measuring coagulation, because it detects enhanced as well as impaired coagulation. It is also a measure of whole-blood coagulation, whereas common laboratory methods of coagulation measurement (activated partial thromboplastin time (aPTT) and prothrombin time (PT)) test plasma only and are not as effective in detecting enhanced coagulation. The TEG® is a point-of-care coagulation test that has recently been applied successfully in cardiac surgery and liver transplantation (10,11). The TEG® pattern is divided into component variables. Reaction time (r time) is the interval between the start of the recording and the time at which the amplitude tracing is 2 mm. It reflects the function of the intrinsic clotting pathway. Coagulation time (r + k) is the time required for the amplitude to reach 20 mm and provides information not only on the intrinsic factors, but also on platelets and fibrinogen, which are also represented by the clot-formation rate. Maximum amplitude (MA) is the greatest amplitude achieved on the TEG® and is a measure of clot

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Table 1. Characteristics of HBOC-201

Hemoglobin: bovine derived, glutaraldehyde polymerized
Ion concentration: Na, 145–160 mEq, Cl, 105–120 mEq; K, 3.5–5.5 mEq; Ca, 0.5–1.5 mEq
Appearance: deep purple
Average molecular weight: 250 kd
pH: 7.6–7.9
Osmolarity: 290–310 mOsm/kg
Colloid osmotic pressure: 25 mm Hg
Viscosity (37°C): 1.3 cP
p50: 43 mm Hg
Administration: peripheral or central vein
Storage: room temperature <37°C for 3 yr

HBOC = hemoglobin-based oxygen carrier; p50 = partial pressure of oxygen at which HBOC-201 is 50% saturated with oxygen.

strength and elasticity; it reflects the properties of platelets and fibrinogen, and also factor XIII.

Methods

Ethical approval for this study was obtained from the University of Cape Town committee on human ethics. Each subject gave oral informed consent and was questioned to eliminate any hemostatic problems or the taking of antiplatelet drugs. On the basis of previous studies from this laboratory of the effects of hemodilution on coagulation (12,13), power analysis suggested that 20 subjects, each acting as his or her own control, should be adequate to demonstrate a 10% change in r time, k time, and α angle induced by Hemopure®, with an α level of 0.05 and a predicted power of 80%.

Blood samples from 20 volunteers from the anesthetic department at Groote Schuur hospital were obtained. Blood was obtained from a free-flowing forearm vein into a 10-mL syringe by using a 2-syringe technique in which the first 2 mL of blood obtained was discarded to avoid contamination with tissue thromboplastins released by venipuncture. With a micropipette technique, precisely measured aliquots of this blood sample were placed into six polypropylene tubes with no anticoagulant. Precisely measured volumes of Hemopure® were added to the blood to obtain mixtures containing concentrations of 0.5, 1, and 2 g/dL of Hemopure® in each blood sample. These mixtures contained 41.5 μL of Hemopure® to 968.5 μL of blood, 83 μL of Hemopure® to 917 μL of blood, and 166 μL of Hemopure® to 834 μL of blood, respectively. Controls consisted of an undiluted aliquot, one dilution with LR of a volume equivalent to the 1g/dL Hemopure® solution, and a second dilution of blood with the volume of LR equivalent to that in the 2 mg/dL Hemopure®, prepared in an identical fashion to the Hemopure® dilutions. These resulted in 9.1% and 19.9% dilution of whole blood by LR. LR was chosen as the control diluent because the solution that makes up Hemopure® (as mentioned above) closely resembles this fluid preparation.

Coagulation of each sample was analyzed with the TEG® 3000 device (Haemoscope Corp., Niles, IL). Specimens from each sample tube (360 μL) were added to the TEG® cuvettes in random order and in random cuvette sequence to minimize the risks of systematic errors. All dilutions and transfers of samples to the TEG® cuvettes were completed within 4 min of blood sampling. The TEG® traces of the normal blood sample and the diluted samples were recorded for 1 h, and the r time, k time, α angle, and MA were measured and recorded for each sample.

TEG® data were captured directly by using the manufacturer's software and were then transferred to an Excel spreadsheet. These data were then analyzed with Statistica Version 6 (StatSoft, Tulsa, OK). Differences between the various dilutions and control samples were analyzed with a one-way analysis of variance, and Fisher's least significant difference test was used to identify significantly different groups. Raw data and differences from the control, undiluted sample for each subject were analyzed.

Results

The mean values from the TEG® data are shown in Table 2, and the differences between the undiluted control and the diluted samples are reflected in the figures. With a Hemopure® concentration of 2 g/dL, r and k times were significantly shortened and the α angle was increased. TEG® with LR dilution with equivalent volume to the 2 g/dL Hemopure® solution also showed significantly shorter r and k times, as well as an increased α angle. Coagulation in samples with Hemopure® at concentrations of 0.5 and 1 mg/dL did not vary significantly from control. However, the k time with an LR equivalent of 1 mg/dL ($\pm$10% hemodilution) was significantly different from that with the undiluted sample.

Similar results were obtained when the differences from the undiluted controls were analyzed (Figs. 1–4). However, both k time and α angle were significantly different from control values at 10% hemodilution with LR, whereas there were no significant differences at this dilution with Hemopure®.

MA did not vary significantly from the undiluted control in any of the tested samples. When the differences from undiluted control were analyzed, the change in MA resulting from hemodilution with Hemopure® at a concentration of 2 g/dL was significantly different from that resulting from dilution with an equivalent volume of LR. However, there were no differences in MA from control values for any of the dilutions.

Table 2. Thrombelastograph Data

	r Time (min)		k Time (min)		α Angle (°)		MA (mm)	
Code	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	15.8	2.1	7.1	1.6	30.5	5.2	51.1	4.9
H1	16.1	2.2	6.8	2.1	30.6	6.7	48.5	7.9
H2	15.5	2.8	6.3	1.8	32.3	7.7	47.6	7.1
H3	13.2*	2.5	5.4*	2.1	37.4*	10.4	47.5	8.8
r2	14.3	2.2	5.8*	1.5	35.2	7.1	51.7	7.4
r3	11.9*	2.2	4.5*	1.3	41.4*	8.0	52.5	9.0

H1 = Hemopure® 0.5 g/dL; H2 = Hemopure® 1 g/dL; H3 = Hemopure® 2 g/dL; r2 = lactated Ringer's solution dilution 9.1%; r3 = lactated Ringer's solution dilution 19.9%; r time = reaction time; MA = maximum amplitude.
* $P < 0.05$ for differences from control. There were no differences between comparable dilutions of either solution.

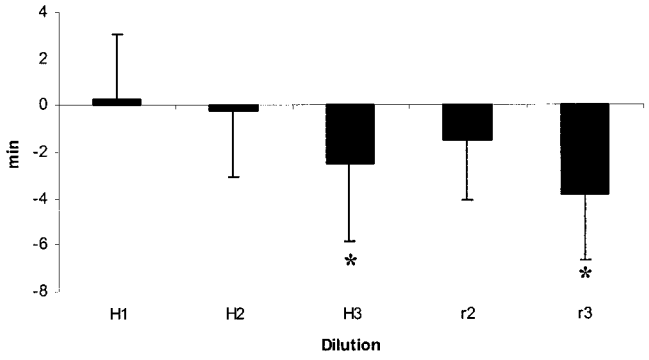

Figure 1. Change of reaction times from control (means and SD) with Hemopure® and lactated Ringer's solutions (LR). H1 = Hemopure® 0.5 g/dL; H2 = Hemopure® 1 g/dL; H3 = Hemopure® 2 g/dL; r2 = LR dilution equal to Hemopure® dilution 1 g/dL; r3 = LR dilution equal to Hemopure® 2 g/dL. *$P < 0.001$ for differences from control.

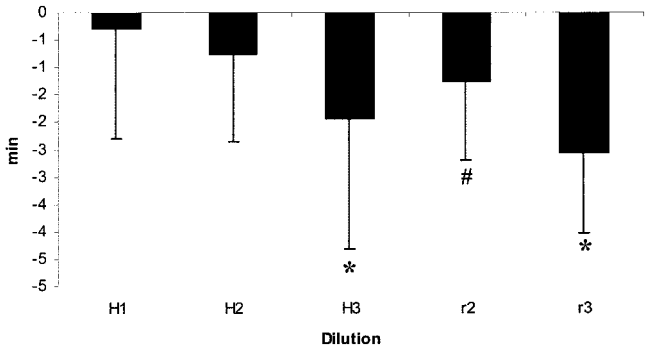

Figure 2. Change in k times from control (means and SD) with Hemopure® and lactated Ringer's solutions (LR). H1 = Hemopure® 0.5 g/dL; H2 = Hemopure® 1 g/dL; H3 = Hemopure® 2 g/dL; r2 = LR dilution equal to Hemopure® dilution 1 g/dL; r3 = LR dilution equal to Hemopure® 2 g/dL. *$P < 0.001$ for differences from control; #$P < 0.05$ for differences from control.

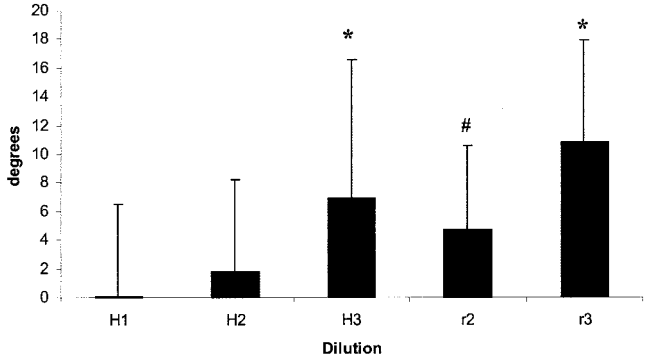

Figure 3. Changes in α angle from control (means and SD) with Hemopure® and lactated Ringer's solutions (LR). H1 = Hemopure® 0.5 g/dL; H2 = Hemopure® 1 g/dL; H3 = Hemopure® 2 g/dL; r2 = LR dilution equal to Hemopure® dilution 1 g/dL; r3 = LR dilution equal to Hemopure® 2 g/dL. *$P < 0.001$ for differences from control; #$P < 0.05$ for differences from control.

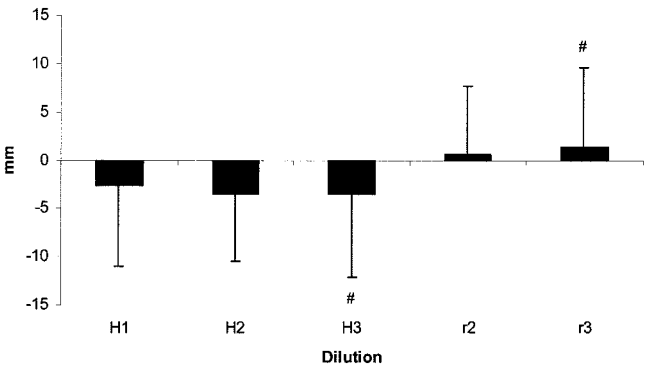

Figure 4. Changes in maximum amplitude (MA) from control (means and SD) with Hemopure® and lactated Ringer's solutions (LR). H1 = Hemopure® 0.5 g/dL; H2 = Hemopure® 1 g/dL; H3 = Hemopure® 2 g/dL; r2 = LR dilution equal to Hemopure® dilution 1 g/dL; r3 = LR dilution equal to Hemopure® 2 g/dL. #$P < 0.05$ for differences between H3 and r3.

Discussion

Hemopure® is a blood substitute with many favorable characteristics that would make it a useful alternative to blood, especially in a setting where fresh blood may not be available. It is a cell-free medium that makes the potential for antigenicity very small. Given its indication for surgically induced anemia, it was important to establish whether it might be expected to interfere with coagulation in clinically relevant concentrations.

Our results revealed a procoagulant effect at a Hemopure® concentration of 2 g/dL. This procoagulant

effect can be ascribed to the effect of hemodilution that was established in previous work on the effect of hemodilution on coagulation. The addition of the various concentrations of Hemopure® tested resulted in an equivalent hemodilution of approximately 5%, 10%, and 20%, and dilutions of 10% and 20% with LR produced the expected enhancement of coagulation that has been widely demonstrated in both laboratory and clinical studies of this level of hemodilution with crystalloid solutions (14,15).

The only statistically significant difference between blood diluted with Hemopure® and that diluted with LR was seen in the change of MA from control values. Because the TEG® does not measure the platelet/endothelial interface, the study does not eliminate the possibility that polymerized hemoglobin molecules may interfere with platelet activity at this level. The small effect of Hemopure® in slightly decreasing MA compared with that of its vehicle, which marginally increased MA, may point to a minor effect on platelet function. Further investigations of the effects of Hemopure® on platelet function are probably justified, but it is unlikely that there will be any clinically significant effect.

One of the difficulties in research related to the effects of IV fluids on coagulation and hemostasis is that *in vitro* hemodilution is different from *in vivo* hemodilution. In the laboratory, there is a straight-line relationship between the volume of diluent added to a given sample and the consequent concentration of the various plasma components. In the clinical scenario, after an initial linear change, hematocrit (HCT) decreases slower than with *in vitro* dilution, and similar changes are likely to occur with platelet concentrations and fibrinogen (16). Reductions of HCT alone may prolong bleeding time in nonsurgical situations (17,18), but a number of studies of normovolemic hemodilution during surgery have failed to demonstrate any association between moderate reductions in HCT ($\pm$30%) and surgical blood loss (19–22). Few studies have addressed quantitative changes in hemostasis during extreme hemodilution, and these studies are frequently confounded by IV fluids used for volume replacement. The overall effect of Hemopure® on hemostasis in a patient who already has marked hemodilution is, therefore, difficult to predict. A mathematical model proposed by Singbartl et al. (16) suggests that, unless hemodilution leads to a reduction in HCT to less than 20% (approximately 60% hemodilution), platelet counts and fibrinogen concentrations should remain within adequate levels to provide acceptable hemostasis. The effect of Hemopure® on coagulation in the presence of extensive surgical blood loss was not determined by this study, but it is unlikely to be significantly different from that of crystalloid solutions such as LR under similar circumstances.

The TEG® suffers from the same limitation as the laboratory-based measures of coagulation in that it assesses the ability of blood to clot in an *ex vivo* situation. It also does not evaluate the platelet/endothelial interaction. Apart from the limitations of the device, the study also examined the effect of Hemopure® on coagulation in normal, undiluted blood. As discussed above, it is possible, but relatively unlikely, that the effects of Hemopure® may be different in a patient with extensive surgical bleeding.

A review of the literature revealed no studies that examined the effects of Hemopure® on coagulation as measured by the TEG®. Several studies have evaluated the effect of HBOC-201 on common laboratory tests of coagulation. Jahr et al. (8) studied the effects of HBOC on coagulation analyzers that perform PT, aPTT, fibrinogen, and antithrombin tests. Mechanical detection methods were less affected by increasing levels than optical detection devices for all the test variables. Ma et al. (23) found that PT, aPTT, and fibrinogen values from the BBL_Fibrometer were unaffected by HBOC <5 g/dL, but optically-based methods exhibited interference at 2 g/dL. Callas et al. (6) tested plasma samples with various concentrations of HBOC-201 up to 6 g/dL and found that tests with a fibrometer for fibrinogen, aPTT, and PT were unaffected by the presence of HBOC. Moreira et al. (9,24) examined the effect of Hemopure® on PT and aPTT in seven coagulation analyzers and found that optical methods of clot detection could not detect clot if the Hemopure® concentration was $\geq$1.3 g/dL but that electromagnetic, light scatter, and mechanical methods were not affected.

Thus, although optically based measures of coagulation may be unreliable in the presence of Hemopure®, mechanically based devices continue to perform reliably, and the product itself appears to have no clinically important effects on coagulation other than those that can be attributed to hemodilution by the LR in which the hemoglobin molecules are suspended.

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