Chapter 34
Effects of Hemoglobin-Based Oxygen Carriers on Blood Coagulation

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34.1 Introduction

There have been at least two major concerns with the use of HBOCs as resuscitation fluids for traumatic hemorrhage: potential coagulopathy and interference with conventional clinical laboratory coagulation measurements. These concerns regarding coagulopathy with HBOCs have long been articulated, as has interference regarding HBOCs and coagulation laboratory equipment not designed for plasma hemoglobins (Jahr et al. 2002).

Possible mechanisms are postulated that include the large molecular size of some HBOCs, as seen with hetastarches and gelatins, nitric oxide scavenging and interference with platelets, and formation of methemoglobin by oxidation of the HBOC and consequent coagulation aberrations (Moallempour et al. 2009). Additional etiologies include decreased plasma calcium concentrations, secondary to volume expansion with non calcium containing solutions.

Hemorrhagic shock and trauma are the leading cause of death in battlefields. Most deaths occur during the first hour from injury, as a result mortality due to trauma and severe hemorrhage may be greatly reduced by early fluid resuscitation. Traumatic hemorrhage associated coagulopathy is partly due to the consumption of both coagulation factors and platelets and partly due to dilutional effect of resuscitative fluids. Hypothermia and acidosis resulting from injury also interfere with clot formation. When a vessel is injured, sequential activation of the coagulation cascade and platelet aggregation results in the formation of hemostatic plug

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at the site of injury to prevent further bleeding, but when bleeding is severe, consumption of coagulation factors followed by infusion of large volume resuscitative fluids devoid of clotting factors and platelets may result in complications such as disseminated intravascular coagulation (DIC), and multi organ failure (Arnaud et al. 2007). Studies suggest that in hemodiluted conditions as such, addition of low doses of recombinant factor VII (rFVIIa) combined with other procoagulants might be of benefit due to its involvement in activation of both intrinsic and extrinsic coagulation mechanisms. Recombinant factor VIIa has been used off-label in combat casualties with severe hemorrhage in an attempt to decrease mortality and the need for blood transfusions (Darlington et al. 2011).

Dilutional coagulopathy with common resuscitative fluids is strongly correlated with the degree of hemodilution regardless of the fluid type used. Studies suggest that the greatest dilutional coagulopathy occurs at hemoglobin less than 6 G/dL, platelets less than 100,000 and fibrinogen concentration at less than 200 mg/dl, threshold indeces that maybe of great value in determining when to transfuse blood or blood products in critically ill patients (Darlington et al. 2011).

Issues specific to blood transfusions, such as transfusion transmitted infections, transfusion reactions, compatibility issues and world wide shortage of donor blood, has led to the development of blood substitutes. Artificial blood substitutes have been the focus of many studies for many decades, in the hope of developing clinically useful products that are universal oxygen carriers with minimum toxicity to manage patients with difficult to control bleeding, especially those situated in difficult to reach areas such as combat zones, mines, etc., where sophisticated medical care, including transfusion of blood or blood products, is not available (Kim and Greenburg 2006; Feola et al. 1988). A number of products have been studied and many of them had to discontinue production due to safety issues including vasoconstriction thought to be related to NO scavenging, GI symptoms, cardiac problems, nephrotoxicity, and death (Johnson and Swiatkowski 2007). Two major issues of the earlier generations of unmodified cell free hemoglobin in HBOCs were their high affinity for oxygen, due to loss of 2,3-diphosphoglycerate (DPG) during purification, and a short intravascular retention time (Kim and Greenburg 2006). To correct these issues led to the production of polymerized (cross-linked) and conjugated hemoglobin/heme based oxygen carriers (Feola et al. 1988). Human gluteraldehyde polymerized hemoglobin (PolyHeme®, Northfield Laboratories, Chicago, IL), HBOC-201 (Hemopure® Biopure Corp/OPK Biotech., Cambridge, MA), and MP4 (Hemospan®, Sangart Corp, San Diego, CA) have been studied in advanced clinical trials to prove safety and clinical benefits to be used as a universal blood substitute in critical care patients. Encapsulated Hbs and perfluorocarbon based oxygen carriers are also in development. HBOCs with built in antioxidants have been developed to reduce oxygen radical mediated damage. Also, with recent advances in recombinant DNA technologies, recombinant HBOCs have been developed and may be modified many ways to achieve desired results (Kim and Greenburg 2006).

A distinct advantage of HBOCs over standard fluids lies in the capability of supplying oxygen to tissues and the lower volume needed to maintain hemostasis

compared to crystalloid solutions in trauma patients with severe hemorrhage with delay in access to hospital care. This may allow ample time for a safe evacuation and transportation of wounded to where definite care is available (Arnaud et al. 2007). Other advantages of red cell substitutes include long shelf life without refrigeration with no type and cross-match requirements. This may result in a better clinical outcome in patients who require immediate transfusion but are situated where blood is not available (e.g., battle fields). If approved, the lower cost, ease of use and universal compatibility of HBOCs will not only change transfusion medicine in USA, but also will greatly affect transfusion medicine all over the world, since 80 % of the world population lives in areas where safe blood or blood products are not available (Kim and Greenburg 2006).

A number of compounds have been studied for coagulation issues, including a number of products no longer available: diaspirin crosslinked hemoglobin, (DCL-Hb/HemAssist®, Baxter), hemoglobin raffimer (Hemolink®, Hemosol), PolyHeme®, (human gluteraldehyde polymerized hemoglobin, Northfield Laboratories). Two products are commercially available from OPK Biotech (formerly Biopure), Cambridge, MA: HBOC-200 (Oxyglobin®, hemoglobin glutamer-200 (bovine), and HBOC-201 (Hemopure®, hemoglobin gluatamer-250 (bovine). A newer product, a zero-linked polymerized hemoglobin, (Oxyvita®, OXYVITA, Inc.), which is currently in preclinical testing, has also been assessed for its potential to cause coagulation abnormalities (Arnaud et al. 2007; Jahr et al. 2012).

This chapter be divided into the following sections: introduction, review of preclinical and clinical coagulation studies with stroma-free hemoglobin (SFH) and other hemoglobin-based oxygen carrier (HBOC) products, coagulation pathways, coagulation testing and commonly used instruments, HBOC effects on platelet function, HBOC and coagulation assessment, potential mechanisms, establishment of need for oxygen delivering resuscitation fluids, potential approaches to development of improved resuscitations fluides, and discussion and summary.

34.2 Review of Preclinical and Clinical Coagulation Studies with Stroma-Free Hemoglobin and Other Hemoglobin-Based Oxygen Carrier (HBOC) Products

Recent generation HBOCs are often evaluated for coagulopathy by TEG as it is a measure of whole blood coagulation. Older studies with stroma-free hemoglobin, and 1st and 2nd generation HBOCs often utilized traditional coagulation tests such as protime (PT), which measures the extrinsic pathway, and activated prothrombin time (aPTT), which measures the intrinsic pathway. Results of older studies are difficult to compare with those with more current HBOC products because they demonstrated varying results, in part due to a large variability in experimental design and less well characterized HBOC preparations used.

Stroma-free hemoglobin (SFH) had a mild prolongation of aPTT (Savitsky et al. 1978). Transfusions of 500 ml of 5–6 % SFH solutions led to a slight decreasing of fibrinogen concentration and activity of clotting factors V, VII, VIII, IX, and X and a prolongation of thrombin time (Uszynski et al. 1976). The prolongation of thrombin time may reflect disturbed polymerization of fibrin. Stroma-free hemoglobin was also studied in vitro and in vivo in rabbits (Browdie and Smith 1975) showing no difference in prologation of PT and aPTT compared to saline. In another study, neither SFH nor polyhemoglobin caused significant changes in PT, aPTT, factor X, fibrinogen, antithrombin III, and antiplasmin levels in rats (Ning and Chang 1990).

Hemoglobin vesicles (Abe et al. 2006), hemoglobin encapsulated in a liposome, were shown to prolong PT and aPTT above the normal range at mixing ratios of 60 %. Complement titers were also examined, and the pegylated hemoglobin vesicle showed less consumption of complement compared to the unpegylated vesicle. It is thought that negatively charged liposomes activate complement and triggers the intrinsic coagulation pathway, but pegylation seemed to decrease complement activation. Polymerized bovine hemoglobin (HBOC-200) had no effect on PT or PTT in ponies (Belgrave et al. 2002). In patients undergoing liver resection, hemodilution with HBOC-201 and 6 % hetastarch did prolong APTT from baseline, but there was no difference between HBOC-201 and 6 % hetastarch (Standl et al. 1998). Pyridoxalated PEG hemoglobin in vitro showed a tendency to prolong thrombin time similar to the saline control (Iwasaki et al. 1986). Albumin-heme in vitro showed no effect on PT and PTT (Huang et al. 2003).

34.3 Coagulation Pathways

The process of preventing blood loss from a vessel or organ of the body is referred to as hemostasis (Lefkowitz 2008). Major constituents of hemostatic system are endothelium, platelets and coagulation factors, which interact and work with each other with the ultimate goal of clot formation and preventing blood loss at the site of an injury, while at the same time controlling clot extension beyond the injury site by antithrombotic mechanisms and finally by clot removal once healing and repair is complete (see Fig. 34.1 for Coagulation Overview (Lefkowitz 2008)).

Vasoconstriction of blood vessels occurs as a first response to blood loss and primary hemostasis takes place by platelet plug formation (Lefkowitz 2008). Von Willebrand Factor secreted from endothelial cells acts as an intercellular glue, binding platelets to one another and to damaged endothelium and forms a plug that temporarily seals the break in the vessel wall (see Table 34.1 for Coagulation Factors (Lefkowitz 2008)).

Secondary hemostasis takes place simultaneously by the formation of a fibrin clot (Lefkowitz 2008). Proteins in the blood plasma, called coagulation factors

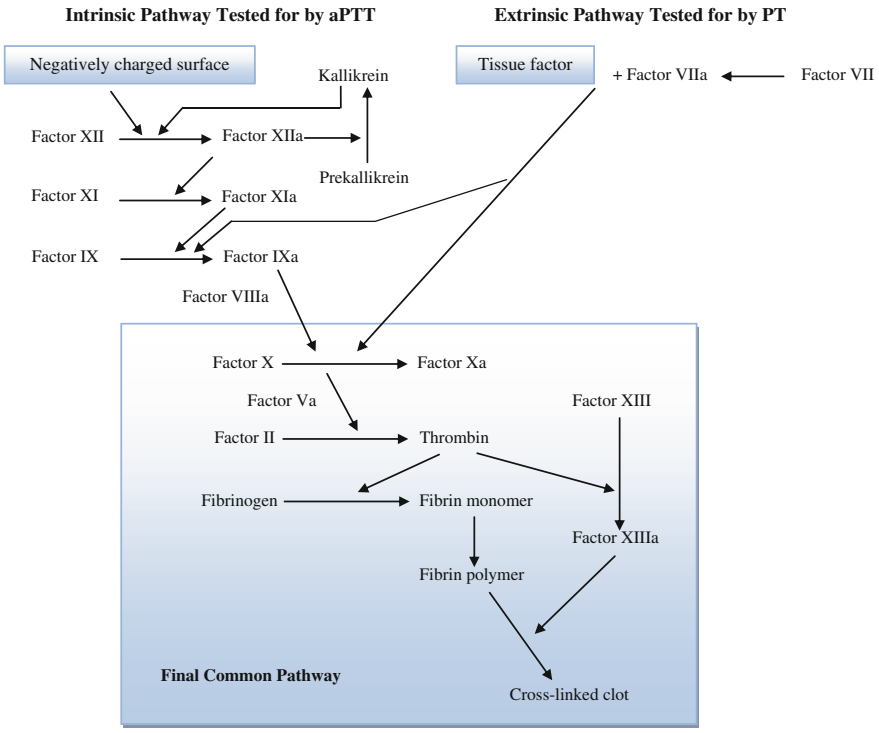

Fig. 34.1 Hemostasis physiology. Modified from Shore-Lesserson L, Committee on Blood Management Monograph on Platelets, American Society of Anesthesiologists, 2012

(Table 34.1), respond in a complex cascade to form fibrin clot, which strengthen the platelet plug. The coagulation cascade has two pathways, the contact activation pathway: Intrinsic Pathway, and the tissue factor pathway: Extrinsic Pathway (see Fig. 34.1). The primary pathway for initiation of coagulation is the extrinsic pathway. Extrinsic pathway mainly generates small amounts of thrombin, the main constituent of the coagulation cascade and thrombin in turn by its feedback activation role, through an intrinsic mechanism generates more thrombin, which in turn forms the definitive clot at the sit of injury.

The intrinsic pathway is initiated by activation of the "contact factors" and can be measured by the activated partial thromboplastin time (aPTT) test (Lefkowitz 2008). The extrinsic pathway is initiated by tissue factor and can be measured by prothrombin [PT] test. The classic coagulation path way is based on in vitro testing and might not be a correct representation of blood clotting in vivo. Various factors are needed for the proper functioning of the cascade such as calcium and phospholipid. Vitamin K is also essential for synthesis of factors II, VII, IX, and X (Lefkowitz 2008).

Table 34.1 Clotting factors: name, description, and function

Name	Description	Function
Fibrinogen (Factor I)	Molecular Weight (MW) = 340,000 daltons (Da); glycoprotein	Adhesive protein that forms the fibrin clot
Prothrombin (Factor II)	MW = 72,000 Da; vitamin K-dependent serine protease	Activated form is main enzyme of coagulation
Tissue factor (Factor III)	MW = 37,000 Da; also known as thromboplastin	Lipoprotein initiator of extrinsic pathway
Calcium ions (Factor IV)	Necessity of Ca^{++} ions for coagulation reactions described in 19th century	Metal cation necessary for coagulation reactions
Factor V (Labile factor)	MW = 330,000 Da	Cofactor for activation of Prothrombin to thrombin
Factor VII (Proconvertin)	MW = 50,000 Da; vitamin K-dependent serine protease	With tissue factor, initiates extrinsic pathway
Factor VIII (Antihemophilic factor)	MW = 330,000 Da	Cofactor for intrinsic activation of factor X
Factor IX (Christmas factor)	MW = 55,000 Da; vitamin K-dependent serine protease	Activated form is enzyme for intrinsic activation of factor X
Factor X (Stuart-Prower factor)	MW = 58,900 Da; vitamin K-dependent serine protease	Activated form is enzyme for final common pathway activation of prothrombin
Factor XI (Plasma thromboplastin antecedent)	MW = 160,000 Da; serine protease	Activated form is intrinsic activator of factor IX
Factor XII (Hageman factor)	MW = 80,000 Da; serine protease	Factor that normally starts aPTT-based intrinsic pathway
Factor XIII (Fibrin stabilizing factor)	MW = 320,000 Da	Transamidase that cross-links fibrin clot
High-molecular-weight kininogen (Fitzgerald, Flaujeac, or William factor)	MW = 110,000 Da; circulates in a complex with factor XI	Cofactor
Prekallikrein (Fletcher factor)	MW = 85,000 Da; serine protease	Activated form that participates at beginning of aPTT-based intrinsic pathway

Modified from Lefkowitz 2008

34.4 Coagulation Testing and Commonly Used Instruments

Two major methods are utilized for detection of coagulation testing: optical or mechanical clot detection. Theoretically, optical may be interfered with if there is a hemolyzed blood specimen or in the case of HBOCs, plasma hemoglobin. Coagulation testing can be delineated by the characteristics of the clotting cascade being assessed (see Table 34.2). Table 34.2 also describes instrument specific methods for detection.

Mechanical instruments may be less susceptible to interference from HBOCs but each instrument/methods must be validated so that accurate clinical interpretation can be made. For example, only the fibrometer was shown to be reliable with HBOC-201 when its concentrations in the sample is greater than 4.8 g/dL (Jahr et al. 2002).

Among the instruments described in Table 34.1, three instruments utilize mechanical clot detection and five instruments use optical clot detection methods for determining prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen (FBG). Antithrombin (AT) values may also be calculated using chromogenic principles on devices that are capable of performing this assay method.

Devices based on thromboelastography or thromboelastometry such as TEG (Haemoscope, Niles, IL (TEG 2005)) and ROTEM (Tem Innovations GmbH, Munich), can monitor the entire process of blood coagulation process using a whole blood sample rather than a plasma sample. Therefore, it can assess qualitative state of hemostasis process that depends on quantity as well as functional status of platelets, plasma clotting factors and fibrinolytic system.

Platelet function can be assessed using thromboelstographic devices. For example, TEG can be used to assess platelet function by utilizing different anticoagulants and differential PLT activators. For example, a standard citrated blood sample with kaolin elicit no effects on PLT function and heparinized blood sample represent blood clot soley based on fibrin as heparin inhibits thrombin and PLT participation. Heparinized blood with ADP (for ADP inhibitor users) or arachidonic acid (for aspirin users) activates PLTs not affected by the drugs (Squires 2002). TEG variables include R (reaction time; time to clot initiation), K and α (rate of clot development), MA (maximum amplitude), and G (maximum clot strength). The measurement R is defined as the latency period between placement of blood in the TEG analyzer and start of clot formation. MA defines the strength of the fibrin/platelet/factor XIII cross-linked aggregate. G is a transformation of MA into units of kdyn/cm2. While both MA and G are reported, G enables results from individual experiments to be more directly compared. The normal range of TEG using sodium citrate and celite/kaolin based on a study of 98 volunteers is reported as: R (2–8 min), K (1–3 min), $\langle(55^{o} - 78^{o})\rangle$, MA (51–69 mm), and G (4.6–10.9 kdyn/cm2) (TEG 2005).

Table 34.2 Coagulation testing

Instrument	Mechanism	Clinical Utility
Thromboelastograph®	Viscoelastic/Thrombin PlateletMapping: ADP, AA	Thrombocytopenia, and anti-GPIIb/IIIa therapy PlateletMapping for aspirin and $P2Y_{12}$ inhibitors
PFA-100®	Closure of a membrane aperture/collagen + ADP, or collagen + epinephrine	vWD, congenital platelet disorder, aspirin therapy
Diagnostica Stago (Parsippany, NJ)	Electromechanical clot detection system; chromogenic capabilities for AT measurements.	PT, aPTT, FBG, and AT
BBL Fibrometer (Becton–Dickinson, Baltimore, MD)	Fibrometer, mechanical clot detection	PT and aPTT
Cardiovascular Diagnostics (Raleigh, NC)	Single-use disposable test cards using the Thrombolytic Assessment System; optical system	PT and aPTT
Dade Behring (Miami, FL)	Optical clot detection; chromogenic capabilities for AT testing	PT, aPTT, FBG, and AT
Hemoliance (Raritan, NJ)	Optical clot detection; chromogenic capabilities for AT testing.	PT, aPTT, FBG, and AT
Organon Teknika Corporation (Durham, NC)	Optical detection system; may correct for lipemia and hemolysis	PT, aPTT, FBG, and AT
Sigma Diagnostics (St. Louis, MO)	Optical clot detection method (Sigma-O) or mechanical clot detection method (Sigma-M)	PT, aPTT, FBG, and AT

Mechanical instruments may be less susceptible to interference from HBOC-201; however, only the fibrometer should be reliably used for PT and aPTT testing with HBOC-201 concentrations greater than 4.8 g/dL (Jahr et al. 2002).

Platelet function may also be measured using the PFA-100 system (Dade Behring, Marburg, Germany). This is a rapid screening tool that is more sensitive in detecting major platelet function defects than the standard bleeding time measurement method (Favaloro 2002). The PFA-100 models high shear stress in vivo conditions by using a capillary apparatus to measure platelet function. Closure time (CT) is defined as the time when blood ceases to flow through the apparatus, implying the formation of a platelet thrombus. Two types of platelet agonists are used to coat the collagen cartridges, epinephrine (cEPI) or adenosine diphosphate (cADP) (Favaloro 2002; Mammen 1998). Nonclosure is defined as CT greater than 300 s. The normal reference ranges were 71 to 118 s for cADP and 94 to 193 s for cEPI (Favaloro 2002). The cEPI cartridge is more sensitive compared to the cADP cartridge, and is especially sensitive to drug-related platelet dysfunction (Newark and Inc 2005; Harrison et al. 2002).

34.5 HBOC Effects on Platelet Function

Platelet function is an important determinant of coagulation. The effect of various HBOCs on platelet function has been studied ex vivo, in animal models, and in humans. In hetastarch solutions, coagulopathy has been shown to correlate with the content of high molecular weight polymers present (Strauss et al. 2002). Studies have suggested that the mechanism involves hetastarch binding to coagulation factors and the surfaces of red blood cells and platelets leading to accelerated clearance of coagulation factors and decreased platelet activation (Huraux et al. 2001; Deusch et al. 2003; Weeks et al. 2008).

As HBOCs are high molecular weight molecules, studies have been done to assess their effect on coagulation. An ex vivo model compared the effects of two HBOCs of different molecular weights, Zero-linked Hb polymer (33 megadaltons) and HBOC-200 (200 kilodaltons), with 6 % hetastarch (670 kilodaltons) on coagulation by thromboelastography (TEG) (Jahr et al. 2008a). TEG analysis of clotting parameters R, K, MA, and G were studied in whole blood diluted by 6 % hetastarch, HBOC-200, or Zero-linked Hb polymer at low (1:11), medium (1:5), high (1:2), and very high (1:1) dilutions. There were no significant differences between Zero-linked Hb polymer and HBOC-200 in any aspect of coagulation at any dilution, despite their difference in molecular weight. This suggests that greater coagulopathy is not inherent with extensive polymerization in HBOC products. Compared to 6 % hetastarch at low and medium dilutions, both Zero-linked Hb polymer and HBOC-200 showed no difference in effect on clot strength (MA, G). However, at high and very high dilutions, Zero-linked Hb polymer and HBOC-200 products exhibited a decrease in clot tensile strength by 33 and 49 %, respectively, compared with 6 % hetastarch. While the mechanism for this difference has not been elucidated, use of Zero-linked Hb polymer at the small volumes recommended by the manufacturer (2–3 ml/kg), which corresponds to a

dilution less than the 1:11 "low dilution", should not increase risk of clinical bleeding.

In an animal model of severe uncontrolled hemorrhage, markers for coagulopathy including platelet function analyzer closure time (PFA-CT) and TEG were compared among groups resuscitated with HBOC-201 or Hextend® (HEX) and a nonresuscitated group (Arnaud and Handrigan 2006). PFA-CT for HBOC-201 and HEX were higher compared to the nonresuscitated group. The peak PFA-CT occurred at 4 h for the HEX group compared to 24 h for the HBOC-201 group. By 48 h, PFA-CT was back to baseline in all groups after blood transfusion. The time to clot formation (TEG-R) was increased in HBOC-201 animals at 24 h compared to HEX animals. Clot strength (TEG-MA) decreased in the HEX group, and to a lesser extent in the HBOC-201 group when compared to the nonresuscitated group. While the nonresuscitated group experienced a post-hemorrhage coagulation pattern that allowed rapid control of bleeding, as evidenced by TEG and PFA-CT parameters as well as prothrombin time, this group also suffered a higher level of mortality. In this study, resuscitation with HBOC-201 did produce a mild dilutional coagulopathy, similar to resuscitation with HEX, when compared to no resuscitation.

An in vivo human study compared platelet function before and after transfusion with either HBOC-201 or packed red blood cell (PRBC) in an adult population undergoing elective orthopedic surgery (Jahr et al. 2010). The PFA-100 system was used to measure closure time. Closure time was measured before transfusion and at several time points after transfusion. In the PRBC group, closure time did not significantly change at any time point. In the HBOC-201 group, closure time was significantly prolonged after transfusion, reaching levels above the upper limit of normal but below the nonclosure time. At Day one after transfusion, closure time for the HBOC-201 group returned to within the normal range. This study echoes the ex vivo and animal studies in illustrating that HBOCs produce mild platelet dysfunction. The clinical significance of these findings, however, is unclear as severe bleeding from thrombocytopenia is rare without concomitant coagulopathy or other defects in the vascular system.

Another mechanism proposed for platelet dysfunction is oxidation of the cell-free hemoglobin in HBOCs to methemoglobin. These oxidative products modify redox-sensitive sites involved in platelet aggregation and activation. In an ex vivo study (Moallempour et al. 2009), previously opened packages of HBOC-200 reached 65 % methemoglobin concentration compared to a 1 % methemoglobin concentration in freshly opened bags. Measuring TEG parameters showed statistically significant impairment in clot propagation and strength in the high methemoglobin samples. This study suggests that HBOC may affect coagulation beyond dilutional effects if methemoglobin is allowed to accumulate.

34.6 HBOC and Coagulation Assessment

Apart from platelet function, coagulopathy is also mediated by changes in the coagulation cascade, commonly measured by PT, aPTT, and AT. The increased hemoglobin concentration in HBOC-201 may interfere with the performance of coagulation analyzers commonly used for PT, aPTT, and AT assays. An in vitro study compared HBOC-201-prepared plasma samples at different concentrations with saline-prepared samples on various coagulation analyzers (Jahr et al. 2002). Interference by HBOC-201 was defined as a difference of >10 % from control. The data showed that at low concentrations of HBOC-201(2.6 G/dL), all instruments (optical and mechanical) reached an accurate and satisfactory result. However, at higher concentrations (3.8 G/dL), optical instruments gave inaccurate results. In these higher concentrations of HBOC-201, the only reliable method was the fibrometer. This demonstrates the importance of choosing the appropriate analyzer when using HBOCs to evaluate for coagulopathy. This will be covered in more detail in the chapter by Smani, et al.

In animal studies, the effect of resuscitative fluids HBOC-201 and a high molecular weight hydroxyethyl starch (Hextend®, Biotime Inc., Berkely, CA) on coagulation was compared to a nonresuscitated group after severe controlled bleeding and a delay to hospital care of 4–24 h. The study showed that the delay of 4–24 h resulted in a lower survival rate for the nonresuscitated group while survival was similar between the HEX and HBOC-201 groups (Arnaud et al. 2007). Coagulopathy was evident in both resuscitated groups due to the dilutional effect of these fluids given in the pre-hospital phase, compared to hemoconcentration in the nonresuscitated group. Transfusions with blood products were given in the hospital phase of the study and showed correction of coagulopathy. Due to the decreased need for blood transfusion in the HBOC-201 group, lab parameters indicating coagulopathy was noted to be higher in this group compared to the other two.

The effect of HBOC-201 (Hemopure®) on different coagulation analyzers has been mostly attributed to interference due to the color change in plasma rather than by the molecule itself. A study designed to further investigate the effect of HBOC-201 on coagulation compared blood samples diluted with either HBOC-201 or lactate ringers (LR) (James 2004). Thromboelastograph (TEG) parameters were analyzed. The advantage of using TEG over common lab methods of measuring coagulation, such as (PT) and (aPTT) is that TEG is a measure of whole blood coagulation not just plasma. At concentrations of 2 g/dl both HBOC-201 and (LR) solutions showed significant decrease in reaction time as well as coagulation times and an increased alpha angle. Therefore, it seems that HBOC-201 and (LR) have similar dilutional effects on coagulation at the comparable clinically relevant concentrations.

34.7 Potential Mechanisms

Many studies have simulated hemodilution during clinical resuscitation of hemorrhagic shock with increasing doses of HBOCs compared to crystalloid or colloid (e.g., hetastarch) fluids. These results suggest a dilutional coagulopathy that seems to be shared by all the above products. For example, when Zero-link Hb polymer, an ultra-high molecular weight HBOC, HBOC-200, 6 % hetastarch, and 0.9 % normal saline were used to dilute whole blood (Jahr et al. 2008a), progressive dilution showed a decrease in TEG parameters for clot strength (MA) and a prolongation of clot kinetics (α). Though the results did not reach statistical significance, crystalloid, colloid, and Zero-link Hb polymer tended to shorten R (clot initiation) at lower dilutions followed by gradual lengthening with higher dilutions. This initial hypercoagulable effect is thought to be mediated by a greater sensitivity of anticoagulants such as antithrombin III to the effects of mild dilution compared with coagulation factors, thrombin, and other factors in the coagulation cascade. This effect has been further described in studies comparing various crystalloids and colloids at increasing dilutions. Up to a 40 % dilution for colloids and 50 % for crystalloids, there was an increase in coagulability (increase in speed of clot formation and clot strength). However, after 70 % dilution, there was evidence of hypocoagulation (Ekseth et al. 2002).

In another study, in vitro dilution with normal saline, polyhemoglobin solution, 5 % bovine albumin at 25 and 50 % dilution did not change TEG parameters r and k significantly (Kim et al. 1992). Stroma-free hemoglobin did show a moderate procoagulant trend at 25 and 50 % dilution with shortened r and k times. A proposed mechanism for this is that stroma-free hemoglobin may be less stable in solution leading to auto-oxidation of hemoglobin to methemoglobin, thereby releasing superoxide radicals that may initiate procoagulant processes such as cell damage and platelet activation. The clot strength parameter MA did decrease in all groups with increasing dilution, suggesting again a mechanism of dilution. Since clot strength does seem to be correlated with hematocrit and platelet counts (Kim et al. 1992), (Table 34.3) a dilutional explanation for this variable is a consistent interpretation.

When low and medium weight hydroxyethyl starches were similarly compared, there was no difference in platelet count or fibrinogen concentration (Jamnicki et al. 2000). However, there was a decrease in factor VIII and von Willebrand

Table 34.3 Potential mechanisms for coagulopathy

Potential mechanisms for coagulopathy with hemoglobin based oxygen carriers
1. Dilutional coagulopathy/hypocalcemia
2. Oxidation to methemoglobin inhibiting platelet aggregation
3. Large molecular weight molecules complexing with von Willebrand factor and speeding its elimination
4. Nitric oxide scavenging

factor (vWF) activity that was more pronounced in the medium weight fluid (HES200) compared to the low weight fluid (HES70). There was also greater increase in aPTT and a more pronounced decrease in MA and α by HES200 compared to HES70. Since these two TEG parameters are influenced by platelet count and function, and there was no difference in platelet count between the HES solutions, the difference may be due to the more pronounced decrease in vWF and its role in platelet linking. The most likely mechanism for the decrease in vWF is enhanced elimination. It is proposed that larger HES molecules may form complexes with the vWF to be cleared from the circulation. Similarly, the greater increase in aPTT may be a reflection of the greater decrease in vWF as well as in factor VIII by the larger molecular weight fluid. As HBOCs are also large molecular weight compounds, there is theoretical concern that similar mechanisms may be present with HBOCs.

Other studies show similar though slightly varying results. HBOC-201, approved in South Africa for treatment of adult surgical patients, was tested in vitro against lactated Ringer's solution and showed no significant difference in TEG parameters at clinically relevant concentrations (James 2004). Whole blood was diluted with HBOC-201 and LR, and at concentration of 2 g/dL, both fluids showed statistically significant shortened R and K times with an increased α angle compared to undiluted control samples. These results suggest a possible procoagulant effect at a HBOC-201 concentration of 2 g/dL, which corresponds to about 20 % hemodilution. In contrast to the above studies, MA did not vary significantly from undiluted control in any of the tested samples. However, the only statistically significant difference between HBOC-201 and LR in this study was a slight decrease in MA by HBOC-201 compared to LR which marginally increased MA. MA reflects the properties of platelets, fibrinogen and factor XIII in contributing to clot strength, so this may reflect a minor effect on platelet function by HBOC-201.

Similarly, in a swine model of hemorrhagic shock (Arnaud et al. 2005), animals were resuscitated with HBOC-201, hydroxyethyl starch or not resuscitated. Parameters for clot kinetics, TEG-R, K, and α were likewise increased while clot strength (MA) was decreased. This study also showed an increase in PFA-CT in the HEX and HBOC group. PFA-CT is increased by low platelet count, qualitative platelet defects, and qualitative/quantitative vWF deficiencies. Notably, PFA-CT is unaffected by coagulation factor deficiencies and hypofibrinogenemia. As the control group did not differ from the HEX and HBOC group in total number of platelets, this result suggests hemodilution of vWF plays a role in the coagulopathic effects of hemodilution with HBOC-201.

Another mechanism posited for abnormal coagulation with HBOC is nitric oxide (NO) scavenging by free hemoglobin resulting in increased hemostasis. NO is synthesized by the vascular endothelium and acts to relax vascular smooth muscle as well as inhibit platelet activation. Hb raffimer or O-raffinose cross-linked hemoglobin (Hemosol, Inc.) decreased bleeding time in anemic and thrombocytopenic rabbits compared to albumin (control) and phenylephrine infusion (to assess vasoconstriction effect comparable to Hb raffimer) (Lee et al. 2000). The in vivo studies in this paper suggest that Hb raffimer did enhance aggregation of

stimulated platelets by abolishing the inhibitory effect of NO. Furthermore, the in vivo study showed a decreased time to carotid artery occlusion by a platelet-rich thrombus in rabbits given Hb raffimer.

However, in an in vitro study with human whole blood, Hb raffimer showed no apparent effect on platelet activation and function. Addition of Hb raffimer to blood samples up to 50 % volume did not cause platelet activation as measured by various markers of platelet activation (CD42b, CD41, PAC-1, CD62, CD63, annexin V and microparticle formation) and PFA-CT (Leytin et al. 2003).

Based on available data (Jahr et al. 2008b; Williams et al. 2002), it appears that clinically tested HBOCs do not produce coagulopathy at a clinically relevant dose. However, only around 2000 subjects have been tested with multiple HBOCs, and post-marketing data on coagulation has not been published, despite approval of one product in South Africa and Russia for over 10 years (HBOC-201).

34.8 Estabishment of Need for Oxygen Delivering Resuscitation Fluids

One of the main areas of interest for application of HBOCs is in the setting of major trauma and hemorrhage. In traumatic hemorrhage, patients may develop an acute coagulopathy which worsens hemorrhage from primary bleeding sites (Hauser et al. 2010). Conventionally, treatment of traumatic coagulopathy involves administration of blood products with a goal of correcting abnormal coagulation test results. However, in major injuries, current blood products can be inadequate to reverse coagulopathy, and HBOCs and other resuscitative fluids may contribute to further dilutional coagulopathy. This leaves room for attempts to develop improved resuscitation fluids that specifically address coagulopathy.

A randomized clinical trial showed that administration of recombinant factor VIIa as an adjunct to direct hemostasis in major trauma reduced the total amount of blood product used (Hauser et al. 2010). However, there was no difference in mortality.

34.9 Potential Approaches to Development of Improved Resuscitation Fluids

As research continues in the area of HBOCs, attempts to improve HBOCs as resuscitation fluids specifically in the area of coagulopathy include supplementing the fluids with coagulation factors and comedicating with procoagulants. One study showed that crosslinking fibrinogen to hemoglobin to form polyhemoglobin-fibrinogen resulted in similar clotting times in vitro and in vivo as whole blood in up to 98 % exchange transfusion (Wong and Chan 2007). In contrast, polyhemoglobin alone increased normal clotting time (1–2 min) to greater than 10 min

after 80 % exchange transfusion in a rat model. Another study showed that administration of recombinant factor VIIa improved PT and aPTT after progressive hemodilution had increased clotting time (Darlington et al. 2011).

34.10 Discussion and Summary

Several of the studies above do suggest that current HBOCs can contribute to mild platelet dysfunction when used in large quantities (Jahr et al. 2008a, 2008b; Arnaud and Handrigan 2006; Jahr et al. 2010). Part of this is the result of a dilutional coagulopathy that is to be expected with large volume resuscitation with any fluid devoid of clotting factors and platelets. However, thus far, the studies indicate that HBOCs do not produce more coagulopathy and platelet dysfunction when compared to hetastarch fluids. Some of the unique properties of HBOCs, such as the risk of oxidation to methemoglobin, have also been studied with respect to effect on coagulation. In an experimental model (Moallempour et al. 2009), large concentrations of methemoglobin (65 %) did impair clot propagation. As fresh samples of HBOC contain only 1 % methemoglobin, this effect may not be ultimately clinically relevant. Thus far HBOCs do not appear to contribute to more platelet dysfunction than other currently acceptable resuscitation fluids. Nonetheless, as HBOCs continue to be developed and refined, careful consideration of their possible unique effects on platelet function and coagulation is prudent.

Allogeneic human blood for transfusions has significant limitations such as short shelf life of blood products, risk of disease transmission, immunomodulation, risk of hemolytic transfusion reactions and logistical constraints (Chen et al. 2009; Jahr et al. 2011). Unmodified stroma free hemoglobin is not safe as a blood substitute as it causes severe adverse effects: short circulation time in vivo, nephrotoxicity, high affinity to nitric oxide (NO) and related vasoconstriction and hypertension Squires (2002; Reid 2003). Multiple modifications of hemoglobin (Hb) based on intra- and intermolecular cross-linking or encapsulation have been tested to find a viable oxygen carrier. Hemoglobin-based oxygen carriers (HBOCs) have been investigated intensively during the last 30 years with the aim to develop an universal blood substitute. Early generation HBOCs such as diaspirin cross-linked Hb, and later generations, with weakly polymerized Hbs like HBOC-201 and Poly PLP-Hb (PolyHeme®, Northfield Corp.) have prolonged intravascular retention time but caused substantial hypertension Chen et al. 2009; Saxena et al. 1999; Zhang et al. 2011. Sakai et al. and Cabrales et al. (2009) demonstrated that the extent of the vasoconstriction and hypertension decreases with increasing size of the HBOC, (Saxena et al. 1999; Zhang et al. 2011). Additionally although not definitively proven, HBOCs with high p50 may release excessive amounts of oxygen into the systemic circulation also inducing vasoconstriction (Lee et al. 2000). Therefore, polymerization of the Hb leading to larger HBOCs with low p50 (high oxygen affinity) may provide a better solution for prevention of vasoconstriction and can lead to suitable blood substitutes (Winslow 2000; Winslow 2003).

However, regarding coagulation, it is hypothesized that unrelated to the oxygen carrying capacity, larger molecules may worsen coagulation abnormalities (Sakai et al. 2002). Free hemoglobin and tetrameric HBOCs have been studied for over 60 years (Jahr et al. 2011; Reid 2003), with first generation having multiple safety issues (renal toxicity, and death) causing trials to be halted (HemAssist®, Baxter, Hemolink®, Hemosol). Second generation HBOCs were studied in great detail, with a number of large clinical trials published (Jahr et al. 2008b). HBOC-201, (OPK Biotech) has been approved for human use in two countries (South Africa and Russia) without evidence of coagulopathy (Williams et al. 2002). Third generation products, such as Zero-link Hb polymer, also have been studied in pre-clinical models and demonstrate no clinically meaningful coagulation (Jahr et al. 2008b). As the studied compound becomes larger to minimize nitric oxide scavenging and hypertension, so may the likelihood of coagulopathies be worsened.

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