Differential Effects of Serial Hemodilution with Hydroxyethyl Starch, Albumin, and 0.9% Saline on Whole Blood Coagulation

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Study Objectives: *To determine by thrombelastography assessed coagulation, the effects of progressive hemodilution with three intravascular volume expanders.*
Design: *Prospective, controlled, whole blood, volumetric ex vivo hemodilution study.*
Setting: *University of Pennsylvania Medical Center Operating Rooms.*
Patients: *60 ASA physical status I and II patients; phlebotomy prior to administration of IV fluids or medications.*
Interventions: *Analysis of whole blood clotting determined by six thrombelastographic channels for control and five volumetric hemodilutions (11%, 25%, 33%, 50%, and 75%) with 0.9% saline, 5% albumin, and 6% hydroxyethyl starch (n = 20 for each diluent group).*
Measurements and Main Results: *Thrombelastographic parameters R (minutes), angle α (°), MA (mm), and lysis (%) were measured and compared to the sample control for each dilution of the same specimen. There was no significant difference between control groups in any thrombelastographic variable (R, angle α, MA, or lysis). No changes were seen in any variable from any diluent at 11% hemodilution. Seventy-five percent hemodilution caused significantly hypocoagulable changes from control for all thrombelastographic parameters for all three diluents. Thrombelastographic indices differed significantly from controls at intermediate hemodilutions. Both colloids caused decreases in measured angle α and MA at lower hemodilution than did 0.9% saline. Albumin 5% caused significant hypocoagulable changes from control values at lower hemodilution than did either 0.9% saline or 6% hydroxyethyl starch for all thrombelastographic parameters. Saline 0.9% increased angle α significantly at 50% hemodilution. Abnormal lysis did not occur at any dilution. Differing ex vivo effects of three different intravascular fluids thrombelastography assessed coagulation are found.*
Conclusion: *No differences were found after 11% hemodilution with any volume expanders. Hemodilution with up to 50% saline maintained thrombelastographic indices. Albumin produced early and profound hypocoagulable effects. Significant hypocoagulability occurred for all three diluents at 75% hemodilution. The study supports the use of albumin in patients at risk for thrombosis, and saline in patients with a need for normal hemostasis.*

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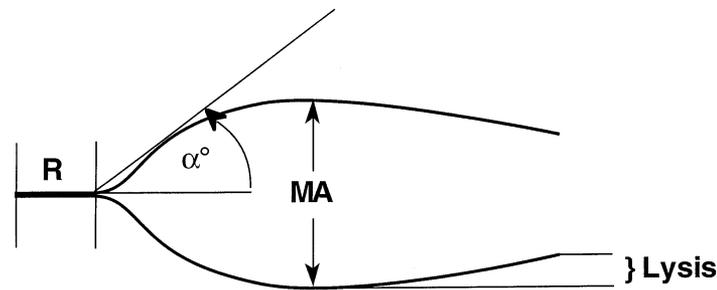
Figure 1. A schematic thrombelastographic (TEG™; Haemoscope Corp., Skokie, IL) trace is composed of four principal parameters. Reaction time (R), clotting initiation time are recorded in minutes, angle α, fibrin polymerization rate, the tangent to the TEG™ curve from the R point, and maximum amplitude (MA), and overall clot strength are recorded in millimeters. Lysis 30 is the percent decrement in the TEG™ amplitude 30 minutes after the acquisition of the MA, a measure of clot's stability.

Keywords: Albumin; blood; coagulation; hemodilution; hydroxythyl starch; saline; thrombelastography.

Introduction

Some advancing level of hemodilution logically must diminish the ability of blood to coagulate. Forty percent normovolemic hemodilution with combined crystalloid and colloid intravascular volume expanders has been shown to prolong both prothrombin (PT) and partial thromboplastin (aPTT) times.[1] Conversely, an intraoperative saline hemodilution of 15% has been suggested to induce "hypercoagulable" changes,[2] and the administration of Hartman's solution has been found to shorten impedance clotting time, predisposing patients to ^{125}I-fibrinogen demonstrable deep venous thrombosis (DVT).[3] Because intravascular (IV) volume expanders are often administered to bleeding, immobilized, or thrombogenic patients, differences in coagulation effects may have clinical importance. This experiment addresses the possibility that three commonly used IV fluids will offer differing effects on *ex vivo* whole blood coagulation. For this purpose we have used the thrombelastograph (TEG™; Haemoscope Corp., Skokie, IL), a whole blood coagulation monitor that gives quantitative and qualitative analysis of pro- and anticoagulation as well as fibrinolysis. *Figure 1* depicts a schematic TEG™. Reaction time (R), recorded in minutes, measures the time from when blood is placed in the TEG™ until the clot attains sufficient gel to deflect the instrument by 1 mm. Thus, R is an index to the initiation of blood clotting, analogous to, though differing from, standard activated plasma gelation tests such as PT and aPTT. Angle α, measured in degrees, is drawn from R to the tangent TEG™ curve and reflects the rate of clot growth through fibrin polymerization. Maximum amplitude (MA), recorded in millimeters, is the maximum deflection, the shear modulus at maximal clot formation. It is an index to maximal clot tensile strength. Lysis is the percent decrement in the TEG™ amplitude 30 minutes after the acquisition of MA. The function of hemostasis is to maintain fluid blood circulating within the vascular space. Effective hemostasis must incorporate adequate response times (reflected in normal R and angle α),

strength (normal MA), and stability (minimal lysis). The advent of plastic disposable reaction cuvettes and computerized analysis have improved TEG™ utility and reproducibility. The R time remains the most sensitive variable to specimen storage and handling.[4] More detailed explanations of the interpretation of TEG™ variables can be found in the literature.[5,6]

Materials and Methods

This prospective controlled whole blood volumetric *ex vivo* hemodilution study design eliminates medications, surgical stress, and tissue factors as additional variables coagulation. Following approval of the protocol by the Institutional Review Board, Committee on Human Studies, University of Pennsylvania, involving human beings, 60 ASA physical status I and II patients scheduled for elective surgical procedures were randomly selected from the daily populations of patients treated in the operating rooms. Selection was based on patient availability prior to the administration of medications or intravenous (IV) fluids. No person with a history of coagulation abnormalities or an abnormal preoperative coagulation profile (PT, aPTT, platelet count) was included for study. Patients taking medications known to alter platelet activity or blood coagulation were excluded from study. All patients had normal renal and hepatic function by laboratory analysis or by history. After application of these criteria, 60 consecutive patients were assigned to each of three hemodilution groups.

Six thrombelastograph channels were prepared using standard calibration equipment for strain, balance, and clearance. Quality control performances using either standardized lyophilized blood or untreated whole blood were performed so as to ensure reproducible results across all six TEG™ channels.

Prior to the induction of anesthesia, 7 ml of whole blood were aseptically obtained after placement of the IV catheter. A two-syringe technique was used; the first 2 ml aliquot was discarded to reduce variable tissue thromboplastin contamination from traumatic venipunctures. A second plastic syringe was filled with 5 ml of whole blood and used as a test tube for pipetting into the TEG™ cups.

The whole blood was always analyzed at 37°C within 4 minutes of collection.

The IV volume expanders studied were 0.9% saline, 6% hydroxyethyl starch (HES; Hespan™, McGaw Pharmaceuticals, Inc., Irvine, CA), and 5% albumin.

Each sample of blood was analyzed as 0% hemodilution (control) or exposed to hemodilutions of 11%, 25%, 33%, 50%, and 75% from one of the three IV volume expanders (n = 20). Each dilution was pipetted into randomly assigned TEG™ cups. A sufficient balance of whole blood from each patient was added to form an end volume of 360 μl. A total of 360 analyses comprised the study. The pins of each TEG™ were partially raised and lowered five times within the cup to insure adequate and uniform mixing of blood with diluent and to standardize agitation and/or activation of the specimen. The morphologies and data for four TEG™ variables (R, angle α, MA, and lysis) were automatically collected and stored on software (cTEG™, Haemoscope Corporation, Skokie, IL) from a chart running at 2 mm/min. Normal values for these variables when using plastic parts are: R = 11.8±2.4 minutes; angle α = 36 ± 7.4 degrees; MA = 54.0 ± 5.6 mm; lysis = 1 ± 1.5%.

In order to study coagulation in relatively unmanipulated blood, no anticoagulant or procoagulant (*ie*, citrate, calcium, or celite) was added to any specimen. Only fresh whole blood was studied with and without dilution for each subject. Control and three treatment groups were subjected to a standardized regimen to minimize operator related effects.

Results were analyzed using analysis of variance for repeated measures and Bonferroni's post hoc test. Dilutions were compared with their identical undiluted control. A stringent P-value of less than 0.01 was considered significant.

Results

Results are summarized in *Figures 2, 3, and 4*. There was no difference among group baselines for any TEG™ variable. Hemodilution of 11% also did not differ significantly from baseline for any IV volume expander in any TEG™ variable. Seventy-five percent dilution caused significantly hypocoagulable changes from baseline for each of the TEG™ parameters for each of the three diluent groups. TEG™ indices differed significantly from control at intermediate dilutions. Both colloids caused earlier and more profound changes than did 0.9% saline in measured angle α and MA. Albumin 5% caused significantly hypocoagulable changes from control values at lower dilutions than did HES for all TEG™ parameters measured. Neither HES nor 0.9% saline altered reaction time at 50% hemodilution. Angle α significantly increased at 50% hemodilution of 0.9% saline. Clot lysis did not occur at any hemodilution of each diluent within 30 minutes after achieving MA. *Figure 5* represents typical serial hemodilutions from the blood of a single patient.
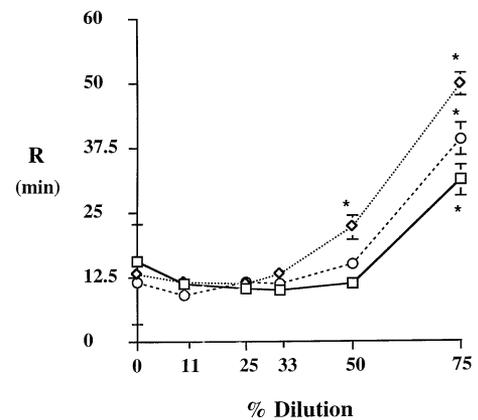

Figure 2. Graphic plots of clot initiation time (R), following progressive hemodilutions of whole blood with (—□—) 0.9% saline, (·····◇·····) 5% albumin, and (·····○·····) 6% hydroxyethyl starch (mean ± 1 standard deviation). *$p < 0.01$ compared with undiluted control.

Discussion

The results presented here demonstrate differential responses of all TEG™ assessed variables of whole blood coagulation to progressive hemodilution with commonly used IV volume expanders.

Albumin produced the earliest and the most profound alterations in coagulation in the present study. An interesting *in vivo* human study of extreme normovolemic hemodilution up to 75% employed only 5% albumin.[7] Significant prolongations of both PT and aPTT were measured at approximately 60% hemodilution. There were concomitant significant reductions in both platelet count and fibrinogen level at 45% HD. Albumin 5% produced more prolongation of PT and aPTT than lactated ringer's solution in an acute hemorrhagic shock/
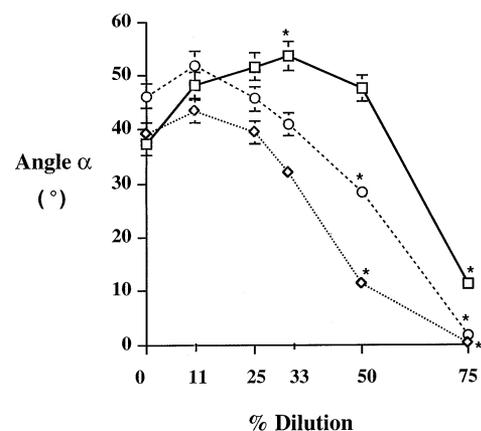

Figure 3. Graphic plots of rate of clot formation (angle α), following progressive hemodilutions of whole blood with (—□—) 0.9% saline, (·····◇·····) 5% albumin, and (·····○·····) 6% hydroxyethyl starch (mean ± 1 standard deviation). *$p < 0.01$ compared with undiluted control.
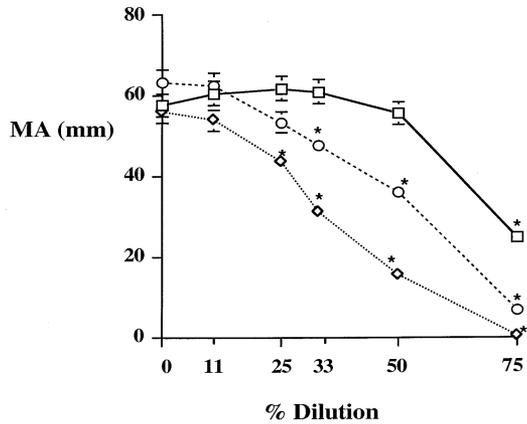
Figure 4. Graphic plot of maximum amplitude (MA), which is an indicator of clot strength, after progressive hemodilutions of whole blood with (—□—) 0.9% saline, (·····◇·····) 5% albumin, and (·····○·····) 6% hydroxyethyl starch (mean ± 1 standard deviation). *$p < 0.01$ compared with undiluted control.

resuscitation dog model.[8] The addition of albumin to a resuscitation regimen in massive transfusion has been suggested to worsen coagulation status, requiring increased need for exogenous blood component therapy.[9]

An inhibitory property of albumin on fibrin polymerization in plasma has been described.[10] The portions of the albumin molecule that bind to fibrin and inhibit fibrin polymerization have been further elucidated.[11] Albumin-exposed fibrin forms smaller diameter fibrils than does control.[12] These phenomena may be implicated in the decrease in clot kinetics and strength evidenced by the diminution in angle α and MA seen with progressive albumin hemodilution in the present study.

The 6% HES employed in this study is a high molecular weight-high molar substitution formulation. A previous study[13] compared the effects of 6% HES with both 5% albumin and 0.9% saline with approximately 20% hemodilution *in vivo* and 50% hemodilution *in vitro*. HES short-

ened thrombin and reptilase clotting times, as well as urokinase activated clot lysis times, in all preparations.[13] This effect is similar to that established for dextran 70, which is known to alter human fibrin morphology[14] and to promote lysis.[15,16] A canine study comparing severe (80%) hemodilution with dextrans and HES suggested that the latter promoted more severe and longer lasting hypocoagulable effects than did either dextran 75 or dextran 40.[17] Unfortunately, it is not clear which molecular weight or molar substitution formulation of HES was used. Lysis was not a feature of the present TEG™ studies after 30 minutes with any of the diluents at any hemodilution. It is possible that early and rapid lysis contributed to the attenuation of the MA, but fibrinogen degradation products were not measured. Well characterized but modest effects of HES on platelet function would also account for some of the decrease in angle α and MA seen with HES.[18]

An *in vivo* study[19] comparing coagulation changes in surgical patients who received either one liter hemodilution with 5% albumin or HES revealed no acute differences between the two diluents. A recent *in vivo* study[20] of patients undergoing hip arthroplasties, each of whom received up to 3 liters of intraoperative 5% albumin or HES (200/0.5), reported abnormally prolonged coagulation parameters after 1500 ml with either colloid. This hypocoagulable state increased with 2000 to 3000 ml administration and persisted into the postoperative period. No significant difference in coagulation profiles were observed between the two colloid volume expanders during the operative course. However, HES's anticoagulant effects outlived those of albumin. A comparison of HES with 5% albumin as a priming fluid for cardiopulmonary bypass revealed no differences in estimated blood loss, transfusion rates, or postoperative chest tube drainage.[21] Similar results were found comparing albumin with 6% HES volume infusion in the treatment of septic shock.[22]

In the present study, hemodilution with 0.9% saline either maintained baseline TEG™ coagulation or demonstrated mild procoagulant changes through 50% hemodilution. Although the TEG™ has been described as a sensitive test for "hypercoagulability",[23,24] we have avoided
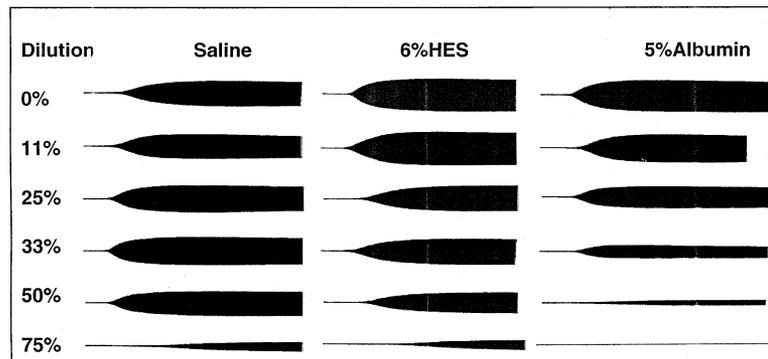

Figure 5. Representative thrombelastographs (TEG™s; Haemoscope Corp., Skokie, IL) of one blood specimen from each study group; control (0%) and five progressive hemodilutions from with 0.9% saline, 6% hydroxyethyl starch (HES), and 5% albumin. As can be seen from these representative tracings, lysis, which is an indication of clot instability, did not occur.

this terminology because there is no evidence that these blood specimens are thrombotic *in vivo*. A preoperative *in vitro* 0.9% saline hemodilution as a provocative test for hypercoagulability identified all study patients who subsequently developed DVT.[25] Janvrin et al.[3] have postulated that IV 0.9% saline predisposes patients to thrombosis. Ng and Lo[23] and Tuman et al.[2] have described procoagulant activity following a modest (10% to 15%) *in vivo* 0.9% saline hemodilution. Similar procoagulant results have been published following a 20% saline hemodilution,[26] and after a 50% dilution with cerebrospinal fluid.[27] We have also previously described procoagulant accentuation of angle α by moderate (25% to 50%) *ex vivo* hemodilution with normal saline.[28] Any procoagulant effect was markedly reversed at 75% hemodilution in both the present and previous studies. These same procoagulant effects following hemodilution have been linked to evidence that antithrombin III is more sensitive to dilution than are the procoagulation factors.[29]

Although there were very obvious differences observed between 25% to 50% hemodilution with 0.9% saline and the colloid IV fluids in this experiment, there are limitations to the extrapolation of these results to the clinical situation. Admixture of the components in this study occurred *ex vivo* and the clotting mechanism was studied *in vitro*. There are *in vivo* rheologic implications that occur with induced decreases in blood viscosity.[30] The TEG™ is a static chamber and the effect of flow is recognized to have implications for coagulation.[31] The TEG™ provides an assessment of global whole blood coagulation but excludes interactions of blood components with endothelial cells, which are important mediators of hemostasis. The main advantages of this protocol are that it eliminates medications, surgical stress, and surgical tissue factors as variables on coagulation.

In summary, no differences were found in TEG™ assessed coagulation after 11% hemodilution with any of the IV volume expanders. Hemodilution with 0.9% saline, up to 50%, maintained TEG™ indices. Albumin demonstrated the earliest and most profound hypocoagulable effects on the TEG™ compared with control. All of the parameters examined confirm highly significant TEG™ assessed hypocoagulability for all three diluents at 75% hemodilution. Future research will involve *in vivo* administration of volume expanders and subsequent coagulation monitoring. The present study, if relevant to *in vivo* effects of 25% hemodilution, supports the use of albumin in patients at risk of thrombosis, and 0.9% saline in patients with need for normal hemostasis.

Addendum

The cost of the albumin 5% 500 ml was $66.00; HES injection 6%, 500 ml was $40.57; and 0.9% saline, 500 ml was $0.49. All figures represent costs to the institution.

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Effects of Progressive Blood Loss on Coagulation as Measured by Thrombelastography

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Abstract

The effects of progressive blood loss on coagulation were studied in 87 adults (age 23–66 yr) undergoing a variety of operations under general anesthesia. None had preoperative alterations in coagulation or liver function and none were receiving anticoagulant or antiplatelet medication. Whole blood coagulation status was quantitated using thrombelastography (TEG). Blood samples for TEG were obtained 5 min before and 15 min after induction of anesthesia, after each increment of blood loss (EBL) equalling 5% of estimated blood volume (EBV), at the end of surgery, and 2 hr postoperatively. Patients with EBL exceeding 0.15 EBV were given packed red cells and crystalloid solution. Patients with EBL less than 0.15 EBV received only crystalloid. Thrombelastography analysis showed a trend toward increased coagulability with progressive blood loss. Two of four patients with 80% loss of EBV maintained normal to enhanced coagulation status, although the other two developed clinical and thrombelastographic evidence of coagulopathy. Thrombelastography allowed rapid intraoperative diagnosis and specific treatment of loss of platelet activity in the latter two patients. We conclude that during moderate to massive blood loss, use of supplemental fresh frozen plasma and/or platelets should be reserved for patients with documented defects in coagulation. Thrombelastography is useful for the detection and management of coagulation defects associated with intraoperative blood loss.