Surface Activation of Whole Blood: Clot Detection with a Thrombelastograph

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Keywords: coagulation tests; surface activation; whole blood

ABSTRACT

Various methods are used to decrease the length of time required to generate useful data from thrombelastography (TEG). The quicker this data can be produced and analyzed the sooner decisions can be made for hemostasis management. The present study was designed to determine the effects of glass compared to plastic with respect to their effect on the activation of the intrinsic pathway of coagulation. Thirty-seven samples of native whole blood (NWB) were drawn via radial artery catheter, internal jugular sheath or by direct venous puncture. Half of each sample was placed in a glass test tube and the other half in a plastic (polypropylene) container, and allowed to sit for periods of 1 minute (n = 4), 1.5 minutes (n = 5), 2 minutes (n = 9), 2.5 minutes (n = 9) and 3 minutes (n = 10). The glass activated samples were placed in plastic TEG cups that had been randomized to one of the two TEG channels, with the plastic activated samples being placed in metal TEG cups in the opposite channel. This resulted in a total of seventy-four TEG profiles.

There were no significant correlations (p < 0.05) between any of the inter- or intra-related TEG parameters, reaction time (R time), kinetic time (K time), maximum amplitude (MA), or alpha angle (AA). However, the R time of the glass activated samples showed a linear decrease in time required to achieve this value. The plastic activated samples did not exhibit this linear decrease in R time. The thrombelastograph index (TI) showed an initial hypocoagulability at 1 min for glass (-1.84 ± 3.46) when compared to plastic (+1.58 ± 0.23). At 1.5 minutes, the TI for glass (-0.12 ± 2.97) approached those for plastic (+0.035 ± 1.68) and remained relatively close until 3 min of activation when the glass samples TI (2.48 ± 3.16) proved to be hypercoagulable when compared to the plastic at 3 min, TI (1.97 ± 2.64). The MA, K time and alpha angle seemed to be largely unaffected by the different activation times.

This study has shown that glass activation may be used effectively to decrease the time required to generate TEG data from NWB samples, and that the glass activation does not seem to affect the TEG parameters significantly other than the R time.

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INTRODUCTION

The thrombelastograph (TEG) was developed by Hartert in 1948 (1). The TEG is used to monitor viscoelastic changes in the whole blood clotting process beginning with initial fibrin formation and ending with clot retraction or lysis. The TEG values and profile of the clot formation may then be used to detect abnormalities in coagulation.

The TEG machine uses either metal reusable or plastic disposable cups and pins. The pins are suspended on a torsion wire and lowered into the cups that have been filled with 0.36 ml of blood that has been heated to 37°C. The cups are maintained at this temperature and are rotated horizontally in an arc of 4° in 45 seconds within a period of 10 seconds. Initially, while the blood is in a liquid state, the pin remains motionless. As fibrin strands begin to form between the cup and pin, the pin begins to rotate with each oscillation. With increased rotation more torque is transmitted to the torsion wire and a shear modulus is created generating a TEG profile on a strip chart or computer.

There are four main parameters generated by the TEG. The reaction time (R time) which reflects initial fibrin formation is measured at a point where the deflection of the TEG profile is 2 mm apart. The normal R time for native whole blood is 21-30 mm. If the R time is prolonged this may indicate the presence of anticoagulants or a factor deficiency. The kinetic time (K time) is when the TEG profile reaches a point 20 mm from the baseline, and reflects rate of clot growth and strength. The normal K time for native whole blood is 35-40 mm. A prolonged K time may be due to a qualitative or quantitative defect in platelets or factors (2). The alpha angle (AA) is similar to the K time in that it represents the speed and strength of clot growth, relating to platelet function and the speed of fibrin formation. The normal AA for native whole blood (NWB) is 30-41°. The fourth parameter measured by TEG is the maximum amplitude (MA) which reflects the absolute strength of the forming clot. The normal MA of NWB is 45-54 mm. The amplitude at 60 minutes beyond MA (A∞) reflects the rate of clot resolution, and is used as an indicator of fibrinolysis.

The TEG offers several advantages over the routine coagulation tests (RCT): prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), bleeding time, fibrinogen and activated clotting time (ACT). This is because the TEG uses a sample of whole blood, rather than plasma fractions as is the case with most of the RCT. Because TEG uses whole blood the information generated takes into account how all the plasma proteins and cellular components in the coagulation process are interacting (2,3). The ACT does use whole blood but the test ends with initial fibrin formation, so limited information can be gathered about platelet function. The bleeding time does take into account the function of platelets, but it has been suggested that since this test looks at a standardized cut in the skin, it is not useful in predicting bleeding from surgical intervention in other areas of the body (4,5).

The TEG also offers the advantage of speed over the RCT because it can be set up and used in the operating room. The TEG values relating to early clot development can be generated in less than 15 min (6), whereas it may take RCT samples this long just to reach the laboratory.

Attempts have been made to compare the RCT values to TEG (3,7-9). Howland, et al., reported that TEG and ACT are better indicators of hypercoagulability than PTT, while the detection of hypocoagulability was best with PTT. The best overall test of hypo- and hypercoagulability was determined from the R time of TEG (8). Spiess, et al., have shown that TEG is a more reliable indicator of postoperative hemorrhage in cardiopulmonary bypass (CPB) patients (87%) than ACT (30%) or RCT (51%) (3).

It appears that TEG is a very reliable device for monitoring coagulation and may be a better test for predicting postoperative bleeding in CPB patients than the RCT (2). With the introduction of disposable cups and pins, it has been reported that TEG results may be more reproducible than with the reusable metal cups and pins (10).

The purpose of this study was to compare glass activated whole blood to the conventionally used plastic activation. This was done in an effort to decrease the length of time between acquiring the native sample and generating TEG data. Because the surface of glass is a hydrophilic surface, as compared to the hydrophobic surface of plastic (polypropylene), it was assumed that the glass would show increased protein adsorption at its surface. Because of these increased adsorption properties, glass
should be a stronger initiator of factor XII and the intrinsic coagulation pathway than plastic (polypropylene) (11,12).

MATERIALS AND METHODS

Native non-heparinized blood samples were drawn from either the sheath of an internal jugular catheter, arterial line, or by direct venous puncture. In the case of the arterial lines 10 ml was aspirated and discarded, then 2.5 ml was removed for the sample. Half the sample was then placed in a 10 ml standard laboratory red top glass test tube and the other half in a 2 ml polypropylene tube. The samples remained in the glass and polypropylene tubes for periods of 1 (n = 4), 1.5 (n = 5), 2 (n = 9), 2.5 (n = 9) or 3 (n = 10) minutes for purposes of activation of factor XII and the intrinsic pathway of coagulation. After the activation time had elapsed, 0.36 ml of the sample was pipetted from the glass tube into the plastic cups and 0.36 ml was pipetted from the plastic container into the metal cups of the TEG machinea. The metal and plastic cups and pins had been randomized to either the left or right channel of the two channel TEG. Once the samples had been placed into the cups, the pins were lowered simultaneously into the samples, and the event markers started. A small amount of mineral oil was then placed on top of each sample to prevent desiccation. The samples were allowed to run until a MA had been reached on each channel. Samples that showed a marked increase in R time, consistent with the presence of large amounts of heparin, were excluded from data that was to be statistically analyzed.

The TEG values obtained were then loaded into a spreadsheet program and grouped by type and time of activation (metal cups and pins with polypropylene and plastic cups and pins with glass). The spreadsheet values were then transferred to a statistical program for analysis of both inter- and intra-related variables. Analysis of variance was performed and when significant F-ratios were achieved, additional multiple comparison tests (Fishers least significant difference) were performed. Statistical significance was accepted at the p < 0.05 level. All data are presented as mean ± standard deviation of the mean.

RESULTS

Thirty-seven samples for a total of 74 TEG profiles were analyzed for statistical significance. The data revealed that the use of glass activation can be used to decrease the length of time needed to acquire TEG data from NWB. The mean R time at 1 minute of glass activation was 48.3 ± 31 mm compared to an a

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a Thrombelastograph, Haemoscope Corp., Morton Grove, IL
mean R time of 16 ± 3.9 mm for 1 minute of plastic activation. At 3 minutes of glass activation the mean R had decreased to 19.3 ± 20 mm while the mean R for plastic had decreased only slightly (Figures 1 and 2) to a mean of (15.25 ± 8.1) mm. The mean K time, MA and AA were for the most part unaffected by different periods of activation time, and did not show large variances for different activation times (Figures 3 through 8). The TI for the glass activated samples was hypocoagulable when compared to the plastic activated samples at 1 minute. At activation times of 1.5 and 2.0 minutes (Figures 9 and 10) the TI were very similar for the glass (-0.12 ± 2.97) and (0.71 ± 3.92) when compared to the plastic (0.035 ± 1.68) and (1.00 ± 2.43). At 3 minutes, the glass activated samples achieved a higher TI (2.48 ± 3.16) compared to the plastic activated samples (1.97 ± 2.64).

DISCUSSION

From the data presented here it seems clear that the use of glass activation as compared to plastic activation of native whole blood is superior for acquiring useful TEG data in a shorter period of time. The current method most widely used in acquiring faster TEG results is the use of a 1% celite solution to enhance factor XII and the intrinsic coagulation pathway (10).

The increased rate of coagulation induced by the glass surface arises from its ability to adsorb plasma proteins and the way these proteins react with its hydrophilic surface (12). Plastic also adsorbs plasma proteins but because of its hydrophobic nature it tends to hold onto these proteins more readily, thus decreasing the number of available sites for the activators of the intrinsic pathway to bind (12-14). Both surfaces are known to exhibit the "Vroman effect," which is the rate at which proteins are competitively removed from a foreign surface (12,13). In other words, there is an initial high concentration of certain proteins that adsorb to the foreign surface. As time passes, the concentration of these proteins declines because they are replaced by other proteins and cofactors of coagulation (13,14). The initial protein layer needs to be partially removed or have gaps created in it, in order for the two main activators of the intrinsic coagulation pathway to begin the contact phase of coagulation (13). These two factors are high molecular weight kininogen (HMWK) and factor XIIa (14). The mean K times in the data in Figure 1 may reflect some of this "Vroman effect." At 1 minute of activation the K time showed a hypocoagulability with glass activation, as compared to 1.5-3.0 minute activation times. This could be due to fewer contact activation sites being available for the factors of the intrinsic pathway. The plastic activated samples did not show this effect (Figures 3 and 4).

By using stainless steel pistons and cuvettes with glass activation the time required to achieve useful TEG data may even become shorter. In this study the glass activated samples were placed into the plastic disposable cuvettes which, in general, appear to be slower activators of coagulation when compared to their stainless steel counterparts (Figures 1 and 2).

The TEG profiles of the glass activated samples analyzed with plastic cups and pins showed hypocoagulability when compared to plastic (polypropylene) activated samples, analyzed with stainless steel cups and pins at the shorter activation times. As the length of activation in both the plastic and glass was increased, the TEG profiles of the metal cups and pins became much more similar to the profiles of the plastic cups and pins. From this observation it may be more beneficial to use glass activation with stainless steel cups and pins or allow for glass activation times to be greater than 3 minutes when using the plastic disposable cups and pins.

CONCLUSION

The expedient acquisition of TEG is important so that specific hemostatic defects can be isolated, and the administration of unneeded homologous blood products can be avoided. From the data presented here it is apparent that the use of glass activation for NWB in TEG can be a useful adjunct. Glass activated samples show a nearly linear decrease in the R time and are able to decrease the length of time required to generate useful TEG data, while plastic activated samples do not exhibit this effect. The remainder of the TEG parameters, K time, alpha angle and MA, were not significantly affected by the glass or plastic activation.

REFERENCES


