Physiol. Res. 51: 145-150, 2002

The Effects of Cardiopulmonary Bypass with Hollow Fiber Membrane Oxygenator on Blood Clotting Measured by Thromboelastography

M. HORÁČEK, K. CVACHOVEC

Department of Anesthesiology and Critical Care Medicine, Charles University, Second Faculty of Medicine and Institute for Postgraduate Medical Education, Prague, Czech Republic

Received February 8, 2001
Accepted August 3, 2001

Summary

In cardiac surgical patients we investigated the effects of cardiopulmonary bypass (CPB) with a hollow fiber membrane oxygenator on blood clotting measured by thromboelastography (TEG). We found only a minimal change in the strength of blood clot described either by the TEG parameter MA (maximum amplitude) or by the shear modulus G calculated from MA. After CPB there was also a significant tendency towards hypercoagulation as defined by shortened parameters R, K and increased α-angle. After comparison with published data obtained in cardiac surgical patients using a bubble oxygenator we conclude that currently used extracorporeal technology exerts a less negative influence on blood clotting than had been conceived previously.

Key words

Blood clot • Thromboelastography • Cardiopulmonary bypass • Bleeding • Oxygenator

Introduction

Cardiac surgical procedures using cardio-pulmonary bypasses (CPB) are inevitably associated with bleeding due to blood clotting impairment. Their causes are multifactorial and immensely complex but the most frequent ones are platelet dysfunction and increased fibrinolysis (Shukri *et al.* 1995). Other contributing factors include hypothermia, heparin, protamine, antiplatelet agents, hemodilution, denaturation or consumption of coagulation factors, preoperative coagulation disturbances and surgical bleeding due to vascular and tissue injuries.

Bleeding has far reaching consequences. It is associated with risks of circulatory instability, tissue hypoxia, multiple transfusions of blood products, surgical reexploration, infection, multiple organ dysfunction syndrome, sepsis and even death. Bleeding also brings about additional costs due to the prolonged need for intensive care and hospitalization (Despotis and Goodnough 2000).

Conventional laboratory methods assessing hemocoagulation do not take into account the interactions between blood cells and plasma factors or the importance of the strength and stability of the blood clot. The aim of these methods is the formation of fibrin fibers or assessment of the number of platelets only. Furthermore,

Fax+420 2 24920590
E-mail physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

the results obtained in the laboratory are not available as readily as is necessary when the patients are bleeding.

Thromboelastography (TEG) is a point-of-care method assessing the formation, strength and stability of the blood clot (Thromboelastograph® Coagulation Analyzer Model 3000 C Operational Manual, Haemoscope Corp., Skokie, Illinois, USA). Although developed by Hartert as early as in 1947 (Hartert 1948), its popularity did not spread until the 1980s when anesthesiologists involved in liver transplantations and open heart surgery realized its clinical potential. It was also found at that time that coagulation occurs on cell surfaces rather than in the plasma (Mann 1984). This finding further supported the value of TEG whole blood analysis as compared to conventional plasma coagulation tests. In addition, TEG offers results much more readily, especially when using activated testing (e.g. celite or tissue factor) (von Kier and Smith 2000).

The purpose of our study was to investigate the effects of current CPB technology with a hollow fiber membrane oxygenator on blood clotting and to compare the results with those obtained after CPB with a bubble oxygenator (Spiess *et al.* 1987, 1994). We tested the hypothesis that the development, strength and stability of blood clots measured by TEG is influenced by CPB with a hollow fiber membrane oxygenator less than by CPB with a bubble oxygenator. This should offer a perspective of easier management of bleeding in cardiac surgical patients after CPB.

Methods

After the approval of the study by the institutional Ethical Committee consecutive adult patients undergoing for the first time heart surgery using CPB between April 1997 and February 1999 were prospectively enrolled. All patients were maintained on their current drug therapy up to the time of the operation except for aspirin withdrawal 7-10 days in advance before elective procedures.

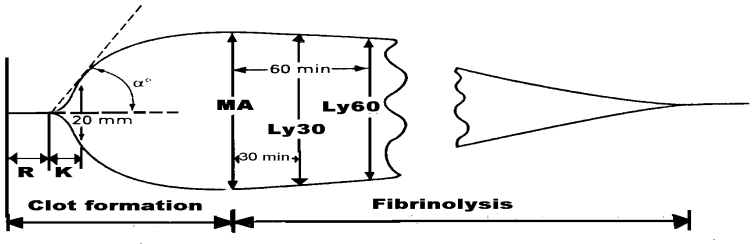

Fig. 1. *Normal TEG tracing*

All anesthetic and surgical procedures were performed in the standard manner by one team of anesthesiologists and surgeons. General anesthesia was induced using a combination of opioids (fentanyl 10-20 $\mu g.kg^{-1}$ or sufentanil 1-2 $\mu g.kg^{-1}$), etomidate 0.2 $mg.kg^{-1}$ and pipecuronium 8 mg. Anesthesia was maintained by nitrous oxide or air with oxygen and isoflurane, sufentanil, midazolam and pipecuronium being supplemented as needed. Before connecting to CPB each patient received 3-4 $mg.kg^{-1}$ bovine lung heparin (Heparin Léčiva inj.) into the central vein to achieve an activated coagulation time (ACT) longer than 400 s. The cardiopulmonary bypass with twin-head roller pumps, Cobe™ capillary membrane oxygenator (Cobe Optima™ Hollow Fiber Sealed Hardshell System), arterial line filtration and non-heparinized circuit was performed using mild hypothermia 33 ^{o}C for coronary artery bypass grafting (CABG) or moderate hypothermia (26-28 ^{o}C) for valve surgery and combined procedures (valve surgery + CABG). The oxygenator was of the hollow fiber type (blood outside fiber) using microporous polypropylene, surface area 1.7 m^2. A cold blood cardioplegia was used for myocardial protection. After successful weaning from CPB protamine (Protamin-Sulfat 1 % inj., Galenika, Yugoslavia) was given in a dose of 1.0-1.5 mg per mg of administered heparin into a peripheral vein to achieve ACT less than 140 s. Inotropic and vasoactive drugs were supplemented as needed. Aprotinin or antifibrinolytics were not used during our study.

Thromboelastography was performed on native blood samples in the standard manner according to the manufacturer´s manual (Thromboelastograph® Coagulation Analyzer Model 3000 C Operational Manual, Haemoscope Corp., Skokie, Illinois, USA). All samples

were withdrawn from the arterial line after at least 5 ml of blood were aspirated and discarded. The baseline TEG assay was performed after the induction of anesthesia and before the start of the operation (TEG 1), the second one after the successful weaning from CPB and after heparin reversal by protamine (TEG 2). All TEG assays were processed by TEG Analysis Program software, version 1.28, supplied by the manufacturer. The results were exported and further processed using Microsoft Excel 97™, statistical evaluation was performed using SPSS Software.

The age and gender of the patients were noted. The following parameters on the TEG tracing were assessed: reaction time R, clotting time K, α-angle, maximum amplitude MA, clot lysis Ly30 and Ly 60 (Fig. 1). Reaction time R corresponds to the whole blood clotting time, i.e. it is the time from the beginning of blood coagulation to the formation of the first fibrin fiber. It is measured from the beginning to the point where the trace is 2 mm wide. Clotting time K corresponds to the time when the blood clot reaches an arbitrarily predefined strength, i.e. the width of the TEG trace equal to 20 mm. The α-angle is the angle between the longitudinal axis and the tangential line through the point where the trace is 2 mm wide. K and α-angle indicate the rate of clot formation. MA (maximum amplitude) is the maximum width of the trace representing the strength of the blood clot. It is a function of platelet number and fibrinogen concentration. It does not rise linearly but curvilinearly with increasing number of platelets and fibrinogen concentration. The differences in the strength of the clot are therefore better seen using the shear modulus of the clot G in $dyn.cm^{-2}$ instead of MA (Spiess *et al.* 1994). G is computed from MA according to the equation (Spiess *et al.* 1994):

$$G = 5000*MA/(100-MA)$$

TEG parameters Ly30 and Ly60 describe the stability of the blood clot. Ly30 (clot lysis 30 min after MA) is a decrease of the amplitude of the curve 30 min after the MA had been achieved, Ly60 (clot lysis 60 min after MA) is a decrease of the amplitude of the curve 60 min after the MA. Both decrements are expressed as a ratio of the maximum amplitude (in %).

Table 1. TEG parameters before and after CPB*

TEG parameter	Normal range	TEG 1	TEG 2	P
R (mm)	19-28	29.9±12.2 (0.5-104; 27)	22.3±9.9 (0-87; 19.5)	<0.05
K (mm)	8-13	15.6±19.8 (4.5-242; 12)	10.5±16.2 (0-255; 8)	<0.05
α-angle (deg.)	29-43	33.5±10.9 (2.5-72; 33)	44.9±13.8 (0-81;46.5)	<0.05
MA (mm)	48-60	53.5±10,2 (3-84; 54)	53.1±10.5 (0-80; 54)	NS
G ($dyn.cm^{-2}$)	4615-7500	6295±2571 (154.6-26250; 5870)	6142±2365 (0-20000; 5870)	NS
Ly30 (%)	≤7.5	2.2±3.5 (0-32; 1.5)	4.8±9.0 (0-74.5; 2)	<0.05
Ly60 (%)	≤15.0	5.0±9.0 (0-64; 3.5)	8.5±14.3 (0-87.5; 5)	NS

TEG 1 before CPB, TEG 2 after CPB. Data are expressed as mean ± standard deviation, (range; median). Tracing speed is 2 mm/min.

The values of all parameters before (TEG 1) and after CPB (TEG 2) given as the means ± standard deviation (S.D.) together with the ranges and the median values were compared using Wilcoxon´s pair test. P value <0.05 was considered statistically significant. The data supplied by the manufacturer of the Thrombo-elastograph® were used for reference.

Results

The TEG assays performed before and after the CPB in 303 patients (227 men [74.9 %] and 76 women [25.1 %]) were evaluated. The age of the patients was 60.0±10.8 years (mean ± S.D.), range 21-78 years, median 63 years.

Parameters acquired from the samples TEG 1 and TEG 2 remained within or close to the reference ranges. The TEG parameters R, K, α-angle and Ly30 before and after the CPB were statistically different, while MA, G and Ly60 were not (Table 1). This means that the TEG assay after the CPB (TEG 2) could generally be described as a tendency towards hypercoagulation (shortened R, K and increased α–angle when compared to prebypass values) and fibrinolysis activation (increased Ly30) with unchanged strength of the blood clot (MA or G).

Table 2. TEG parameters in patients requiring reoperation for bleeding after CPB (bleeders) and in patients without reoperation (non–bleeders)*

TEG parameter	Normal range	TEG 2 – bleeders (n=14)	TEG 2 – non-bleeders (n=289)	P
R (mm)	19-28	20.7±9.4 (0-39.5; 18.8)	22.4±9.9 (0-87; 19.5)	NS
K (mm)	8-13	9.0±4.8 (0-18.5; 8)	10.6±16.5 (0-255; 8)	NS
α-angle (deg.)	29-43	39.3±15.8 (0-62; 42.3)	45.2±13.7 (0-81; 46.5)	NS
MA (mm)	48-60	50.9±15.9 (0-64; 54.8)	53.2±10.2 (0-80; 54)	NS
G (dyn.cm^{-2})	4615-7500	5816±2255 (0-8889; 6066)	6158±2373 (0-20000; 5870)	NS
Ly30 (%)	≤7.5	5.6±10.9 (0-41; 2.5)	4.7±9.0 (0-74.5; 2.0)	NS
Ly60 (%)	≤15.0	10.0±19.8 (0-70.5; 2.5)	8.4±14.1 (0-87.5; 5.0)	NS

**TEG 2 after CPB. Data are expressed as mean ± standard deviation, (range; median). Tracing speed is 2 mm/min.*

Comparing the TEG parameters after the CPB (TEG 2) in patients requiring reoperation for excess bleeding (bleeders, n=14) and in patients without bleeding (non-bleeders, n=289) no statistically significant differences were found in any TEG parameter.

Discussion

What is happening when heparinized blood comes in contact with a synthetic non-heparinized surface of CPB tubing, oxygenator, reservoir and filters? Exposure of blood to synthetic surfaces has been considered the primary source of „blood activation" (Hsu 1997). Immediately after commencing CPB plasma proteins, mainly factor XII, von Willebrand factor, fibronectin, trombospondin and fibrinogen are absorbed on the foreign surface. Platelets adhere to surface-bound fibrinogen within 1-2 minutes and their concentration in circulating blood rapidly decreases to a variable extent (as much as 30-50 %) (Hyde *et al.* 1998). Platelets also release the content of their α-granules (platelet factor 4, β-thromboglobulin, platelet-derived growth factor and others) so that the concentrations of these factors in the circulating blood increase (Hyde *et al.* 1998). The adhesion of platelets does not continue further because fibrinogen undergoes conformational changes or is displaced by high-molecular-weight kininogen. The synthetic surface thus becomes unreactive to platelets (passivated) shortly after CPB with the membrane oxygenator has commenced but factors already released can influence haemostasis even further into the period following CPB. When the bubble oxygenator is used each bubble creates a new surface and traumatization of the blood (platelet loss and protein denaturation) in such a system is higher (Edmunds *et al.* 1991, DiNardo 1998), i.e. the deterioration of blood coagulation is more profound. Much less traumatic membrane oxygenators are generally preferred for open heart surgery today, especially for long cases with CPB times longer than 1-2 hours.

In our study the strength of the blood clot desribed either by the TEG parameter MA (mm) or expressed as the shear modulus of blood clot G (dyn.cm^{-2}) did not significantly change after the CPB. Contrary to the usual assumption of hypocoagulability due to platelet dysfunction (i.e. decrease of MA or G) we found that there is a tendency towards hypercoagulation after the CPB (i.e shortened R, K with pertinent values

close to the lower limit of normal ranges, increased α-angle slightly above the upper limit and unchanged, normal MA or G).

Our study enrolled unselected cardiac surgical patients operated in our institution. Our group of patients should not differ from the population of Czech cardiac surgical patients from the late 90s in which there was also a prevalence of men (75.6 %) in their sixties (60.7±9.7 years [mean±S.D.]) (http://www.medicon.cz). Similarly, the most common surgical procedure in our group was a coronary artery bypass grafting (CABG) due to coronary artery disease. None of the patients were operated for cyanotic congenital heart disease which could influence blood coagulation.

Our study group (303 patients) is larger than the one published by Spiess *et al.* in 1987 (39 patients) and is almost equal to the group from the other study published by Spiess *et al.* in 1994 (377 patients altogether). Although the mean or median age was not mentioned in either of these studies, we might assume our patients were of similar age.

Table 3. TEG parameters before (TEG 1) and after CPB (TEG 2) in the study published by Spiess *et al.* in 1987 (n=39)

TEG parameter	TEG 1	TEG 2
R (mm)	30.0±16	24.0±24
K (mm)	18.9±26	11.3±23
α-angle (deg.)	44.1±20	35.6±20
MA (mm)	57.5±11	42.0±11
Ly60 (mm) *	50.2±17	54.7±22

** Normal value: Ly60 > MA – 5*

When the results of TEG analysis before the CPB in our study are compared with that of Spiess *et al.* from 1987 (Table 3) it can be concluded that the TEG pattern before the CPB in both studies was quite similar and that blood coagulation was essentially normal.

Similarly, in the second study published by Spiess *et al.* in 1994 (Table 4), the coagulation situation before the CPB also conformed to the published reference values although TEG parameters R and K were slightly shorter.

Table 4. TEG parameters before (TEG 1) and after CPB (TEG 2) in the study published by Spiess *et al.* in 1994

TEG parameter	TEG 1 CABG (n = 107)	TEG 1 AVR (n = 50)	TEG 1 MVR (n = 36)	TEG 1 Transplant (n = 16)
R (mm)	17.1±11.9	13.3±4.4	13.6±3.7	19.4±12.3
K (mm)	5.9±5.9	4.5±2.5	4.8±2.4	8.3±6.9
α-angle (deg.)	60.2	63.4±10.8	61.4±9.1	52.3±16.8
MA (mm)	54.4±8.6	55.1±6.9	53.8±6.7	48.7±8.0
G (dyn.cm^{-2})	6321±1950	6421±1862	6001±1379	4956±1459
Ly60 (mm) $^{\&}$	51.2±8.0	51.8±6.4	50.7±6.4	45.2±6.9
TEG parameter	**TEG 2 CABG (n = 222)**	**TEG 2 AVR (n = 69)**	**TEG 2 MVR (n = 39)**	**TEG 2 Transplant (n = 18)**
R (mm)	13.3±6.3	12.9±4.7	12.8±2.7	11.8±3.0
K (mm)	10.1±7.5†	11.0±7.1†	11.6±8.9†	7.9±4.0
α-angle (deg.)	47.0±11.9†	46.6±10.4†	45.0±12.0†	50.9±10.5
MA (mm)	39.7±8.5†	39.2±8.9†	39.2±8.9†	44.6±7.2
G (dyn.cm^{-2})	3552±1149†	3365±867†	3271±1166†	4138±1326†
Ly60 (mm) $^{\&}$	37.8±9.1†	37.7±6.3†	38.0±8.7†	43.4±7.3

CABG = coronary artery bypass graft, AVR = aortic valve replacement, MVR = mitral valve replacement, Transplant = heart transplantation. $^{\&}$ *Normal value: Ly60 > MA – 5.* † *P < 0.05 between times within groups*

However, a comparison of the results after CPB is quite different. In our patients, the values of MA as well as of G stayed almost at the same level as before CPB (MA decreased by 0.75 %, G decreased by 2.4 %), whereas in both studies reported by Spiess *et al.* MA and G decreased strikingly (MA by 27 % in the study from 1987, by 28.1 % in the study from 1994 and G by 43.6 % in 1994). The α-angle in our patients increased but in Spiess´s studies it decreased. This means that the clot in our patients was being formed more rapidly (wider α-angle) and was stronger (wider MA and higher G) than in patients reported repeatedly by Spiess *et al.*

TEG is considered as a method capable of predicting postoperative hemorrhage and the need for

reoperation even better than other coagulation tests (Spiess *et al.* 1987). We could not confirm this opinion in our study. All cases of bleeding in our patients could be surgically corrected and were not caused by impaired blood coagulation.

To elucidate the differences in the TEG pattern after CPB between our study and the studies published by Spiess *et al., it could be* assumed that main reason is due to the different type of oxygenator used (Spiess used bubble oxygenators) (personal communication, Spiess 2001).

In conclusion, we have demonstrated an almost negligible effect of CPB with a hollow fiber membrane oxygenator Cobe Optima™ on the strength of the blood clot as described by the TEG parameter MA or expressed as the shear modulus G. Our finding differs from the previous results obtained repeatedly by Spiess *et al.*, who demonstrated a significant decrease in MA or in G. Comparing the TEG from pre- and post-CPB assays in our study, there was a statistically significant tendency towards hypercoagulation. Such data have not been available in the literature till now. The use of hollow fiber membrane oxygenators has been demonstrated to be clinically advantageous as it presents less opportunities for derangement of hemostasis.

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Reprint requests

Dr. M. Horáček, Department of Anesthesiology and Critical Care Medicine, Motol University Hospital, V Úvalu 84, 150 06 Prague 5, Czech Republic.