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Similarities in Thromboelastometric (ROTEM®) Findings between Humans and Baboons

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ABSTRACT

Introduction: Interest in visco-elastic testing in different clinical scenarios has increased but few data are available on thromboelastometric findings in primates.
Materials and Methods: Blood cell count (hemoglobin, hematocrit, platelet count), coagulation parameters (prothrombin time, International Normalized Ratio, fibrinogen), and ROTEM® (Tem International GmbH, Munich, Germany) variables were analyzed using blood from 25 anesthetized male baboons and 21 non-anesthetized healthy volunteers. The platelet component of the clot was calculated as the difference in maximum clot elasticity (MCE) between the whole blood clot (EXTEM test) and the fibrin-based clot (FIBTEM test). In subgroups of each species, 10 μg abciximab was added to the regular FIBTEM reagent (cytochalasin D) for additional platelet inhibition.
Results: Blood cell count was comparable between humans and primates. Both fibrinogen concentration ($p<0.0001$) and maximum clot firmness (MCF) in FIBTEM assays were significantly lower in baboons ($p>0.0001$, and $p=0.006$, respectively). PT, INR, and clotting time in NATEM assays were significantly prolonged in humans compared with baboons. MCF in NATEM, EXTEM and INTEM assays was not different between baboons and humans. Clot lysis in NATEM, EXTEM and INTEM assays was significantly higher in humans ($p<0.0001$). In contrast FIBTEM clot lysis was significantly higher in baboons ($p=0.01$). Addition of abciximab into the FIBTEM assay resulted in a significant reduction in MCF and MCE ($p<0.001$) and, consequently, the platelet component increased similar in both humans and baboons ($p<0.001$).
Conclusion: Activated ROTEM® tests revealed broad similarities between humans and baboons. ROTEM® assays developed for use in humans can also be used in baboons.

Introduction

Animal experiments provide important insights into basic and experimental hemostasis and thrombosis research [1]. However, the relevance of routinely used animal models, such as mice, rats or pigs, in translating these results to humans is still under discussion [2]. Significant differences between human and animals have been reported for standard coagulation parameters like prothrombin time (PT) and activated partial thromboplastin time (aPTT), and also for more advanced coagulation tests such as thrombin generation [3]. For example, the initiation of the coagulation process in rats is much more accelerated than in humans [3]. Other studies reported very short aPTT in pigs compared with humans [4,5].

Baboons have been used as an important animal model to study coagulation abnormalities in different experimental settings [6–9]. Coagulation profiles of primates have revealed many similarities to humans, making these animals more suitable as study subjects than other species [10].

In recent years, point-of-care monitoring techniques such as thrombelastography (TEG®) and thromboelastometry (ROTEM®) have gained increasing attention in a variety of clinical scenarios as well as in experimental research [11–13]. ROTEM® provides a comprehensive overview of the whole clotting process, measuring the initiation of coagulation, the speed of clot formation, the final clot quality, and clot lysis [14]. However, until now only limited data describing the normal values of ROTEM® parameters in primates have been available [15].

The aim of this study was to perform normal thromboelastometric testing in baboons and to compare the results with those in humans. The hypothesis was that thromboelastometric measurements in baboons are comparable to those in humans.

Abbreviations: BW, body weight; CFT, clot formation time; CT, clotting time; EDTA, ethylenediaminetetraacetic acid; EXTEM, extrinsically activated test; FIBTEM, extrinsically activated test with cytochalasin D; Hb, hemoglobin; Hct, hematocrit; INTEM, intrinsically activated test; INR, International Normalized Ratio; LI, lysis index; MCE, maximum clot elasticity; MCF, maximum clot firmness; ML, maximum lysis; NATEM, non-activated test; PT, prothrombin time; SD, standard deviation.

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Materials and Methods

The experimental protocol as part of another study was approved by the Institutional Animal Care Use Committee at Free State University (Bloemfontein, South Africa). All experiments were performed under the conditions described in the Guide for the Care and Use of Laboratory Animals as defined by the National Institutes of Health.

Data from twenty-five healthy male Chacma baboons of the strain *Papio ursinus*, with a median weight of 17.2 (15.8–21.75) kg, and age between 3 – 10 years were included in the study performed for additional purposes. To ensure good quality conditions the animals were quarantined for 3 months before the study. The primates were fasted overnight before experiments but had free access to water.

The human subjects included in the study (21 healthy adult volunteers, 8 male and 13 female with a mean age of 41.9 ± 7.3 years) donated blood for thromboelastometric measurements performed with the aim to establish internal reference ranges and run quality controls according to the manufacturer´s recommendations. After informed consent, blood samples were collected from previously healthy Caucasian volunteers. Only subjects without any history of bleeding or venous thromboembolism were selected as blood donors. Furthermore the volunteers did not take any medication influencing platelet function or the hemostatic system.

Premedication, Anesthesia and Instrumentation of the Animals

Premedication of the baboons was performed by intramuscular injection of 6–8 mg/kg BW ketamine (Ketalar®, Pfizer, Vienna, Austria). After placing in supine position, the right cubital vein was canulated and anesthesia was induced using 5 mg/kg BW sodium pentobarbital (Sandoz GmbH, Kundl, Austria). Afterwards, the trachea was intubated and the animals were attached to a respirator and ventilated in a pressure-controlled mode (Evita 2, Dräger, Lübeck, Germany).

Anesthesia was maintained by continuous infusion of 0.8 mg/kg/h sodium pentobarbital, 0.8 μg/kg/h sufentanil (Janssen, Vienna, Austria), and 1 mg/kg/h rocuronium (Organon, Oss, Netherlands). The fraction of inspired oxygen was kept at 30% and the arterial partial pressure of CO_2 was maintained at 35–45 mmHg. Body temperature was kept at 37 °C.

For blood pressure monitoring an arterial catheter was inserted in the right femoral artery using Seldinger technique. The cephalic vein was used for fluid therapy.

Blood Sampling from Animals

After establishing stable hemodynamic conditions, baseline blood samples were drawn from the cubital vein. The first 2 mL of blood drawn were discarded. For blood cell count (hematocrit [Hct], hemoglobin [Hb] concentration and platelet count) blood was collected in 3 mL K_3EDTA tubes containing 1.6 mg/mL ethylenediaminetetraacetic acid (EDTA; Vacuette®, Greiner Bio-One GmbH, Linz, Austria). For standard coagulation tests and ROTEM® analyses, 2.7 mL blood were collected in propylene tubes containing 0.3 mL of buffered 3.2% trisodium citrate, resulting in a citrate:blood volume ratio of 1:9 (Vacuette®, Greiner Bio-One GmbH, Linz, Austria).

Blood Sampling from Human Controls

Blood samples from humans were drawn after minimum stasis from the cubital vein. The first 2 mL of blood were discarded, then 3 mL blood were collected in K_3EDTA tubes containing 1.6 mg/mL EDTA (S-Monovette®, Sarstedt AG & Co, Nümbrecht, Germany) for full blood cell count. Blood samples for standard coagulation tests and ROTEM® measurements were collected in 3 mL propylene tubes containing 0.3 mL buffered 3.2% trisodium citrate, giving a citrate:blood volume-ratio of 1:9 (S-Monovette®, Sarstedt AG & Co. Nümbrecht, Germany).

ROTEM® Analyses

Four ROTEM® analyses were performed within minutes after sample collection: a non-activated test (NATEM), which was recalcified with no further modification; an extrinsically activated assay using recombinant tissue factor (EXTEM); an intrinsically activated test using kaolin (INTEM); and an extrinsically activated test using recombinant tissue factor with cytochalasin D (FIBTEM) added. Cytochalasin D inhibits platelet function by blocking the platelet cytoskeleton; therefore, this test provides information on the fibrin-based component of the clot. Lang et al. reported that the combination of abciximab, a potent glycoprotein IIb/IIIa receptor antibody, and cytochalasin D resulted in further significant inhibition of platelet function [16]. Consequently, we performed in a subset of 5 animals and in 10 human blood samples an additional analysis using the standard FIBTEM and 10 μg of abciximab, a monoclonal antibody which inhibits platelet glycoprotein receptor IIb/IIIa, *in vitro* in order to enhance platelet inhibition. All ROTEM analyses in humans and baboons were performed by the same investigator (HS).

The following parameters were measured using ROTEM®: clotting time (CT [s], the time from the start of the assay until formation of a clot with an amplitude of 2 mm); clot formation time (CFT [s], time from the end of CT [amplitude of 2 mm] until a clot firmness of 20 mm is achieved); maximum clot firmness (MCF [mm], the peak strength of the clot, resulting from the interaction of fibrin, activated platelets and factor XIII [FXIII]); the lysis index at 30, 45 and 60 minutes (LI 30, LI 45, LI 60 [%], clot firmness at 30, 45 or 60 minutes after CT, as a percentage of MCF, indicating the speed of fibrinolysis); and maximum lysis (ML [%], the maximum reduction in clot firmness observed after MCF has been reached, given as a percentage of the MCF value). Hyperfibrinolysis was defined as a reduction of the MCF > 15% [14].

MCF, which is commonly used to asses clot strength, does not reflect the physical properties of clot strength according to Hooke's law (i.e., the relationship between MCF and maximum clot elasticity [MCE] is curvilinear). Thus, MCE is more appropriate to calculate clot strength according to the following equation: MCE = (MCF × 100)/100-MCF [17].

Table 1
Blood cell counts and coagulation parameters of baboons and humans.

Parameters	Baboon	Human	p
Hemoglobin level (g/L)	14.65 (14,23 - 15.38)	14.3 (13.25 - 15.55)	ns
Hct	44.8 (42.53 - 46.08)	42.45 (40.5 - 44. 58)	ns
Platelet count (G/L)	261 (222–306.5)	242.5 (209.5 - 306.3)	ns
PT (sec)	9.95 (9.33 - 10.88)	11.95 (11.48 - 12.68)	< 0.0001
INR	0.82 (0.77 - 0.90)	0.89 (0.87 - 0.98)	0.0031
Fibrinogen concentration (mg/dL)	102.6 (78.5 - 123.6)	291 (260.3 - 317.5)	< 0.0001

Data are expressed as median (interquartile range). aPTT, activated partial thromboplastin time; Hb, hemoglobin; Hct, hematocrit; INR, International Normalized Ratio; PT, prothrombin time; ns, not significant.