Thrombosis Research 147 (2016) 115–121

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Platelet function in baboons and humans — A comparative study of whole blood using impedance platelet aggregometry (Multiplate®)☆·☆☆

Martin Ponschab, MD [a,b,1], Martijn van Griensven, PhD [c], Stefan Heitmeier, PhD [d], Volker Laux, PhD [d], Christoph J. Schlimp, MD [a], Andreas Calatzis, MD [e], Soheyl Bahrami, PhD [a], Heinz Redl, PhD [a], Herbert Schöchl, MD [a,f,*,1]

[a] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Vienna, Austria
[b] Department of Anaesthesiology and Intensive Care, AUVA Trauma Hospital Linz, Academic Teaching Hospital of the Paracelsus Medical University, Salzburg, Austria
[c] Experimental Trauma Surgery, Department of Trauma Surgery, Klinikum rechts der Isar, Technical University Munich, Germany
[d] Bayer Pharma AG, Acute Care Research, Wuppertal, Germany
[e] Verum Diagnostics, Munich, Germany
[f] Department of Anaesthesiology and Intensive Care, AUVA Trauma Centre, Salzburg, Austria

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 28 September 2016
Accepted 5 October 2016
Available online 6 October 2016

Keywords:
Baboons
Humans
Coagulation
Platelets
Platelet aggregometry

ABSTRACT

Background: Platelets play a pivotal role in coagulation, inflammation and wound healing. Suitable animal models that have the potential to mimic human platelet function are limited. The objective of the current study was to compare platelet aggregation response in the whole blood of baboons and humans using impedance aggregometry.

Methods: Blood was drawn from 24 anesthetised male baboons and 25 healthy volunteers. The platelet aggregation response was determined by impedance aggregometry (Multiplate®). Platelets in the hirudinised whole blood samples were stimulated with four different activators: adenosine diphosphate (ADP), collagen (COL), thrombin receptor activating peptide-6 (TR1AP), and activation of PAR-4 thrombin receptor subtype (TR4AP) at standard concentrations. Higher than standard concentrations were tested in a subgroup of the animals.

Results: The cell counts showed no differences between baboons and humans. The platelet aggregation response was significantly lower in baboons compared to humans when stimulated with the platelet agonists ADP ($p < 0.0001$), COL ($p = 0.021$) and TR4AP ($p < 0.0001$). TR1AP did not stimulate platelet aggregation in the baboon blood. Doubling the concentration of ADP and of TR4AP significantly increased the AUC compared to the standard concentration. In contrast, increased COL levels did not further increase the AUC.

Conclusion: The current study revealed that testing the platelet function in baboon blood by impedance aggregometry is feasible with ADP, COL and TR4AP, but not with TR1AP. Compared to humans, the aggregation response is lower in baboons. Considering the limitations in accordance to these results, baboons might represent a potential species for further platelet research.

1. Background

Platelets play a pivotal role in coagulation, inflammation and wound healing [1]. Thrombocytes are activated by sub-endothelial collagen, which causes changes in platelet morphology, promotes the expression of glycoprotein receptors on their surface and stimulates the release of platelet granule content [1,2]. This complex mechanism facilitates the initial adhesion of platelets to the damaged sub-endothelium and the subsequent platelet aggregation. Moreover, platelets provide a matrix for the assembly of coagulation proteins, resulting in a significant thrombin burst [3]. Furthermore, platelets have been identified as drivers of vascular inflammation. Platelets recruit leucocytes to the injured vessel wall and promote the release of microvesicles and cytokines with inflammatory properties [4–9]. Thus, platelets are involved

☆ Level of evidence: IV.
☆☆ Study type: Observational animal in vivo study.

* Corresponding author at: Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Centre, Vienna, Austria. Department of Anesthesiology and Intensive Care Medicine, AUVA Trauma Centre Salzburg, Academic Teaching Hospital of the Paracelsus Medical University, Salzburg, Dr. Franz Rehrl Platz 5, 5020 Salzburg, Austria.
E-mail addresses: martin.ponschab@auva.at (M. Ponschab), Martijn.vanGriensven@tum.de (M. van Griensven), stefan.heitmeier@bayer.com (S. Heitmeier), volker.laux@bayer.com (V. Laux), christoph.schlimp@trauma.lbg.ac.at (C.J. Schlimp), andreas.calatzis@roche.com (A. Calatzis), soheyl.bahrami@trauma.lbg.ac.at (S. Bahrami), heinz.redl@trauma.lbg.ac.at (H. Redl), herbert.schoechl@auva.at (H. Schöchl).
[1] The authors all contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.thromres.2016.10.005
0049-3848/

in both haemostasis and inflammation [6–9]. There is growing interest in the crucial role that platelets play in many physiological and pathophysiological processes [9].

Platelet function testing is essential to diagnose inherited and acquired platelet disorders [10,11]. Among the various techniques to detect and monitor platelet reactivity, impedance aggregometry by Multiplate® (multiple platelet function analyzer) has earned increasing interest over the last decade [12–15]. Multiplate® was initially developed as a point of care device for the rapid assessment of platelet aggregation in whole blood [13]. This method allows for the evaluation of platelet function under low shear conditions; thus, platelets can interact with other blood cells [16,17].

For investigators, it is of fundamental interest to have adequate experimental animal models available that allow for the translation of results of animal studies into the context of the human environment. However, there are substantial differences in the physiological and pathophysiological mechanisms of different species e.g., rodents or pigs, compared to human subjects with respect to their platelet function [18,19]. Non-human primates, such as baboons, represent an interesting species that is genetically closely related to humans. Recent research has revealed close similarities between humans and baboons in terms of both platelet function and visco-elastic test results [20,21]. However, also significant differences have been observed [22].

The aim of the current study was to investigate whether using Multiplate® to measure the platelet aggregation following stimulation with different platelet agonists delivers comparable results between humans and baboons.

2. Methods

The experimental protocol was approved as part of another study by the Institutional Interfaculty Animal Ethics Committee at Free State University (Bloemfontein 9300, South Africa) under protocol number 03/2010. All of the 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-four healthy male Chacma baboons of the strain *Papio ursinus* were included in the study. The animals were quarantined for 3 months before the study to ensure that they were exposed to high quality living conditions. The primates received no food overnight before experiments but had free access to water.

2.1. Premedication, anaesthesia and instrumentation of the animals

The animals received premedication by intramuscular injection of 6–8 mg/kg BW ketamine (Ketalar®, Pfizer, Vienna, Austria). The baboons were placed in the supine position, and the right cubital vein was cannulated. Anaesthesia was delivered as a 5 mg/kg BW sodium pentobarbital (Sandoz GmbH, Kundl, Austria) injection. 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). Anaesthesia 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 mm Hg. The body temperature was maintained at 37 °C. The cephalic vein was used for fluid therapy. An arterial catheter was inserted into the right femoral artery for blood pressure monitoring.

2.2. Blood sampling from animals

After establishing stable haemodynamic conditions, baseline blood samples were drawn from the cubital vein. The first 2 mL of blood drawn were discarded. For the blood cell counts (haematocrit [Hct], haemoglobin [Hb] concentration and platelet count [Plt]), blood was collected in 3 mL K3EDTA tubes containing 1.6 mg/mL ethylenediaminetetraacetic acid (EDTA; Vacuette®, Greiner Bio-One GmbH, Linz, Austria). The blood samples for platelet aggregation analyses were collected in hirudin-coated 3 mL tubes (Roche Diagnostics International Ltd., Rotkreuz, Switzerland).

2.3. Blood sampling from human controls

Following the approval from the Ethics Committee of the Austrian Worker's Compensation Board (application number 15/2013), 25 healthy Caucasian volunteers were included in the study after signing informed consent. Only subjects without any history of bleeding or venous thromboembolism and those not receiving any anticoagulant or antiplatelet medication within the last 14 days were selected as blood donors. Blood samples were drawn after minimum stasis from the cubital vein, and the first 2 mL of were discarded.

The same blood collection systems were used for humans as described for baboons.

2.4. Cell count measurements

The blood cell counts were measured with a Cell-Dyn 3700® Hematology Analyzer (Abbott GmbH und Co. KG, Abbott Diagnostics Europe, Wiesbaden, Germany).

2.5. Impedance aggregometry (Multiplate®) measurements

Impedance aggregometry was performed using multiple electrode aggregometry (Multiplate®, Roche Diagnostics International Ltd., Rotkreuz, Switzerland).

Human and baboon platelets were stimulated simultaneously with four different activators: adenosine diphosphate (ADP), collagen (COL), thrombin receptor activating peptide-6 (TRAP) for PAR-1 receptor, and a PAR-4 thrombin receptor subtype (PAR4) agonist. The TRAP-6 agonist provided by the company is a specific agonist that strongly activates the PAR-1 receptor, while the PAR-4 receptor agonist is selective for the PAR-4 receptor only. For easier understanding, we named the TRAP-6 agonist Thrombin-receptor-1-activating-peptide (TR1AP), and the PAR-4 receptor agonist Thrombin-receptor-4-activating-peptide (TR4AP). The baboon blood samples were collected in hirudin-containing tubes. For the human controls, the recommended agonist concentration was used. For the baboons, we used the standard concentration, and in some animals, we used 1.5-fold and 2-fold increased concentrations for the ADP and COL. Thus, the final concentrations for the ADP were 6.5 μM, 9.75 μM and 13 μM. For COL we used bovine-type-collagen I. Collagen type I is a triple helix consisting of one alpha-2 chain and two alpha-1 chains, alpha-1 is 139 kDa and alpha-2 is 129 kDa. So the trimeric form is ~400 kDa. The final concentration was 3.2 μg/mL, this means that expressed in molarity we used a concentration of 8 nM, 12 nM and 16 nM. After the measurement of samples from six baboons, standard thrombin-receptor-1-activating peptide (TR1AP) testing was terminated as it did not result in platelet activation. For the remaining 18 baboons, we initiated the activation of the PAR-4 thrombin receptor subtype by a selective PAR-4 receptor agonist (TR4AP). We investigated four different concentrations of PAR-4 agonists: TR4AP with 397 μM (the company's recommended concentration), with twice the concentration (794 μM), with 4 times the concentration (1588 μM), and with 8 times the concentration (3176 μM). All of the reagents were obtained from Verum Diagnostica GmbH, Munich, Germany.

To ensure adequate resting time, the Multiplate® measurements were performed approximately 30 min after the collection of the blood samples. All of the analyses were performed according to the manufacturer's recommendations. Normal saline 0.9% solution was used to dilute the agonists to their final concentration. The platelet function was assessed in single-use test cells that incorporated two independent sensor units, on which the platelet aggregation occurred. After