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Composition of growth factors and cytokines in lysates obtained from fresh versus stored pathogen-inactivated platelet units

Felix Sellberg [a], Erik Berglund [b], Martin Ronaghi [a], Gabriel Strandberg [a], Helena Löf [a], Pehr Sommar [c], Norbert Lubenow [a], Folke Knutson [a], David Berglund [a,*]

[a] Department of Immunology, Genetics and Pathology, Section of Clinical Immunology, Uppsala University, Sweden
[b] Department of Transplantation Surgery, Division of Transplantation Surgery, CLINTEC, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden
[c] Department of Molecular Medicine and Surgery, Section of Plastic Surgery, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden

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ABSTRACT

Background: Platelet lysate is a readily available source of growth factors, and other mediators, which has been used in a variety of clinical applications. However, the product remains poorly standardized and the present investigation evaluates the composition of platelet lysate obtained from either fresh or stored pathogen-inactivated platelet units.
Materials and Methods: Platelet pooled units (n = 10) were obtained from healthy blood donors and tested according to standard procedures. All units were pathogen inactivated using amotosalen hydrochloride and UVA exposure. Platelet lysate was subsequently produced at two separate time-points, either from fresh platelet units or after 5 days of storage, by repeated freeze-thaw cycles. The following mediators were determined at each time-point: EGF, FGF-2, VEGF, IGF-1, PDGF-AB/BB, BMP-2, PF4, TGF-β isoform 1, IL-1β, IL-2, IL-6, IL-10, IL-12p70, 1L-17A, TNF-α, and IFN-γ.
Results: The concentration of growth factors and cytokines was affected by time in storage. Notably, TGF-β, PDGF-AB/BB, and PF4 showed an increase of 27.2% ($p < 0.0001$), 29.5% ($p = 0.04$) and 8.2% ($p = 0.0004$), respectively. A decrease was seen in the levels of IGF-1 and FGF-2 with 22% ($p = 0.041$) and 11% ($p = 0.01$), respectively. Cytokines were present only in very low concentrations and all other growth factors remained stable with time in storage.
Conclusion: The composition of mediators in platelet lysate obtained from pathogen-inactivated platelet units differs when produced from fresh and stored platelet units, respectively. This underscores the need for further standardization and optimization of this important product, which potentially may influence the clinical effects.

Abbreviations: BC, buffy coat; BMP-2, bone morphogenic protein-2; CPD, citrate phosphate dextrose; ELISA, enzyme-linked immunosorbent assay; EGF, epidermal growth factor; FGF-2, fibroblast growth factor-2; HGF, hepatocyte growth factor; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor-1; IL, interleukin; MBA, multiplex bead assay; PL, platelet lysate; PDGF-AB/BB, platelet-derived growth factor-AB/BB; PF4, platelet factor 4; PRP, platelet-rich plasma; TPC, thrombocyte particle concentration; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

* Corresponding author. Dag Hammarskjölds väg 20, Rudbeck Laboratory, Uppsala 751 85. Fax: +4618505261.
E-mail address: david.berglund@igp.uu.se (D. Berglund).

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1. Introduction

The clinical use of platelet units is well established for the prevention and treatment of bleeding. Over the last decades, platelets have also been clearly demonstrated to be of use in the field of regenerative medicine. Soluble factors stored in platelets, such as growth factors and chemokines, can readily be obtained by e.g. repeated freeze-thaw cycles [1], direct platelet activation [2], sonication [3] or solvent/detergent treatment [4] to obtain platelet lysate (PL). Clinical data are emerging that demonstrate the effectiveness of platelet-derived growth factors to treat a variety of conditions, including chronic wounds [5,6], burn injuries [7], osteoarthrosis [8], as well as tendon [9] and nerve injuries [10]. The rationale for using PL to improve the healing and regeneration of human tissues is further supported by studies demonstrating that PL efficiently propagates several cell types when cultured *in vitro*, ranging from leukemia and epithelial tumor cell lines to mesenchymal stromal cells and endothelial colony forming progenitor cells [11–13]. Indeed, PL is being extensively used in GMP-compliant culturing of cellular therapeutics [14,15]. However, preparations of PL are poorly standardized and no consensus is available how to produce PL and define its composition.

Large quantities of "off-the-shelf" PL can be produced from allogeneic donors, whereas the use of autologous PL imposes both practical challenges as well as limitations on the quantity that can be obtained. Arguably, the only advantage of using autologous PL is to avoid the risk of transmitting donor-derived pathogens. With the use of pathogen-inactivation techniques, such as the Intercept® system [16–18], the risk-benefit assessment is shifted further in favor of using allogeneic PL. In particular, local blood banks are well suited to conveniently produce PL from healthy blood donors, and it is attractive to use out-dated platelet units to obtain PL. However, little is known about the composition of growth factors in PL produced from fresh versus stored platelet units, in particular when pathogen-inactivation techniques have been applied. We hypothesized that the composition of PL may change over time and investigated the composition of growth factors and cytokines in PL produced from either fresh or stored pathogen-inactivated platelet units.

2. Materials and methods

2.1. Preparation of platelet units

The study was conducted within the quality system of the Uppsala University Hospital blood bank after a decision in the department board. The selection of donors and the blood donation procedure followed the Swedish Guidelines for Blood Banks. Briefly, whole blood (450 ± 45 mL) was collected in 63 mL citrate phosphate dextrose (CPD) solution from healthy blood donors, mean age 46 ± 2.6 years with 57% female donors. Whole blood was cooled to room temperature (22 ± 2 °C) on 1,4-butanediol plates and stored overnight. Buffy coats (BC) were subsequently obtained by centrifugation followed by separation of erythrocyte concentrates and plasma according to validated protocols using the Macopress Smart REVO device (MacoPharma International GmbH, Tourcoing, France) with bottom-and-top separation. Individual platelet units were produced from seven units of pooled ABO-identical BC within 24 hours of the original blood collection using a sterile docking device. Pooled BC were supplemented with PAS-E additive solution and centrifuged in the Terumo Automated Centrifuge and Separator Integration System (TACSI®, Terumo Europe, Tuttlingen, Germany) to obtain leukoreduced platelet concentrates subsequently pooled to obtain platelet units, with a residual plasma concentration of approximately 35%.

2.2. Photochemical pathogen inactivation

Platelet units ($n = 10$) were pathogen-inactivated using the Intercept® system [19] (Cerus Europe BV, Amersfoort, Netherlands) immediately after platelet processing, according to the manufacturer's instructions. Briefly, using a sterile docking device, the platelet unit, amotosalen hydrochloride (A-HCl) container, and the illumination container were connected in series. The leukoreduced platelet unit was passed through the container with A-HCl and transferred into the illumination container. After thorough mixing, air was expelled from the illumination container before separating it by heat seal. The container was subsequently exposed to a 3 J/cm^2 treatment with UVA light (320–400 nm, Intercept UVA Illuminator, Cerus) under agitation (60 cycles/min). After illumination, a compound adsorption device (CAD, Cerus) was sterile-connected to eliminate A-HCl residues, where the UVA-treated platelets were transferred into the CAD and stored at room temperature under agitation for 6 hours. Finally, the platelet unit was transferred into a plastic storage container.

2.3. Quality assessment and lysis of platelets

Platelet units were stored at room temperature (22 ± 2 °C) under agitation on a flat-bed shaker. Samples were obtained for quality assessment and lysis from the same platelet units ($n = 10$) at two time points, either within 24 hours of production (fresh platelets) or after five days of storage at room temperature (stored platelets). The quality assessment included the determination of pH, glucose level and lactate content analyzed by OMNI S Cobas b221 system (Roche Diagnostics GmbH, Mannheim, Germany). The thrombocyte particle concentration (TPC) was measured using ABX Micros ES 60 (Horiba Medical, Montpellier, France). In addition, visual inspection for the swirling phenomenon was performed on each platelet unit from which samples were taken, graded on a three level scale as no swirling, intermediate or full swirling. Platelet units were lysed by freezing at −70 °C and thawing at 37 °C using a water bath for two consecutive cycles to obtain PL.

2.4. Growth factor and cytokine profiles

The composition of growth factors and cytokines in PL were analyzed using multiplex bead (MBA) or enzyme-linked immunosorbent assays (ELISA). PL were analyzed for the following growth factors and cytokines using the Luminex xMAP MBA technology (Merck Millipore, Darmstadt, Germany) on a MAGPIX instrument (Luminex