VIA MEDICA

CONGRESS REPORT

Journal of Transfusion Medicine
2023, vol. 16, no. 1, 31–34
DOI: 10.5603/JTM.2023.0003

ISSN 1689-6017
e-ISSN 2080-1505

Innovative applications of platelet derivatives in light of information presented during the 2022 virtual congress of the International Society of Blood Transfusion (ISBT); selected issues

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Platelet concentrates (PCs) have long been in use in the hemotherapy of thrombocytopenia and accompanying symptoms of bleeding disorder. In the 1980s, the stimulating effect of platelet-derived growth factors on cell metabolism was observed in animals. The factors include: platelet-derived growth factor (PDGF-aa, PDGF-ab and PDGF-bb), transforming growth factor beta (TGF-B1 and TGF--B2), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF). The important role of biological mediators that regulate early proliferation and participate in the differentiation of all cell types relevant for regeneration of soft and hard tissues has also been confirmed. Since then, the interest in platelet-based products has significantly increased, and the use of platelet-rich plasma or PC, has become the subject of a growing number of publications not only in the field of regenerative medicine (e.g. limb ulcers, osteoarthritis or bone graft fixation), but also in plastic surgery or cosmetology. Frequent reports on the clinical use of platelet derivatives in the form of lysates have been appearing. Since the 1990s, there has also been growing interest in products containing autologous serum, e.g. for patients with dry eye syndrome (DES).

During the virtual congress of the International Society of Blood Transfusion (ISBT) held on June 4–8, 2022, several papers on the unconventional use of platelet derivatives were presented. The related issues were accessible during poster sessions: "Blood products — The versatility of platelets and their products" and "Cellular therapies — New avenues of cellular therapy" [1–3].

In the paper "Although expired, platelets are now starting a new life in medicine", Burnouf' (Taipei University of Medical Sciences) discusses an alternative use of expired PCs. The study results demonstrate that allogeneic platelets in expired PCs can be a valuable material for preparation of human platelet lysates (HPL) or platelet biomaterials for clinical use in cell therapies and regenerative medicine. The scientific rationale for these clinical applications is the large amount of trophic factors in platelets (growth factors, cytokines, chemokines, antioxidants, inflammatory and anti-inflammatory factors, etc.) which are indispensable for coordination and promotion of cell growth and tissue regeneration. Lysates obtained from expired PC are stored frozen in blood establishments and delivered to the HPL manufacturer under previously agreed conditions. The process of lysate preparation consists in freezing and thawing of PCs in order to promote platelet lysis. The result is the release of biologically active substances from platelet granules. The obtained protein solution is subjected to centrifugation (or filtration) to remove residual morphotic elements. The final step involves sterile filtration, bottling, freezing and storage. Lysates are used, i.a. to supplement culture media for mesenchymal stromal cells (MSC) (bone marrow, adipose tissue, Wharton's jelly or tooth pulp), as alternative to the most common fetal bovine or horse serum. With zoonotic serum there is always the higher risk of both culture contamination and transfer to the medium of: xenoproteins, toxins, growth inhibitors and zoonotic infectious agents such as bovine herpesvirus Type-1 (BHV-1) which

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causes infectious bovine rhinotracheitis (IBR/IPV) or bovine viral diarrhoea virus (BVDV) [1].

Such risk is eliminated if human lysate is used. Experimental studies have also confirmed the effectiveness of adding platelet lysate to the growth medium of corneal epithelial cells, chondrocytes, fibroblasts and endothelial cells.

The potentially beneficial effect of platelet lysates in regenerative medicine was based on the extensive clinical experience with allogeneic in autologous platelet rich plasma (PRP) in orthopedic surgery (e.g. osteoarthritis), soft tissue wound healing (e.g. resistant ulcers), maxillofacial surgery, dentistry and implantology, sports medicine and ophthalmology. Allogeneic PCs are also used in preparation of eye drops of human origin (EDHO) in the form of serum or platelet lysates used for the management of the dry eye syndrome. In the preclinical stage are currently studies on the assessment of neuroprotective and neuro-regenerative effects of lysates in the management of neurodegenerative disorders and brain tissue injuries [4–6].

As emphasized in another study — "Preparation and neuroprotective activity of nanofiltered human platelet lysate in Parkinson's disease and traumatic brain injury models" — which was presented during the Session: Cellular therapies — New avenues of cellular therapy, platelet lysates may be the future of biotherapy of neurodegenerative diseases and injuries of the central nervous system. A homogeneous series of lysate are obtained by pooling PC and introducing pathogen inactivation methods to ensure product safety. Burnouf et al. presented studies evaluating the impact of nanofiltration during the process of lysate preparation from pooled PCs on the effectiveness of therapy of Parkinson's disease and brain tissue damage. The PC pool was obtained from 50 PC units first subjected to initial filtration (0.2 and 0.1 μm) and then to nanofiltration (0.001 m^2 Planova 20 N (19 nm)) at a constant flow rate of 0.1 ml/min and under controlled pressure. The mouse parvovirus (MVM) model and the immuno-qPCR method were used to assess the degree of viral reduction. Total protein content was determined by the Bradford method, and the quantification of neurotrophic factors was determined by ELISA tests. Platelet-derived extracellular vesicles (PEVs) were studied by dynamic light scattering (DLS) and nanoparticle tracking (NTA). Proteomic analysis was performed with liquid chromatography and mass spectrometry. In vivo cell models were used to evaluate the neuroprotective and anti-inflammatory functions of platelet lysates subjected to nanofiltration. Differentiated human midbrain dopamine (DA) neurons were used as a model for Parkinson's disease (PD). At the same time, an in vivo mouse model was used to perform a mild traumatic brain injury to assess whether intranasal administration of human platelet lysates (nanofiltred) reduces the expression of pro-inflammatory mRNA markers (RT-PCR). The studies revealed that nanofiltration can be implemented in the process of obtaining platelet lysates in order to increase viral safety with no effect on the neuroprotective and anti-inflammatory effects of these products [2].

In the same session — New avenues of cellular therapy, Le et al. presented the results of multi-center studies performed in China and France ("Proteomics studies of human platelet lysates for optimized applications in cell therapies and regenerative biotherapies"). The paper emphasizes the fact that no standard method of platelet lysate preparation has been developed so far, and the differences in the method of preparation may affect the protein content and biological function, of the product and therefore its safety and clinical effectiveness. The aim of the study was to compare 7 methods of preparing human platelet lysate (HPL):

1. Freeze-thaw platelet lysate (FTPL): PC was frozen and thawed to release platelet content into plasma compartment.
2. Serum-Converted Platelet Lysate (SCPL): calcium chloride added to PC to convert fibrinogen into fibrin.
3. Heat treated Serum-Converted Platelet Lysate (HSCPL): SCPL was heat-treated (56°C, 30 min).
4. Platelet Pellet Lysate (PPL): isolated plasma-free platelets lysed by freeze/thaw.
5. Heat-treated-Platelet Pellet Lysate subjected to heating (56°C, 30 min) (HPPL).
6. Micro-Filtered-HPPL (HPPL0201): a 0.2–0.1 μm filtration sequence was used to remove large molecules or particles.
7. Nanofiltered-HPPL (HPPL0201): HPPL0201 was filtered through Planova 20 N, a 19-virus removal filter.

Proteomics studies identified 1441 proteins in various types of lysates. Some plasma proteins were too abundant and masked platelet-derived proteins. Removal of the 'interfering proteins' made it possible to evaluate the platelet proteome. The protein composition of platelet lysates was found dependent on the preparation method used, and particularly on the additional steps such as plasma removal, heating and filtration. Differences

in the proteome composition of platelet lysates may therefore affect their functions and in consequence their effectiveness for cell therapy and regenerative medicine [3].

The latest reports on the use of platelet lysates

Research-outcome on platelet lysates presented at the ISBT congress confirms the rising interest in these products. There is a growing number of literature reports on both the methods of preparation and application. One study demonstrated that platelet lysates used in growth media enhanced the clonogenic properties of mesenchymal stem cells (MSC) from bone marrow and adipose tissue. The same study showed the slight effect of the preparation method on the pluripotent cells (e.g. on the differentiation potential). Because of the lack of uniform methods of obtaining platelet lysates, they are not often used for the production of growth media. It may however be expected that promising research outcome as well as safe methods used for preparation of PC as starting material (e.g. virus detection, pathogen inactivation techniques) will render lysates a more appreciated addition to growth media) [4, 7, 8].

Apart from participation in the coagulation process, platelets also play an important role in other physiological mechanisms. Platelets have been found to secrete pro-neurogenic factors and communicate with the brain tissue to affect the cognitive functions of the brain (the ability to process environmental stimuli). This is closely related to the presence of neurotrophic factors in the platelet α-granules such as brain-derived neurotrophic factor (BDNF), platelet factor 4 (PF4) stimulator of neurogenesis, as well as other aforementioned proteins involved in the mechanisms of growth and regeneration (e.g. EGF, VEGF or PDGF). These reports have raised interest in the potential use of lysates in neuro-regenerative processes. The results of both in vitro and in vivo studies in such models of diseases as: amyotrophic lateral sclerosis (ALS), traumatic brain injury (TBI), Parkinson's and Alzheimer's confirmed the supportive role of lysates in the optimal structure and function of neurons (neuroprotective effect). In the ischemic stroke model, also the stimulating effect on nerve cell and blood vessel formation was confirmed. For the above mentioned disease entities, the induction of nerve cell division is of crucial importance and a great chance for health improvement. In advanced cases of traumatic brain injury (TBI), which may cause physical, cognitive, socio-emotional impairment or even death, palliative (i.e. symptomatic) management seems to be the only approach. Lysate therapy, due to the wide spectrum of proteome proteins (a set of proteins present in the cell at a given moment), may be one of the very few accessible therapeutic options. For the maximal safety of studies, HPLs subjected to nanofiltration and inactivation by heating (approx. 55°C for 0.5 h) (Heat-treated Inactivated Platelet Pellet Lysate; IHPPL) are used. Membrane filtration removes viruses. High temperature prevents the activation of thrombin and factor XI and minimizes thrombosis risk. A 5% solution of IHPPL after a week of incubation with microglia and neurons showed no toxicity towards them and did not induce inflammatory activity (as marked by no expression of inflammatory factors — tumor necrosis factor; TNF and anti-cyclooxygenase, COX-2). With support of research on neuron division, there is hope for implementation of lysates to clinical procedures for management of the disorders of the nervous system [6, 9, 10].

Due to the biologically active substances, platelet-derived products have already found their way to the therapy of numerous diseases, such as: hard-healing wounds and degenerative joint diseases. The current research may open the pathway for future clinical use of these products in neurodegenerative diseases and the regeneration of bones, tendons, neurons etc.

Conflict of interest: none declared

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