RESEARCH ARTICLE

DOI: 10.4274/tjh.galenos.2023.2022.0445
Turk J Hematol 2023;40:43-49

Oxidative and Fibrinolytic Mechanisms: Two Important Processes to Consider in Platelet Storage

Oksidatif ve Fibrinolitik Mekanizmalar, Trombositten Zengin Plazmadan Elde Edilen Trombositlerde Dikkate Alınması Gereken İki Önemli Süreç

Jesus Javier Garcia-Bañuelos[1,*], Marina Galicia-Moreno[1,*], Hugo Christian Monroy-Ramirez[1], Jose Navarro-Partida[2], Blanca Estela Bastidas-Ramirez[3], Arturo Santos[2], Juan Armendariz-Borunda[1,2]

[1]Instituto de Biología Molecular en Medicina y Terapia Génica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, Mexico

[2]Tecnologico de Monterrey, Escuela de Medicina y Ciencias de la Salud, Jalisco, Mexico

[3]Instituto en Investigación en Enfermedades Crónico Degenerativas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, Mexico

Abstract

Objective: Platelet-rich plasma (PRP) is used in multiple coagulation disorders. Its therapeutic effectiveness relies on technical procedures related to PRP procurement and preservation because free radicals induce platelet activation and aging. This work aims to elucidate the oxidative mechanisms involved in activation of platelets obtained from PRP during storage.

Materials and Methods: One hundred ten PRP batches were obtained from healthy donors and kept under stirring at a temperature of 20-24 °C. Protein extraction was performed from platelet homogenates and plasma at different times of storage from day 1 to 20. The activities of antioxidant markers such as catalase (CAT), superoxide dismutase, and ceruloplasmin, as well as fibrinolytic protein activity metalloproteases 2 and 3, plasmin, and urokinase plasminogen activator, were analyzed by zymography assays. Oxidized proteins were also determined.

Results: Significant activity of antioxidant enzymes and fibrinolytic molecules was observed on day 5 of storage in PRP homogenates, which increased over time and was concomitantly correlated with oxidized protein levels. Reverse enzymatic activity patterns were observed in plasma, except for CAT, which remained unchanged.

Conclusion: Storage conditions of platelets from PRP for up to 5 days induced in vitro platelet activation by oxidative damage and proteolysis. This finding confirms the need for proper management of these blood products to preserve their viability and functionality.

Keywords: Platelet storage, Platelet activation, Platelet-rich plasma, Oxidative damage, Metalloproteases

Öz

Amaç: Trombositten zengin plazma (TZP) pek çok koagülasyon bozukluğunda kullanılır. TZP'nin etkinliği, üretimi ve saklanması sırasındaki teknik işlemlere bağlıdır; çünkü serbest radikaller trombositin aktivasyonunu ve yaşlanmasını tetikler. Bu çalışma, TZP'den elde edilen trombositlerin, depolama sırasındaki aktivasyonunda yer alan oksidatif mekanizmaların aydınlatılmasına odaklanmıştır.

Gereç ve Yöntemler: Sağlıklı donörlerden 110 TZP örneği hazırlandı ve karıştırıcı eşliğinde 20-24 ˚C'de tutuldu. Trombosit homojenatından ve plazmadan, depolamanın 1. gününden 20. gününe kadar farklı zamanlarda protein ekstraksiyonu yapıldı. Katalaz, superoxit dismutaz ve seruloplazmin gibi antioksidan belirteçlerin aktiviteleri yanı sıra metaloproteaz 2, ve 3, plazmin ve ürokinaz plasminojen aktivatör gibi fibrinolitik proteinlerin aktiviteleri, zimografi testiyle analiz edildi; ayrıca oksidize olmuş proteinler belirlendi.

Bulgular: TZP homojenatının depolama süresinin 5. gününde, antioksidan enzimler ve fibrinolitik moleküllerde belirgin bir aktivite gözlendi; bu durum zamanla daha da arttı ve oksidize protein düzeyleri ile eş zamanlı bir ilişki gösterdi. Plazmada ise katalaz haricinde tersine bir enzimatik aktivite paterni gözlendi; katalaz değişmeden kaldı.

Sonuç: TZP'deki trombositlerin depolama koşulları, 5 güne kadar in vitro trombosit aktivasyonunu, oksidatif hasar ve proteoliz yoluyla tetikler; bu bulgu bu kan ürününün canlılığının ve fonksiyonlarının korunması için kurallara uygun davranılması gerekliliğini desteklemektedir.

Anahtar Sözcükler: Trombosit depolanması, Trombosit aktivasyonu, Trombositten zengin plazma oksidatif hasar, Metaloprotezlar

*These authors contributed equally to this work.

Address for Correspondence/Yazışma Adresi: Juan Armendariz-Borunda, PhD, Instituto de Biología Molecular en Medicina y Terapia Génica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Jalisco, Mexico
E-mail : armdbo@gmail.com ORCID: orcid.org/0000-0002-7101-9943

Received/Geliş tarihi: October 5, 2022
Accepted/Kabul tarihi: January 26, 2023

Introduction

The use of platelets is a therapeutic tactic widely used to resolve hemostasis disorders [1]. These methods include the use of platelet concentrate preparations or platelet-rich plasma (PRP) with buffy coat [2]. The second of these options, PRP, offers higher concentrations of platelets compared to plasma and, due to its autologous origin, does not produce immunogenic adverse reactions [3]. In transfusion medicine, platelets have been used to manage bleeding events due to severe thrombocytopenia caused by medullar aplasia, leukemia, or hemorrhages during surgery [4]. The correct preservation of platelets obtained from PRP is vital to obtain the desired therapeutic aims. The standard technical procedure for the conservation of platelets from PRP includes gentle agitation in proper aseptic containers at room temperature of 20-24 °C [5,6]. Under these conditions, storage is limited to 5 days since bacterial growth is favored [7].

Platelets obtained from PRP activation, regardless of the technique used, undergo a process related to several cellular signaling pathways. Recently, it has been suggested that oxidative stress has an important role in platelet activation [8]. During regular platelet stimulation, redox imbalance can arise. Platelet aggregation is related to high oxygen consumption and an increase in oxidized glutathione levels [9]. It has also been reported that hydrogen peroxide at micromolar concentrations is able to support both platelet aggregation and disintegration [10,11]. Numerous cellular antioxidant systems have been described in platelets, and enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase help maintain the redox balance in these cells [9]. On the other hand, platelets also release fibrinolytic proteins, such as urokinase-type plasminogen activator (uPA) and matrix metalloproteinases (MMPs) 1, 2, and 9. These enzymes exert diverse responses in regulating platelet functions, such as adhesion and aggregation [12]. Interestingly, MMP2 and MMP9 are translocated to the platelet surface during aggregation, favoring contact with cellular surface proteins [13]. Moreover, MMP translocation and releasing processes are linked to platelet activation and downregulated by endogenous antioxidant inhibitors of platelet aggregation such as nitric oxide (NO) and prostacyclin 12 [13,14].

Since free radicals induce platelet activation, this research was planned with the aim of elucidating the effect of oxidative damage in the activation of platelets obtained from PRP during storage by analyzing activity and fibrinolytic markers.

Materials and Methods

PRP Procurement and Preservation

Platelets from PRP were isolated from blood samples collected into acid citrate dextrose from 110 healthy volunteers from a blood transfusion medical service. Informed consent was provided according to the protocol of Official Mexican Standard NOM 003 [15] and international regulations [16,17]. Platelets from PRP were obtained by centrifugation of whole blood (400-450 mL) at 200x g for 10 min at room temperature, followed by a second centrifugation at 1200x g for 20 min to pellet the platelets (45-60 mL per sample). These samples were subsequently kept at room temperature of 20-24 °C under constant stirring (no more than 20x g) for a period of 1 to 20 days, with each sample containing approximately $5.5x10^{10}$ platelets and $1x10^{7}$ leukocytes [18]. Platelet-poor plasma was aspirated, transferred to an empty satellite bag, and stored at -80 °C for later use. The platelet pellets were allowed to settle for 90 min and were then resuspended manually and stored on a horizontal flat shaker.

To ensure that bacterial contamination was not a factor influencing the in vitro biological effects, platelet samples were tested for bacterial growth using a conventional hemoculture technique [16].

Platelet Protein Isolation

Protein isolation from platelets from PRP was performed according to Leoncini et al. [19]. Briefly, for each platelet sample, centrifugation was performed at 1200x g for 20 min and the pellet was washed twice in 36 mM citric acid buffer containing 100 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 5 mM glucose at pH 6.5 [18]. The platelet pellet was then minced in 700 µL of protein extraction buffer [20], the obtained homogenate was centrifuged at 12000x g for 5 min at 4 °C to remove particulate matter, and the supernatant was stored at -70 °C [19]. The Bradford method was used to measure the protein concentration of each sample [20]. Five samples were used for each study group.

Determination of CAT, SOD, and Ceruloplasmin Biological Activity

Platelet homogenates with approximate concentrations of $1x10^{8}$ platelets/mL were used for analyzing CAT and SOD, while ceruloplasmin (Cp) activity was evaluated in plasma derived from PRP using the method introduced by Leoncini et al. [19]. Activity in gel slabs was quantified in all tests using the Kodak 1D 3.5 image analyzer (Eastman Kodak Co., Rochester, NY, USA), and represented in area (relative units).

Oxidized Protein Quantification

Oxidized proteins were quantified using a Western blot assay (OxyBlot Kit, Chemicon International, Temacula, CA, USA). Briefly, 25 µg of total protein from platelets obtained from PRP was denatured by adding 12% SDS and 10% β-mercaptoethanol followed by incubation at room temperature by 15 min. Proteins were then separated in 12% SDS-PAGE and transferred to

PVDF membranes, blocked with blocking/dilution buffer, and incubated with rabbit primary anti-2,4-dinitrophenylhydrazone (2,4-DNP, 1:150) for 1 h at 18-25 °C with gentle shaking. Later, the membrane was rinsed three times with 1X PBS-T and incubated with a secondary antibody (1:300) in blocking dilution buffer for 1 h at 18 °C with gentle shaking. The membrane was then rinsed three times with 1X PBS-T. Blots were washed and protein was revealed using BM Chemiluminescence Kits (Roche Diagnostics, Indianapolis, IN, USA). Densitometric analysis was performed with the Kodak 1D 3.5 image analyzer (Eastman Kodak Co., Rochester NY, USA).

Zymography for Detecting Plasmin, MMP2, and MMP3MMP2 and MMP3 enzymatic activity was evaluated by zymography assays of total protein from platelet homogenates or plasma obtained from PRP as described previously [21]. Briefly, 30 µg of total protein from platelets or plasma was loaded onto 10% SDS-PAGE gel containing either 1 mg/mL gelatin for plasmin and MMP2 (Bio-Rad Laboratories, Hercules, CA, USA) or 2 mg/mL casein for MMP3; after electrophoresis, the SDS was removed from the gel by incubation in Triton X-100 at room temperature and samples were subsequently washed to remove the detergent and incubated at 37 °C for 48 h in a development buffer (BRIJ35, Sigma-Aldrich, St. Louis, MO, USA) [18]. Casein was used as a substrate for MMP3 with development buffer containing 0.1 mol/L glycine at pH 8.3 [21]. This gel was stained with the Coomassie technique, followed by destaining in the same solution without dye. Proteinase activity was detected as unstained bands on a blue background representing areas of gelatin digestion. Degradation bands were observed at 66 kDa (MMP2), 80 kDa (MMP9), and 55 kDa (MMP3). Gels were digitized and analyzed using the Kodak 1D 3.5 image analyzer (Eastman Kodak Co., Rochester NY, USA) and represented in area (relative units).

Zymography for uPA Activity

Activity of uPA was analyzed in total protein or platelet homogenates by zymography assays as described previously [21]. At the end of the technique, the gel was stained with 0.5% Coomassie blue. The proteolytic activity of uPA (54 kDa) was detected based on a white zone in a dark field [22]. Activity in the gel slabs was quantified using the Kodak 1D 3.5 image analyzer (Eastman Kodak Co., Rochester NY, USA), and represented in area (relative units).

Statistical Analysis

Results were analyzed by one-way ANOVA followed by the Dunnett post hoc test. Bivariate correlations were evaluated using Pearson correlation analysis. Significance was accepted at $p<0.05$ and results are given as mean ± standard deviation values.

Results

Increased CAT Activity in Platelets Obtained from PRP in Conventional Storage

To monitor the activity of antioxidant enzymes in PRP during storage, zymography assays were carried out. CAT and SOD were tested in both plasma and total proteins from platelets obtained from PRP. CAT activity was found to be significantly increased on day 7 of storage in platelet homogenates ($p<0.0001$) and it remained increased throughout the remainder of the study period (Figure 1A). Interestingly, the CAT activity in plasma was increased for the first 2 days (Figure 1B) and then showed a small but significant decrease from day 3 of storage ($p<0.0001$) that persisted throughout the remainder of the study. Regarding SOD activity, this antioxidant protein was not detected (data not shown).

Ceruloplasmin Activity Is Altered in Plasma Derived from PRP and Oxidied Proteins Are Generated in Platelets from PRP During Storage

Cp was detected from the first day of storage (Figure 1C), with a small but significant decrease on day 7 ($p=0.041$). To corroborate oxidative damage in thrombocytes, western blot assays (OxyBlot) for oxidized proteins were also carried out. As shown in Figure 1D, abundant oxidized proteins from PRP were detected from day 5 of storage ($p<0.0001$); however, a negative trend was then observed up to day 10. This finding reflects the phenomenon of oxidative damage that platelets undergo during storage.

Activation of Fibrinolytic Proteins on Platelets Obtained from PRP During Storage

Fibrinolytic proteins studied in platelet homogenates showed intense activity beginning as early as day 4 of storage. MMP-activating molecules such as uPA and plasmin displayed the same behavior patterns as MMPs in platelet lysates, with significantly increased activity at day 4 ($p<0.001$) that remained high for the rest of the study period (Figures 2A and 2C). Interestingly, in plasma, these markers showed an inverse pattern with decreased activity from day 5 of storage (Figures 2B and 2D). On the other hand, MMP2 had increased activity from day 5 of storage ($p<0.0001$) (Figure 2E) and MMP3 had increased activity from day 4 of storage ($p<0.0001$), remaining high throughout the rest of the observation period (Figure 2G). Similarly to the behavior of plasma uPA and plasmin, the plasma activity of MMP2 and MMP3 was decreased from day 4 and day 7, respectively, and these responses persisted throughout the remainder of the study (Figures 2F and 2H).

Interestingly, the increasing activities of MMP2, MMP3, uPA, and plasmin significantly correlated with the increasing levels of oxidized proteins ($r=0.729$, $r=0.629$, $r=0.578$, and $r=0.803$, respectively, with $p<0.01$ for all).

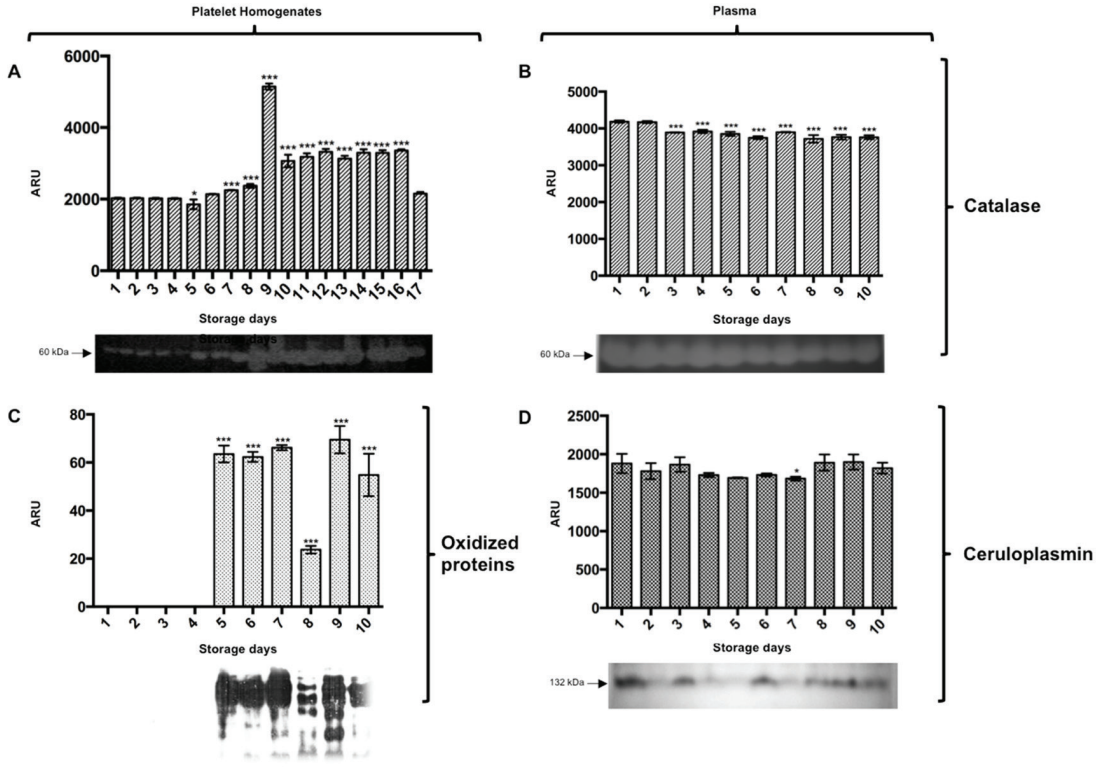

Figure 1. Platelets obtained from platelet-rich plasma undergo oxidative damage and show activation of catalase during standard storage processes: antioxidant activity of catalase in platelet homogenates (A) and plasma (B); (C) western blot assay for oxidized proteins (C); and zymography assays for ceruloplasmin with densitometric analysis and representative gel (D). Values are mean ± standard deviation of 3-5 samples per group. Asterisks indicate that values differ significantly from the first day of the observation period. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

The studied fibrinolytic proteins exhibited reversed patterns of activity in plasma. In the first days of observation, the activity was higher, but it decreased significantly throughout the course of storage with the aging of the platelets (Figure 2).

Discussion

PRP is a blood derivative containing platelet concentrations of at least 1,000,000 platelets/µL suspended in plasma [23]. Historically, platelet concentrates have been used to treat bleeding events due to thrombocytopenia caused by bone marrow aplasia, acute leukemia, or significant blood loss, and in recent years, these blood components are also widely used in clinical practice to stimulate human tissue healing. Platelets obtained from PRP have also recently experienced a surge in clinical usage for sports medicine, bone regeneration, plastic surgery, and other medical conditions due to their potential healing properties through the recruitment, proliferation, and differentiation of cells [24,25,26]. Several studies have focused on improving storage conditions for platelet concentrates, but little is known about the homeostasis of platelets obtained from PRP under storage conditions. In the present study, we have established that thrombocytes from PRP undergo oxidative damage in storage conditions, specifically on day 5 of storage. This oxidative damage correlates with the antioxidant activity found in platelets from PRP. CAT, which allows the conversion of H_2O_2 into H_2O and O_2, exhibited increased activity in platelet lysates obtained from PRP from day 6 of storage, just 1 day after oxidative damage was documented in the thrombocytes obtained from platelet concentrates. Taken together, these findings confirm that platelets experience oxidative stress in the routine methods of storage and preservation.

Variations in redox balance reduce the efficacy and impact the viability of platelets, and these processes are seen during normal platelet stimulation. It has been documented that superoxides are produced by platelets [9] and hydrogen peroxide is generated by thrombocytes in response to thrombin stimulation [27]. This response could also be present in platelets from PRP. The pathway by which platelets produce intracellular reactive oxygen species seems to be catalyzed by nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) oxidase, and their activation is associated with gp91phox-dependent enzyme activation [28,29].

On the other hand, during platelet activation, large amounts of superoxide anions are generated, a response that is counteracted by administering antioxidants in vitro or by the

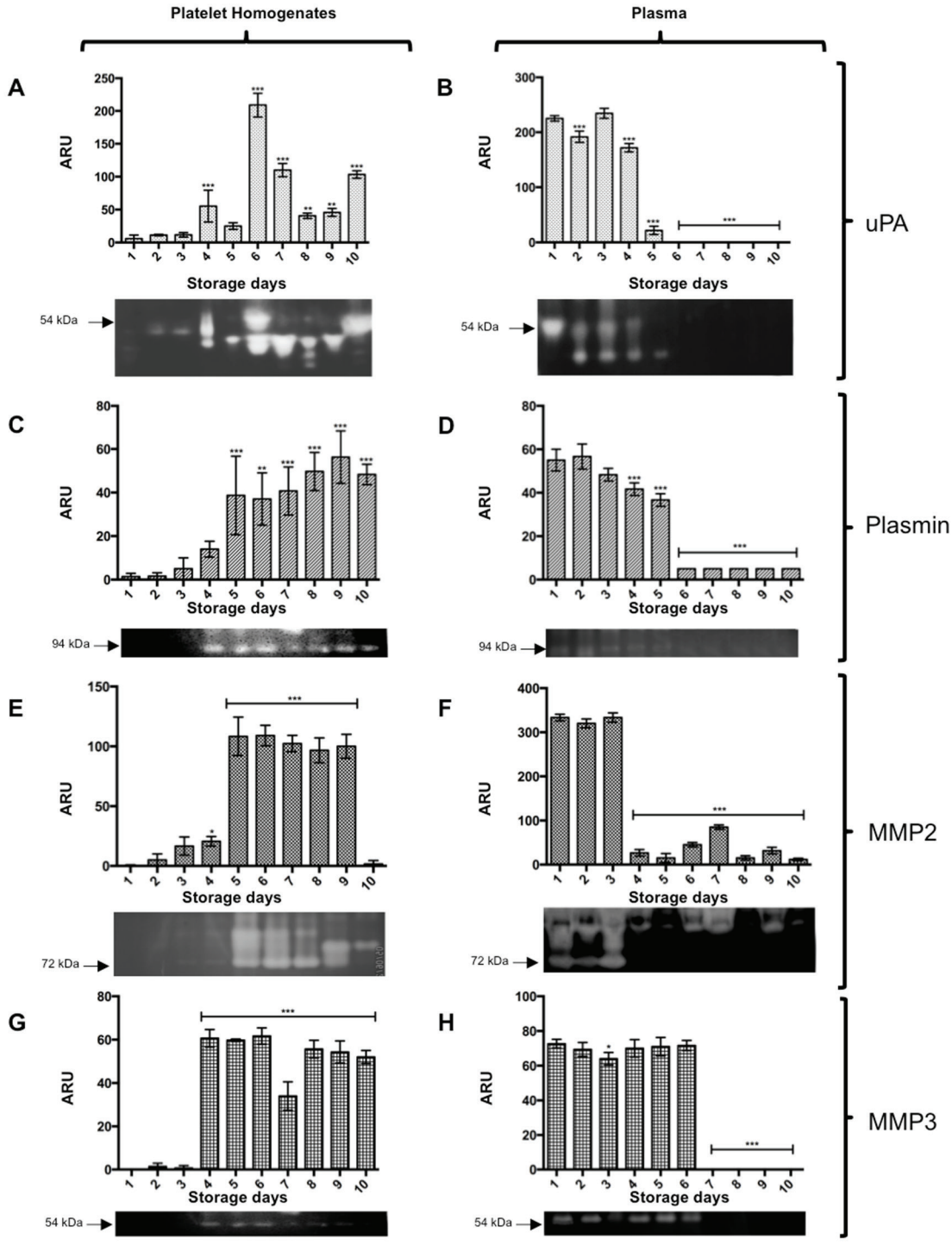

Figure 2. Increasing activity of fibrinolytic proteins was detected in platelets obtained from platelet-rich plasma during standard storage. Zymography assays were performed for (A, B) urokinase plasminogen activator (uPA), (C, D) plasmin, (E, F) metalloprotease-2 (MMP2), and (G, H) metalloprotease-3 (MMP3). Densitometric analyses and representative gels are presented. Values are mean ± standard deviation of 3-5 samples per group. Asterisks indicate that values differ significantly from the first day of the observation period. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

activity of endogenous platelet antioxidants such as SOD [30]. In our study, we were unable to demonstrate differences in SOD activity in platelet lysates, and this failure may have been due to the fact that the concentration of SOD in this blood component is approximately 1 fg of SOD per platelet with 77% in the Cu/Zn isoform, while the rest is in the Mn-SOD isoform, and this is insufficient for evaluation by zymography. However, Faria et al. [31] evaluated the enzymatic activity of

SOD using two different techniques and concluded that there were differences in the evaluation results of SOD activity as well as alcohol dehydrogenase between the two methodological techniques used. Better sensitivity of enzymatic evaluations by spectrophotometric techniques was reported [31,32,33].

In our work, the activity of fibrinolytic proteins was also analyzed in platelet lysates and plasma from PRP. Recently, MMPs and their inhibitors and activators have been the subject of in-depth research in the field of platelet biology, but there are still gaps of information regarding whether their functions are altered in platelets obtained from PRP. It has been established that platelet activation and aggregation are mediated by MMP2, which interacts with integrin $\alpha_{IIb}\beta_3$ [34], and it seems that MMP9 may counteract the pro-aggregatory effect of MMP2 [35]. The latter enzyme inhibits phospholipase C activation via NO production and cyclic GMP in human platelets, resulting in platelet aggregation inhibition [36]. We found that MMP2 and MMP3 were activated in platelet lysates from PRP between days 4 and 5 of storage, interestingly at the same time that oxidative damage was documented.

Besides the increased activity of MMPs, heightened activity of uPA and plasmin in platelet homogenates from PRP was also found. uPA is a serin protease that promotes plasmin activation both in vivo and in vitro [37]. It has been reported that plasmin mediates the activation of thrombocytes by cleavage of protease-activated receptor 4 [38]; thus, our finding regarding the activity of plasmin in platelets strongly suggests thrombocyte activation during the storage of platelet concentrates. In addition to the effect of plasmin on platelet activation, this enzyme directly activates pro-MMP1, pro-MMP9, pro-MMP10, and pro-MMP13, and uPA can also activate pro-MMP2 [39]. This could explain the increase in the activity of MMP2 found in our platelet lysates from PRP during storage.

Interestingly, an opposite pattern of activity was observed for fibrinolytic proteins in plasma. This finding may be the result of the internalization of proteins that occurs with tissue factor and other protein contents in alpha granules, a response that is independent of GPIIb and GPIIIa [40].

Conclusion

The application of appropriate preservation and storage methods for PRP allow for platelet activation, presumably by oxidative stress, before the generally accepted expiration date established for platelet concentrates. In the present study, oxidative damage, as well as increased activities of antioxidant and fibrinolytic proteins, appeared in thrombocytes starting from day 4 of storage. These results provide evidence of the processes that may be activated in packaged platelets under standard handling and storage conditions. Additional translational research is needed to obtain more precise data on the possible clinical implications of these changes generated in platelets obtained from PRP.

Acknowledgments

Special thanks to Dr. Jaime González Cuevas, who died before the publication of these results, for his significant contributions to the realization of this project.

Ethics

Ethics Committee Approval: The procedure was approved by the institutional review board.

Informed Consent: All participants provided written informed consent, and the study was carried out according to the provisions of the Declaration of Helsinki.

Authorship Contributions

Concept: J.J.G-B., J.A-B.; Design Concept: J.J.G-B., J.A-B.; Design: J.J.G-B., M.G-M., B.E.B-R.; Data Collection or Processing: J.J.G-B.; Analysis or Interpretation: J.J.G-B., M.G-M., H.C.M-R., J.N-P.; Literature Search: A.S.; Writing: J.J.G-B., M.G-M., B.E.B-R.: J.J.G-B., M.G-M., B.E.B-R.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was supported in part by Programa de Fortalecimiento de Institutos, Centros y Laboratorios de Investigación 2022, Universidad de Guadalajara, to Juan Armendariz-Borunda.

References

1. Etulian J. Platelets in wound healing and regenerative medicine. Platelets 2018;29:556-568.
2. Ojha S, Tirlotkar A, Gupta AM, SH S, Chavan P, Poojary M. Comparative analysis of platelet concentrates prepared after two hours and overnight storage of buffy coat at room temperature. Transfus Apher Sci 2021;60:103014.
3. Verma R, Kumar S, Garg P, Verma YK. Platelet-rich plasma: a comparative and economical therapy for wound healing and tissue regeneration. Cell Tissue Bank (in press).
4. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27:158-167.
5. Dohan Ehrenfest DM, Andia I, Zumstein MA, Zhang CQ, Pinto NR, Bielecki T. Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: current consensus, clinical implications and perspectives. Muscles Ligaments Tendons J 2014;4:3-9.
6. Braathen H, Sivertsen J, Lunde THF, Kristoffersen EK, Assmus J, Hervig TA, Strandenes G, Apelseth TO. In vitro quality and platelet function of cold and delayed cold storage of apheresis platelet concentrates in platelet additive solution for 21 days. Transfusion 2019;59:2652-2661.
7. Jimenez-Marco T, Castrillo A, Hierro-Riu F, Vicente V, Rivera J. Frozen and cold-stored platelets: reconsidered platelet products. Platelets 2022;33:27-34.

8. Fuentes E, Gibbins JM, Holbrook LM, Palomo I. NADPH oxidase 2 (NOX2): a key target of oxidative stress-mediated platelet activation and thrombosis. Trends Cardiovasc Med 2018;28:429-434.
9. Freedman JE. Oxidative stress and platelets. Artherioscler Thromb Vasc Biol 2008;28:s11-s16.
10. Canoso RT, Rodvien R, Scoon K, Levine PH. Hydrogen peroxide and platelet function. Blood 1974;43:645-656.
11. Maselli E, Pozzi G, Vaccarezza M, Mirandola P, Galli D, Vitale M, Carubbi C, Gobbi G. ROS in platelet biology: functional aspects and methodological insights. Int J Mol Sci 2020;21:4866.
12. Gresele P, Falcinelli E, Sebastiani M, Momi S. Matrix metalloproteinases and platelet function. Prog Mol Biol Transl Sci 2017;147:133-165.
13. Santos-Martínez MJ, Medina C, Jurasz P, Radomski MW. Role of metalloproteinases in platelet function. Thromb Res 2008;121:535-542.
14. Jurasz P, Sawicki G, Duszyk M, Sawicka J, Miranda C, Mayers I, Radomski MW. Matrix metalloproteinase 2 in tumor cell-induced platelet aggregation: regulation by nitric oxide. Cancer Res 2001;61:376-382.
15. Centro Nacional de la Transfusión Sanguínea. NORMA Oficial Mexicana NOM-253-SSA1-2012, Para la disposición de sangre humana y sus componentes con fines terapéuticos. Government of Mexico, Mexico City, 2012.
16. Slichter S. Extension of Platelet Storage: Fact or Fantasy: Food and Drug Administration Blood Products Advisory Committee Meeting. FDA, Silver Spring, 2002.
17. Council of Europe. Guide to the preparation, use and quality assurance of blood components. Recommendation No. R (95) 15. 20th edition. Council of Europe, Brussels, 2020.
18. Dejouvencel T, Doeuvre L, Lacroix R, Plawinski L, Dignat-George F, Lijnen HR, Anglés-Cano E. Fibrinolytic cross-talk: a new mechanism for plasmin formation. Blood 2010;115:2048-2056.
19. Leoncini G, Maresca M, Colao C. Oxidative metabolism of human platelets. Biochem Int 1991;25:647-655.
20. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-254.
21. González-Cuevas J, Bueno-Topete M, Armendariz-Borunda J. Urokinase plasminogen activator stimulates function of active forms of stromelysin and gelatinases (MMP-2 and MMP-9) in cirrhotic tissue. J Gastroenterol Hepatol 2006;21:1544-1554.
22. Monvoisin A, Neaud V, De Ledinghen V, Dubuisson L, Balabaud C, Bioulac-Sage P, Desmoulière A, Rosenbaum J. Direct evidence that hepatocyte growth factor-induced invasion of hepatocellular carcinoma cells is mediated by urokinase. J Hepatol 1999;30:511-518.
23. Gupta S, Paliczak A, Delgado D. Evidence-based indications of platelet-rich plasma therapy. Expert Rev Hematol 2021;14:97-108.
24. Grambart ST. Sport medicine and platelet-rich plasma: nonsurgical therapy. Clin Podriat Med Surg 2015;32:99-107.
25. Badran Z, Abdallah MN, Torres J, Tamimi F. Platelet concentrates for bone regeneration: current evidence and future challenges. Platelets 2018;29:105-112.
26. Ghname AA, Trisliana Perdanasari A, Davis MJ, Reece EM. Platelet-rich plasma: principles and applications in plastic surgery. Semin Plast Surg 2019;33:155-161.
27. Vavaev AV, Buryachkovskaya LI, Uchitel IA, Tishchenko EG, Maksimenko AV. Effects of hydrogen peroxide and catalase derivatives on functional activity of platelets. Bull Exp Biol Med 2012;152:307-312.
28. Stokes KY, Russell JM, Jennings MH, Alexander JS, Granger DN. Platelet-associated NAD(P)H oxidase contributes to the thrombogenic phenotype induced by hypercholesterolemia. Free Radic Biol Med 2007;43:22-30.
29. Pignatelli P, Sanguigni V, Lenti L, Ferro D, Finocchi A, Rossi P, Violi F. gp91phox-dependent expression of platelet CD40 ligand. Circulation 2004;110:1326-1329.
30. Fidler TP, Rowley JW, Araujo C, Boudreau LH, Marti A, Souvenir R, Dale K, Boilard E, Weyrich AS, Abel ED. Superoxide dismutase 2 is dispensable for platelet function. Thromb Haemost 2017;117:1859-1867.
31. Faria G, Mendes Guimarães R, Carvalho E, Sousa D. Methods for the assessment of isoenzymes activity in sunflower seeds. J Seed Sci 2015;37:211-217.
32. Meng YY, Trachtenburg J, Ryan US, Abendschein DR. Potentiation of endogenous nitric oxide with superoxide dismutase inhibits platelet-mediated thrombosis in injured and stenotic arteries. J Am Coll Cardiol 1995;25:269-275.
33. Wilkesman J, Kurz L. Zymography principles. Methods Mol Biol 2017;1626:3-10.
34. Choi WS, Jeon OH, Kim HH, Kim DS. MMP-2 regulates human platelet activation by interacting with integrin $\alpha_{IIb}\beta_3$. J Thromb Haemos 2008;6:517-523.
35. Santos-Martinez MJ, Medina C, Gilmer JF, Radomski MW. Matrix metalloproteinases in platelet function: coming of age. J Thromb Haemost 2008;6:514-516.
36. Sheu JR, Fong TH, Liu CM, Shen MY, Chen TL, Chang Y, Lu MS, Hsiao G. Expression of matrix metalloproteinase-9 in human platelets: regulation of platelet activation in in vitro and in vivo studies. Br J Pharmacol 2004;143:193-201.
37. Whyte CS, Mutch NJ. uPA-mediated plasminogen activation is enhanced by polyphosphate. Haematologica 2021;106:522-531.
38. Quinton TM, Kim S, Derian CK, Jin J, Kunapuli SP. Plasmin-mediated activation of platelets occurs by cleavage of protease-activated receptor 4. J Biol Chem 2004;279:18434-18439.
39. Lijnen HR, Van Hoef B, Lupu F, Moons L, Carmeliet P, Collen D. Function of the plasminogen/plasmin and matrix metalloproteinase systems after vascular injury in mice with targeted inactivation of fibrinolytic system genes. Arterioscler Thromb Vasc Biol 1998;18:1035-1045.
40. Lopez-Vilchez I, Diaz-Ricart M, Galan AM, Roque M, Caballo C, Molina P, White JG, Escolar G. Internalization of tissue factor-rich microvesicles by platelets occurs independently of GPIIb-IIIa, and involves CD36 receptor, serotonin transporter and cytoskeletal assembly. J Cell Biochem 2016;117:448-457.