Evaluating the Safety of Intravenous Delivery of Autologous Activated Platelet-rich Plasma

Karina Karina1,2,3,4,5.*, Krista Ekaputri1, Johannes Albert Biben1, Ratna Herawati Purwoko1, Tommy Partunggul Sibueva1, Sarah Listyo Astuti1, Anastasia Maria Loho1, Yuliardy Limengka1, Nelfidayani1, Agustini S1, Grady Krisandi2,3, Azza Maryam1, Imam Rosadi2,6, Iis Rosliana2, Siti Sobariah2, Wismo Reja Subroto2, Irsyah Afini2, Tias Widyastuti2, Alfida Zakiyah2, Difky Ernanda2, Noor Aini2, Jusryanti2, Sulaeah AD1, Sristin Indah Prestiani1, Indah Mustika Donna1, Habibi1, Meyla Shinta Mutiara1

1 Klinik Hayandra, Yayasan Hayandra Peduli, Jakarta, Indonesia, 2 Hayandra Lab, Yayasan Hayandra Peduli, Jakarta, Indonesia, 3 Faculty of Medicine, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia, 4 Universitas Paramadina, Jakarta, Indonesia, 5 Universitas Paramadina, Jakarta, Indonesia, 6 Pusat Kajian Stem Cell, Universitas Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia, 7 Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Indonesia

ABSTRACT

Introduction: Autologous platelet-rich plasma (PRP) has been a growing trend in the field of medicine due to its broad range of application and is considered safe from bloodborne diseases. Furthermore, various studies have tried to optimize the use of autologous PRP through various preparation protocols, including PRP activation. However, most of the studies available have not evaluated the safety for intravenous delivery of PRP, especially autologous activated PRP (aaPRP). Therefore, this study aimed to evaluate the safety of intravenous delivery of aaPRP.

Methods: Blood was drawn from each patient and aaPRP was isolated through calcium activation and light irradiation. Each aaPRP was administered intravenously to all patients. Adverse events were documented and analyzed.

Results: Six hundred eleven patients participated in this study with a total of 4244 aaPRP therapies. Quality control of aaPRP showed no platelets present after both calcium activation and light irradiation. No adverse events such as allergic reaction, infection, and coagulation problems were observed on all patients over the course of the study.

Conclusion: Our results showed that intravenous administration of autologous aaPRP is safe even in patients with various pathological conditions.

Keywords: Intravenous infusion, platelet-rich plasma, safety

INTRODUCTION

Platelet-rich plasma (PRP) is a high platelet concentrate extracted from the processed autologous plasma of the whole blood (1). A total of more than 1100 proteins have been found in PRP with different functions, ranging from enzymes, growth factors, and messengers of the immune system (2). These proteins and many bioactive factors are mainly secreted by three types of granule (alpha, delta, lambda) within the platelets, with alpha granules being the most abundant. Platelet activation is required for the release of these proteins and bioactive factors by alpha granules (3). Secreted proteins and bioactive factors upon activation are found to take part in various biological processes, including cellular proliferation and differentiation, matrix remodeling, and angiogenesis. These biological processes are found to enhance wound healing and tissue regeneration (2).

Various protocols of PRP preparation exist with the basic steps consist of: (1) Blood collection, (2) centrifugation, (3) plasma aspiration, (4) potential second centrifugation, (5) selected supernatant removal, (6) mixing/resuspension of platelets, (7) activation, and (8) application (4). Among all of the basic steps, platelet activation is a crucial step in PRP preparation. Through activation, degranulation of alpha granules, which releases growth factors, will be more optimal and leads to higher availability of bioactive molecules (5).

PRP is a growing trend in medical field, with the application ranging from cardiovascular to ocular disease (6). In plastic surgery, the clinical application of PRP to date includes wound healing, fat grafting (7), bone grafting, skin and face rejuvenation, and hair restoration (4,8). With such broad popularity and range of application, the safety of PRP therapy becomes a crucial topic. Since PRP therapy is autologous, it is safer with no concern of bloodborne
intravenously with blood transfusion set tube to remove
activated PRP until clots were formed. Clots were then elimi-
ated and 10 mL of NaCl 0.9% was added. The PRP was
activated PRP . Calcium chloride (0.15 mL) was added and
mixed with the inactivated PRP. Calcium
plasma in the tube (2.5 mL) which was considered as inac-
tivated PRP. The pellets of platelets were resuspended in the remaining
platelet count of 1328 × 10^3/μL post-activation with
platelet count to nearly zero.

Quality control of PRP
Around 200 μL of PRP aliquot was moved into 1.5 mL sterile microtubes. Analysis of sample was done using Sysmex KX-21 (Sysmex Corporation, Japan) automated hematolgy analyzer that has been calibrated before analy-
zing the platelet counts of PRP. Each aaPRP batch was
analyzed twice in every processing stage for platelet count
measurement.

Data collection and analysis
A retrospective analysis was performed on aaPRP-treated
patients with various pathological conditions from
January 2016 to December 2020. Included variables were
gender, age, number of PRP treatment, pathological con-
dition, and incidence of adverse reactions. Adverse events
include side effects or patient discomforts or complaints,
while serious adverse events include life-threatening con-
ditions that require major intervention or hospitaliza-
data. Data was further analyzed descriptively, as shown
in the figures.

RESULTS
Patient demographics and PRP administration
Among 611 patients, 284 (46.48%) patients were male and
327 (53.52%) patients were female. The median age was
49 years old. The youngest patient was 19 years old and
the oldest patient was 75 years old. A total of 4244 aaPRP
therapy was done throughout the study.
Diabetes mellitus was the most common pathological
condition found in the patients, followed by osteoarthri-
tis, hypertension, stroke, post-cardiac stenting, anti-aging,
and other pathological conditions. The distribution per-
centage of patient’s pathological conditions is shown in
Figure 1.

Quality control of PRP
The blood of the patient was analyzed for blood cells and
platelets count. The patient’s platelets were counted in each
stage of PRP preparation and shown in Figure 2. High
platelet count of 1328 × 10^3/μL was found in PRP but
significantly decreased to 5 × 10^3/μL post-activation with
calcium activator. Further photo-activation of PRP reduced
platelet count to nearly zero.
Leukocytes were also counted in each stage of PRP prep-
ration and shown in Figure 3. PRP had leukocyte count of
0.6 × 10^3/μL and significantly reduced to zero with calcium
activation and photo-activation.

Safety analysis
Among all patients, no allergic reactions, infections, and
coagulation problems were observed. No serious adverse
events that caused life-threatening condition that required
hospitalization or urgent interventions happened in this
study. Overall, no aaPRP-related adverse events were
reported among all patients that participated in the study.
Improper processing and administration showed that there were no statistically significant differences in platelet morphology and, therefore, was more sensitive to small processing error. The important point is that in the above trial, therapeutic PRP was produced only by the double centrifugation protocol (16).

In our study, calcium activator-light irradiation-activated PRP was used due to better efficacy and safety. The rationale behind the use of calcium activation was to activate platelets so growth factors would be secreted from the α-granules without the need for the patient’s body to activate the platelets (8,18). Furthermore, activation leaves no platelets in the activated PRP which prevents potential thromboembolic events (19). It has been shown that photo-activation of PRP with low-level light decreases the concentration of pro-inflammatory cytokines, such as interleukin-2 (IL-2) and IL-6, and increases the concentration of leukocyte-derived anti-inflammatory factors, such as IL-1 receptor antagonist (IL-1RA) and IL-2RA (20,21,22).

Two case studies by Freitag et al. showed that the patients treated with activated PRP demonstrated improvement in clinical outcome of osteoarthritic patients (23,24). In contrast, a clinical trial in osteoarthritis cases done by Paterson et al. showed that there were no statistically significant improvements in the activated PRP group when compared to the control (hyaluronic acid) group (11). However, all these studies demonstrated no side effect or complication of activated PRP; hence, it may be considered as a safe treatment option (11,23,24). Furthermore, with increased concentration of anti-inflammatory factors, aaPRP may also act as an anti-aging agent that helps reduce chronic systemic inflammation which is one of the major markers of aging (25). Our study confirmed that the activation step of our processing technique successfully activate the PRP as indicated by the significant decline of the platelet count.

Possible reported adverse events of PRP through intravenous administration include allergic reactions, infections, and coagulation problems. Allergic reactions may happen due to the substances used to prepare PRP as reported by Michal et al. (26). Improper processing and administration of PRP may also cause infections (15). The presence of platelets in PRP has also raised concern for causing thrombosis (27). As for this study, no adverse events were reported in all 611 patients.

There were no allergic reactions related to aaPRP administration in our patients as an autologous therapy generally has a low risk of allergic reaction complication. Our study also did not find any infection-related complications due to the administration of aaPRP as Reddy et al. mentioned (15). The preparation of our aaPRP was handled with aseptic and antiseptic technique to maintain its sterility from the time of blood withdrawal until its administration to the patient. No coagulation problems such as thromboembolic events were observed in this study. This can be explained because, during the processing period, all clots had been removed meticulously. In addition to that, the PRP was administered through blood transfusion set tube so that any possible remnant of clots would be filtered out before it entered the circulation. The activation of PRP with calcium activator and light irradiation reduced the number of platelets to nearly

**FIGURE 1.** Distribution of patient’s pathological conditions.

**FIGURE 2.** Platelet count from venous blood until final autologous activated platelet-rich plasma product.

**FIGURE 3.** Leukocyte count from venous blood until final autologous activated platelet-rich plasma product.

**DISCUSSION**

Previous studies have evaluated the safety of PRP for localized administration. However, the safety for intravenous PRP administration has not been evaluated (10). To our knowledge, this study is the first study involving large number of patients to evaluate the safety of intravenous administration of aaPRP in various pathological conditions.

Double centrifugation protocol was used in our study. Through sterile centrifugation process, platelets will be separated from red blood cells and sequestrate platelets in high concentration without causing damage or lysis of platelet to avoid triggering premature release of growth factors (16). The optimal or “therapeutic” platelet concentration of PRP should be 4- to 5-fold greater than that of the whole blood (17). According to the animal trial done by Nagata et al., between double-centrifugation and single-centrifugation protocol, the former one yielded higher platelet concentrations, but the setback was it caused alterations in platelet morphology and, therefore, was more sensitive to small processing error. The important point is that in the above trial, therapeutic PRP was produced only by the double centrifugation protocol (16).

In our study, calcium activator-light irradiation-activated PRP was used due to better efficacy and safety. The rationale behind the use of calcium activation was to activate platelets so growth factors would be secreted from the α-granules without the need for the patient’s body to activate the platelets (8,18). Furthermore, activation leaves no platelets in the activated PRP which prevents potential thromboembolic events (19). It has been shown that photo-activation of PRP with low-level light decreases the concentration of pro-inflammatory cytokines, such as interleukin-2 (IL-2) and IL-6, and increases the concentration of leukocyte-derived anti-inflammatory factors, such as IL-1 receptor antagonist (IL-1RA) and IL-2RA (20,21,22).

Two case studies by Freitag et al. showed that the patients treated with activated PRP demonstrated improvement in clinical outcome of osteoarthritic patients (23,24). In contrast, a clinical trial in osteoarthritis cases done by Paterson et al. showed that there were no statistically significant improvements in the activated PRP group when compared to the control (hyaluronic acid) group (11). However, all these studies demonstrated no side effect or complication of activated PRP; hence, it may be considered as a safe treatment option (11,23,24). Furthermore, with increased concentration of anti-inflammatory factors, aaPRP may also act as an anti-aging agent that helps reduce chronic systemic inflammation which is one of the major markers of aging (25). Our study confirmed that the activation step of our processing technique successfully activate the PRP as indicated by the significant decline of the platelet count.

Possible reported adverse events of PRP through intravenous administration include allergic reactions, infections, and coagulation problems. Allergic reactions may happen due to the substances used to prepare PRP as reported by Michal et al. (26). Improper processing and administration of PRP may also cause infections (15). The presence of platelets in PRP has also raised concern for causing thrombosis (27). As for this study, no adverse events were reported in all 611 patients.

There were no allergic reactions related to aaPRP administration in our patients as an autologous therapy generally has a low risk of allergic reaction complication. Our study also did not find any infection-related complications due to the administration of aaPRP as Reddy et al. mentioned (15). The preparation of our aaPRP was handled with aseptic and antiseptic technique to maintain its sterility from the time of blood withdrawal until its administration to the patient. No coagulation problems such as thromboembolic events were observed in this study. This can be explained because, during the processing period, all clots had been removed meticulously. In addition to that, the PRP was administered through blood transfusion set tube so that any possible remnant of clots would be filtered out before it entered the circulation. The activation of PRP with calcium activator and light irradiation reduced the number of platelets to nearly
zero and leukocytes to zero. This implies that when aaPRP was administered to the patient, there were virtually no platelets and leukocytes left that may potentially cause thrombosis (19).

A systematic review and meta-analysis have found that PRP which is rich in wound healing-related growth factors, such as vascular endothelial growth factor and platelet-derived growth factor, helps in patients with diabetic ulcer (28,29). PRP has also been reported to be beneficial for ischemic stroke, osteoarthritis, and chronic ulcers. There have been no previous reports regarding the adverse event of PRP use in those cases or its combination with stomal vascular fraction (11,12,30,31,32,33). Our study showed that the intravenous administration of aaPRP in patients with various pathological conditions did not cause any adverse event. Thus, aaPRP for therapy in various pathological conditions is safe for patients and might even be beneficial to treat their pathological condition.

The limitation of this study is the absence of objective evaluation to measure the efficacy of aaPRP as a therapy for various pathological conditions. Although aaPRP is proven to be safe, further prospective study is required to objectively evaluate the efficacy of aaPRP as a therapy for various pathological conditions.

CONCLUSION
The use of aaPRP intravenously in this study showed no allergic reactions, infections, and coagulation problems despite various patients pre-existing conditions. This suggests that intravenous injection of aaPRP is safe with no adverse effects.

COMPETING INTERESTS
The authors declare no conflicts of interest.

REFERENCES
1. Wu PIK, Diaz R, Borg-Stein J. Platelet-rich plasma. Phys Med Rehabil Clin N Am 2016;27(4):825-33. https://doi.org/10.1016/j.pmr.2016.04.008.
2. Du R, Lei T. Effects of autologous platelet-rich plasma injections on facial skin rejuvenation. Exp Ther Med 2020;19(4):3024-30. https://doi.org/10.3892/etm.2020.8531.
3. Pavlovic V, Cinc M, Jovanovic V, Stojanovic P. Platelet-rich plasma: A short overview of certain bioactive components. Open Med (Wars) 2016;11(1):242-7. https://doi.org/10.1515/omew-2016-0048.
4. Abu-Ghname A, Perdanasari AT, Reece EM. Principles and applications of fat grafting surgery. Semin Plast Surg 2019;33(3):147-54. https://doi.org/10.1055/s-0039-1650343.
5. Cavallo C, Roffa A, Grigo J, Mariani E, Prattelli L, Merli G, et al. Platelet-rich plasma: The choice of activation method affects the release of bioactive molecules. Biomed Res Int 2016;2016:6591717. https://doi.org/10.1155/2016/6591717.
6. Choi J, Minn KW, Chang H. The efficacy and safety of platelet-rich plasma and adipose-derived stem cells: An update. Arch Plast Surg 2012;39(6):555-92. https://doi.org/10.5999/aps.2012.39.6.555.
7. Gentile P, Garvochic S. Systematic review: Adipose-derived mesenchymal stem cells, platelet-rich plasma and biomaterials as new regenerative strategies in chronic skin wounds and soft tissue defects. Int J Mol Sci 2021;22(4):1-14. https://doi.org/10.3390/ijms22041538.
8. Gentile P, Garvochic S. Autologous activated platelet-rich plasma (AA-PRP) and non-activated (A-PRP) in hair growth: A retrospective, blinded, randomized evaluation in androgenetic alopecia. Expert Opin Biol Ther 2020;20(3):327-37. https://doi.org/10.1080/14712598.2020.1728491.
9. Safdar A, Shaaban H, Tibayan R, Miller R, Boairdo R, Gurun G. The clinical efficacy of using autologous platelet-rich plasma in hip arthropathy: A retrospective comparative study. J Nat Sci Biol Med 2015;6(1):49-55. https://doi.org/10.4103/0976-9698.149077.
10. Masei-Campbell AL, Ismail A, Reynolds KA, Poon E, Semano L, Grushchak S, et al. A systematic review of the safety and effectiveness of platelet-rich plasma (PRP) for skin aging. Arch Dermatol Res 2020;312(5):301-15. https://doi.org/10.1007/s00403-019-1999-9.
11. Paterson KL, Nichols M, Bennett KL, Bates D. Intra-articular injection of photo-activated platelet-rich plasma in patients with knee osteoarthritis: A double-blind, randomized controlled pilot study. BMC Musculoskelet Disord 2016;17(1):1-9. https://doi.org/10.1186/s12891-016-0920-3.
12. Cook CS, Smith PA. Clinical update: Why PRP should be your first choice for injection therapy in treating osteoarthritis of the knee. Curr Rev Musculoskelet Med 2018;11(4):583-92. https://doi.org/10.1007/s12178-018-0524-x.
13. da Silva FA, Rodrigues BL, Huber SC, Júnior JL, Lana JF, Montalvillo SA, et al. The use of platelet-rich plasma in the treatment of refractory Crohn’s disease. Int J Clin Exp Med 2017;10(8):7533-42.
14. Alcaraz J, Oliver A, Sánchez JM. Platelet-rich plasma in a patient with cerebral palsy. Am J Case Rep 2015;16:486-92. https://doi.org/10.12699/ajcr.893805.
15. Huilman S, Reddy R, Reddy R, Babu NC, Ashok GN. Stem cell therapy and platelet-rich plasma in regenerative medicine: A review on pros and cons of the technologies. J Oral Maxillofac Pathol 2018;22(3):367-74. https://doi.org/10.4103/jomp.jomp_93_18.
16. Nagata MJ, Messora MR, Futalaneto FA, Fucini SE, Bosco AF, Garcia VG, et al. Effectiveness of two methods of preparation of autologous platelet-rich plasma: An experimental study in rabbits. Eur J Dent 2010;4(4):395-402. https://doi.org/10.5535/ejds.16.80.001.
17. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62(4):489-95.
18. Gentile P, Cole JP, Cole MA, Garvochic S, Biedi A, Scioli MG, et al. Evaluation of not-activated and activated PRP in hair loss treatment: Role of growth factor and cytokine concentrations obtained by different collection systems. Int J Mol Sci 2017;18(2):1-18. https://doi.org/10.3390/ijms18020408.
19. Tomaulucci M, Brass LF, Slater TJ. Regulation of platelet activation and coagulation and its role in vascular injury and arterial thrombosis. Invest Cardiol Clin 2017;6(1):1-12. https://doi.org/10.1016/j.iccl.2016.08.001.
20. Moheddin A, Lewis P, Coudhury K, Sadiq B. Clinical outcome of photoactivated platelet-rich plasma in the treatment of knee osteoarthritis. Rheumatol Orthop Med 2019;4(1):1-4.
21. Zhvegai NA, Samolovka KA. Pro and anti-inflammatory cytokine content in human peripheral blood after its transcutaneous (in vivo) and direct (in vitro) irradiation with polychromatic visible and infrared light. Photomed Laser Surg 2006;24(2):129-39. https://doi.org/10.1899/ps/.2006.24.129.
22. Sigmundsdottir H, Johnston A, Gudjonsson JE, Valdimarsson H. Narrowband-UVB irradiation decreases the production of pro-inflammatory cytokines by stimulated T cells. Arch Dermatol Res 2005;297(1):39-42. https://doi.org/10.1007/s00403-005-0655-9.
23. Freitag J, Ben, Barrand A. To evaluate the effect of combining photo-activation therapy with platelet-rich plasma injections for the novel treatment of osteoarthritis. BMJ Case Rep 2013;2013:bcr2013037463. https://doi.org/10.1136/bcr-2012-007463.
24. Freitag J. The effect of photoactivated platelet-rich plasma injections in the novel treatment of shoulder osteoarthritis. Int J Case Rep Images 2014;5(8):546. https://doi.org/10.5343/ijcri.2014.96-cr-10407.
25. Chung HY, Kim DH, Lee EK, Chung KW, Chung S, Lee B, et al. Redefining chronic inflammation in aging and age-related diseases: Proposal of the senoinflammation concept. Aging Dis 2019;10(2):367-82. https://doi.org/10.14336/ad.2019.0324.
26. Latalski M, Walczyk A, Fatyga M, Rutz E, Szponder T, Bielecki T, et al. Evaluation of activated and non-activated platelet-rich plasma on the irradiation decreases the production of pro-inflammatory cytokines by stimulated T cells. Adv Wound Care 2019;8(7):298-308. https://doi.org/10.1177/1559051519849766.
27. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet activation: The mechanisms and potential biomarkers. Biomed Res Int 2016;2016:1-16. https://doi.org/10.1155/2016/6591717.
28. Malesci-Campbell AL, Ismail A, Reynolds KA, Poon E, Semano L, Grushchak S, et al. A systematic review of the safety and effectiveness of platelet-rich plasma (PRP) for skin aging. Arch Dermatol Res 2020;312(5):301-15. https://doi.org/10.1007/s00403-019-1999-9.
29. Karina KA et al., et al. Journal of Health Sciences XXXX;X(X)1-5.
30. Zhang Y, Ying Q, Ren C, Ji Zhang Y, Borlongan CV, Zhang J, et al. Administration of human platelet-rich plasma reduces infarction volume and improves motor function in adult rats with focal ischemic stroke. Brain Res 2014;1594:267-73. https://doi.org/10.1016/j.brainres.2014.10.035.

31. Vina M, Camozzi L, Spitaleri MI, Reinchisi G. Hyperconcentrated platelet-rich plasma (High-PRP) for the treatment of a non-healing ulcer of the lateral malleolus: A case report and literature review. CellR4 2020;8:e2873.

32. Karina K, Rosliana I, Rosadi I, Schwartz R, Sobariah S, Afini I, et al. Safety of technique and procedure of stromal vascular fraction therapy: From liposuction to cell administration. Scientifica 2020;2020:2863624. https://doi.org/10.1155/2020/2863624.

33. Moegni KF, Rosliana I, Remelia M, Rosadi I, Sobariah S, Afini I, et al. Stromal vascular fraction (SVF) therapy for treatment of various diseases: Delivering safety of the first patented SVF technique in Indonesia. Cytotherapy 2019;21(5):S86. https://doi.org/10.1016/j.jcyt.2019.03.511.