Research article

Evaluation of plasma PDGF and VEGF levels after systemic administration of activated autologous platelet-rich plasma

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ABSTRACT

Introduction and Aim: Activated autologous platelet-rich plasma (aaPRP) is becoming a popular therapy to accelerate healing in the field of plastic surgery. Platelets, which are abundant in aaPRP, can release many growth factors including platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). This study aims to examine the plasma levels of PDGF and VEGF in healthy subjects after intravenous administration of aaPRP.

Materials and Methods: Nine healthy patients with no prior history of metabolic disease were divided into two groups (control and experiment group). The treatment group which consists of six patients received intravenous aaPRP treatment. The preparation of aaPRP starts with the collection of 24 mL of whole blood in sodium citrate tubes followed a two-step centrifugation procedure and subsequent chemical activation. aaPRP was then administered intravenously to patients. Meanwhile, the control group received no intervention. Venous blood samples were taken before and one week after the treatment and the plasma PDGF and VEGF levels were determined by enzyme-linked immunosorbent assay (ELISA).

Results: The treatment group showed statistically significant increase in VEGF after 7 days compared to before aaPRP administration. However, the change in PDGF levels of the treatment group was unnotable.

Conclusion: The present findings indicate that intravenous administrations of activated aaPRP may increase plasma VEGF level up to 1 week after aaPRP administration.

Keywords: Activated autologous platelet-rich plasma (aaPRP); platelet derived growth factor (PDGF); vascular endothelial growth factor (VEGF).

INTRODUCTION

Autologous activated platelet-rich plasma (aaPRP) is processed autologous plasma. It is enriched in platelet concentration, hence it is abundant in bioactive molecules such as growth factors in comparison to whole blood (1, 2). These molecules exert numerous positive effects on the body and because of its potential benefit, aaPRP has been of great academic interest over the past few years (1, 3). Apart from that, aaPRP has also been increasingly replacing fetal bovine serum in mesenchymal stem cell cultures. Animal-to-human transmission of disease and immunogenetic reactions are some of the concerns supporting the shift to non-animal material such as aaPRP (4).

aaPRP and similar platelet concentrate products has been utilized for many treatments, including wound healing (5), psoriasis (6), and musculoskeletal medicine (7, 8). Furthermore, aaPRP therapy increases proliferation rate and induces chondrogenic differentiation of adipose-derived stem cells (9). Our previous study showed that intravenous (IV) administration of aaPRP combined with stromal vascular fraction (SVF) was safe to treat 421 patients of various pathologies and demographics (10). It was reported that both PRP and aaPRP improved tendon healing rates, pain levels, and functional outcomes significantly higher than platelet-rich fibrin (PRF; 8); while another study demonstrated that PRP in other preparation methods, such as advanced platelet-rich fibrin (A-PRF) and concentrated growth factors (CGF), stimulated periosteal cell proliferation (11). A recent meta-analysis has also documented various applications of CGF, ranging from facial rejuvenation to cartilage grafting and facial peripheral nerve injury (12).

In practice, PRP therapy aims to mediate regeneration through growth factors. Among the diverse favorable molecules present in PRP are platelet-derived growth factor (PDGF), which is abundant in platelet, and vascular endothelial growth factor (VEGF); These growth factors are both responsible for the positive effects of PRP (13). PDGF and VEGF are known to play a role in angiogenesis, wound healing, and regulation of cell growth (1-3, 13).

PRP activation is a procedure commonly performed to induce degranulation and release of beneficial bioactive molecules. Activation of PRP can be done by means of chemical agents such as calcium activator or thrombin, mechanically such as friction or freeze-thawing, or by photoactivation (10, 14). It is estimated that 70% of total growth factor is released within 10 minutes of platelet activation, while the total amount is released about an hour post-activation (2).

There are only a limited amount of research studying the effects of IV aaPRP administration, let alone its influence on plasma growth factor. Therefore, this study aimed to compare PDGF and VEGF concentration in blood plasma between healthy patients treated and not treated with systemic aaPRP injection.

MATERIALS AND METHODS

This prospective cohort study observed PDGF and VEGF levels after aaPRP administration in healthy patients. Ethical clearance for the study was acquired from Health Research Ethics Committee, University of Indonesia, and Cipto Mangunkusumo Hospital. After informed consent was obtained, subjects without any history of metabolic diseases are considered as healthy and included in the study. Six patients were administered aaPRP and regarded as the treatment group, while three volunteers were recruited as the control group and received no intervention. Prior to aaPRP therapy, all patients were sampled for plasma PDGF and VEGF levels. Plasma growth factor levels were then re-evaluated 7 days after administration of systemic aaPRP.

Platelet-rich plasma preparation

aaPRP was prepared using a method which was invented and developed by Hayandra Lab. Samples of 24 mL whole blood were collected by venous puncture from each patient into eight sodium citrate tubes and centrifuged (Thermo ST40) at 1,000 rpm for 10 minutes. Blood plasma was then aspirated and subjected to another centrifugation at 3,000 rpm for another 10 minutes until platelets were concentrated at the bottom of the tube. The top portion of the tube was discarded until each tube contains 2.5 mL of plasma, which was reckoned as inactivated PRP. The eight tubes of PRP are then merged into four tubes each containing 5 mL aaPRP and activated with 0.15 mL calcium activator (H-Remedy, Hayandra Lab,

Indonesia) for each tube. Subsequent clots are then removed, followed by addition of 10 mL NaCl 0.9% and photoactivation (AdiLight-1, AdiStem Ltd., Hong Kong) afterwards. Finally, the 15 mL aaPRP is suspended in 100 mL of NaCl 0.9% and administered intravenously to the subjects for 15 minutes.

PDGF and VEGF analysis

PDGF and VEGF levels were determined using FineTest PDGF (EH3531) and VEGF (EH0327) enzyme-linked immunoassay kits (Wuhan Fine Biotech Co., Ltd, Wuhan, Hubei, China). Both kits employ quantitative sandwich enzyme immunosorbent assay technique. After blood was collected, the sample was centrifuged and resulting supernatant was immediately collected and assayed. Sample dilution is performed, and incubation is done at 37°C for 90 minutes. Absorbance is then read in microplate reader at 450 nm both kits in accordance with the manufacturer's instructions. ELISA results were expressed in pg/mL.

Statistical analysis

Plasma PDGF and VEGF levels were compared between treatment and control groups before and after administration of aaPRP with 7 days interval. Normality tests were done, followed by independent samples t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. Meanwhile, comparison of in-group data before and after the 7 days were done with paired samples t-test for normally distributed data and Wilcoxon test for non-normally distributed data. A p-value of less than 0.05 is considered statistically significant.

Ethical statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by Health Research Ethics Committee, University of Indonesia and Cipto Mangunkusumo Hospital with issued letter of approval No. 628/UN2.F1/ETIK/2016.

RESULTS

The characteristics of the nine patients enrolled in this study are summarized in Table 1.

Table 1: Characteristics of the patients in both treatment group and control group

Parameters	Treatment	Control
Sample size	6	3
Male	2 (33%)	1 (33%)
Female	4 (67%)	2 (67%)
Median of age	49.5	31

Fig. 1 Shows mean plasma PDGF and VEGF levels between untreated control and treatment group respectively before and after administration of IV aaPRP. PDGF levels before (6478.81 ± 300.08 vs 8265.63 ± 5841.28 pg/mL, p = 0.63) and after treatment (7298.76 ± 1457.39 vs 6931.94 ± 2210.37 pg/mL, p = 0.81); and VEGF levels before

(41.25 ± 1.61 vs 40.12 ± 6.68 pg/mL, *p* = 0.36) and after treatment (77.68 ± 52.96 vs 86.64 ± 27.29 pg/mL, *p* = 0.41) showed no significant nor notable mean differences between untreated and aaPRP-treated patient groups.
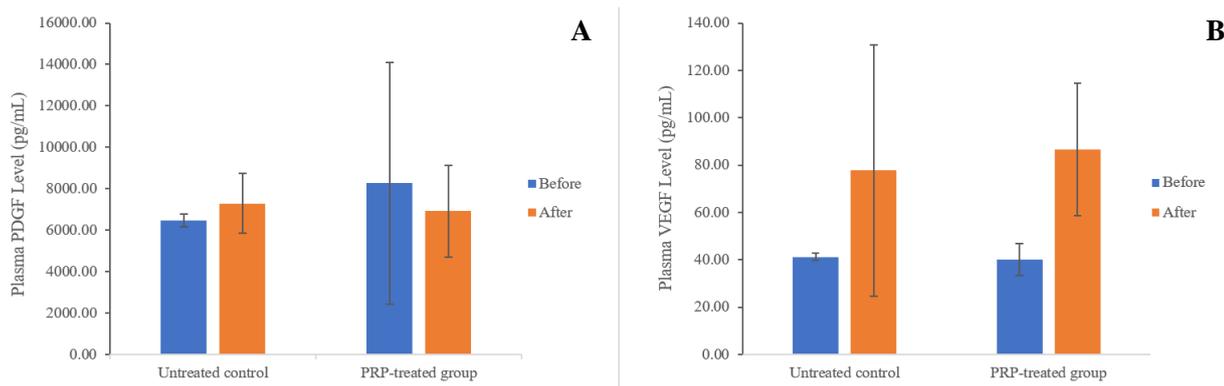

Fig. 1: Comparison of mean plasma PDGF (A) and VEGF (B) levels in untreated control and PRP-treated group.

DISCUSSION

Both platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are found in platelets, which is the main component of prepared PRP, along with various levels of leukocytes (depending on the protocol used), and low to absent amount of red blood cells (1-3,13). Prepared PRP can be administered without prior activation (inactivated PRP), or activated exogenously (aaPRP) before administration with various methods in order to induce rapid secretion of aforementioned growth factors from the platelets (2).

In our study, we activated our PRP with calcium activator and laser before intravenous administration. Photoactivation of platelets by a low-frequency light source results in controlled and prolonged release of growth factors (15). The usage of photoactivated PRP has been reported in studies with positive results (7,10,16). It is also shown that platelet activation method affects the release of bioactive molecules and its kinetics, hence the type of PRP activation method should also be considered with regards to what procedure is being performed and the desired biological outcome (17).

PDGF regulates cell growth and division, it also plays a role in angiogenesis. It is also an effective chemoattractant and mitogen for mesenchymal cells (13). As PRP contains high levels of PDGF, it is used to enhance mesenchymal stem cell (MSC) performance in stromal vascular fraction (SVF) therapy (4,9,10). In animal models, PDGF-BB has also been found to prevent aberrant angiogenesis in therapeutic angiogenesis with high VEGF levels. PDGF has been shown to prevent vascular tumor and instead yields normal mature capillaries (18). Other growth factors have also been found to regulate each other, exhibiting inhibitory and synergistic interactions.(19) As such, it can be inferred that growth factors are dynamic and interdependent. Based on our findings, IV aaPRP did not alter plasma PDGF level significantly. This result can be attributed to maintenance of homeostasis which resulted in appropriate growth factor levels.

VEGF plays an important role in the initiation of angiogenesis through migration and proliferation of endothelial cells (13). In renal podocytes, VEGF-A and VEGF-C levels are maintained at a balance and affects podocyte survival and apoptosis. Its levels are also indicated to influence progression of glomerular diseases, such as diabetic nephropathy (20,21). This indicates that regulation of growth factor levels is important for normal bodily functions to occur. Previous *in vivo* study involving intradermal injections of PRP improved rat wound closures with less inflammation, more skin appendages, and blood vessels-leading to a higher rate of hair growth (5). Our previous study also revealed that platelet concentration in diabetic donors had higher whole blood platelet concentration, lower total protein, and higher VEGF concentrations than non-diabetic PRP, making it a promising treatment for diabetic foot ulcer (14). Our in-group data showed that IV aaPRP supplementation significantly increased plasma VEGF levels within normal levels 7 days after administration, showing the potential of IV aaPRP therapy for diabetic foot ulcer and tissue regeneration, among others.

To the best of our knowledge, our study is the first to explore the plasma growth factor levels after IV aaPRP infusion. Our results suggests that IV aaPRP significantly increased plasma VEGF concentration up to one week after administration, while the change of PDGF levels were not statistically significant. However, our study contains some limitations. As our research only measured a few of the numerous growth factors present in the body, other unmeasured growth factors may have affected the growth factors we measured. The small pool of sample could also cause the study to become underpowered, leading to a statistically insignificant result. The lack of randomization meant that we could not properly control confounding factors such as age, fitness activity, pre-existing comorbidities, and many other

factors. Therefore, future studies with a larger subject pool and better study designs are needed to confirm these findings.

CONCLUSION

As seen in the data procured, we conclude that IV administration of aaPRP may increase plasma levels of VEGF up to 7 days after administration. However, future studies are still needed to confirm this finding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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