Changes of Vascular Endothelial Growth Factor and Platelet-derived Growth Factor Concentrations in Platelet-rich Plasma After Preparation

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Abstract: Platelet-rich plasma (PRP) is plasma that contains platelets concentrated by centrifugal separation of a blood specimen. Advances in the practical applications of PRP have enabled regenerative therapies for hard tissue. Blood contains many growth factors. Therefore, to more effectively use PRP in the clinical setting, it is important to evaluate how growth factor levels change after PRP preparation. The objective of the present study was to observe how the concentrations of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) in PRP change after preparation. PRPs were prepared from 17 healthy consenting male and female subjects aged 25-32 years. Enzyme-linked immunosorbent assay (ELISA) was used to measure the VEGF and PDGF concentrations at 15, 30, 60, 120 and 1,440 min after PRP preparation. We found that VEGF and PDGF concentrations remained high, and exhibited their highest levels at 24 h after PRP production. This persistence suggests that VEGF and PDGF might remain active in tissue regeneration, even 24 h after PRP is prepared.

Key words: PRP, Growth Factor concentration, VEGF, PDGF

Introduction

Regenerative medicine aims to regenerate tissue or organs that are not fully functional because of illness or injury; this field is also expected to be applicable to the development of new drugs1). The clinical applications of hard tissue regeneration therapy have been reported for autogenous or artificial bone grafts in dental practice2), guided tissue regeneration3), and growth factors4,5). Autogenous bone grafts are generally the safest option6) for bones compromised after the removal of a benign tumor or the planned implant at a dental site; however, these grafts may invade healthy parts of the body and can sometimes pose clinical risks. Artificial bone materials such as calcium phosphate or ß-tricalcium phosphate are often used to avoid such issues7). However, because artificial bones cannot undergo osteoinduction, the sites at which they can be used are limited. Growth factors promote angiogenesis, new bone formation, and wound healing, and when used together with artificial bones, they help to promote osteoinduction to the artificial bone and regeneration of bone and blood vessels at compromised bone sites8). Thus, growth factors are recommended for use in clinical applications.

In 1998, Marx et al.9) detected multiple growth factors in platelet-rich plasma (PRP); PRP contains platelets concentrated by centrifugal separation of an individual’s blood. These workers combined an autogenous bone graft with PRP to treat bones that had been compromised by tumor excision. They reported an early-stage increase in the maturity of bone grafts in the presence of PRP, relative to autogenous bone grafts without PRP. Since then, clinical studies have explored the potential uses of PRP in regenerative medicine.

PRP is manufactured by the centrifugal separation of collected blood specimens, which increases platelet concentration by 2–8-fold. Reports of regenerative therapy advancements involving the clinical use of PRP are numerous; however, few studies have evaluated how growth factor levels, which promote healing, change over time10,11). The present study thus produced PRP from blood specimens, and investigated how PRP growth factor abundances changed following preparation. Among the growth factors contained in PRP, we focused on platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which contribute to the formation of new blood vessels for the delivery of nutrients important for tissue healing and regeneration.
Materials and Methods

Collection and storage of samples

With approval from the Ethics Committee of Osaka Medical College (No. 1753, A Study Involving Measurement of Growth Factor Concentration in Platelet-Rich Plasma (PRP)), blood specimens were acquired from 17 informed and consenting individuals in good health, aged 25-32 years (10 males, 7 females, average age of 26.3 years). Blood samples were extracted from the median cubital vein using a 22G syringe, and immediately mixed with EDTA-2Na powder to prevent coagulation in the collection tube. Furthermore, the ELISA kit used to quantify VEGF and PDGF was confirmed to not be affected by EDTA. After the samples were mixed, they were transferred to plastic test tubes and centrifuged for 10 min at 2,750 rpm, 1,000 x g in a centrifuge (Centrifuge 416G, Morita, Osaka, Japan).

After centrifuging, each specimen had separated into the three layers of blood cells, PRP, and plasma (Fig. 1). Growth factors are known to be released after platelets are activated and á-granules are destroyed. To observe how the abundances of the growth factors VEGF and PDGF changed over time, we collected 500 µl PRP samples from the 10 ml whole blood specimens. We detected VEGF and PDGF concentrations at five time points over the duration following the end of centrifugal separation: 15, 30, 60, 120 and 1,440 min after preparation. At each observation point, 100 µl was extracted from each 500 µl sample and cryopreserved at -80°C. Freezing of the samples ceased any further changes to the proteins.

Growth Factor Quantification (ELISA)

ELISA is an analytical method for quantifying small amounts of protein using highly specific antigen-antibody reactions, antibody-linked reporter-enzymes, and chromogenic reporter-enzyme substrates. To determine how the concentrations of the growth factors VEGF and PDGF changed after PRP preparation, ELISA was conducted using a Human VEGF Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA), and a Human PDGF-AB Quantikine ELISA Kit (R&D Systems), according to the manufacturer’s instructions. PDGF was measured in a 50-fold dilution of the collected sample. Absorbance was measured at wavelengths of 450 nm and 540 nm using a spectrophotometer (Corona SH-1000 absorbance grating microplate reader, Hitachi High-Tech Science Corporation, Tokyo, Japan). A standard curve was generated from standard solutions at each concentration to calculate protein concentrations.

Statistical Analysis

Using Microsoft Excel 2008 (Redmond, WA, USA) and ystat 2008 (Igakutosho-shuppan, Tokyo, Japan) for statistical analysis, significant differences were detected by comparing groups using one-factor analysis of variance (ANOVA). The Bonferroni method was used for multiple comparison testing. Statistical values are reported as means ± standard deviations; for each test, p < 0.05 was considered indicative of a significant difference.

Results

VEGF

Figure 2 shows mean VEGF concentrations for the samples...
Whether changes in growth factors over time vary based on patient time, although these authors used a different production method.

A significant increase in the amounts of VEGF and PDGF over time was observed by Su et al. These results suggested that the concentrations of VEGF, PDGF, and transforming growth factor-$
\beta$(TGF-$
\beta$) significantly increased over time; thus, these actions should also be considered in the clinical application of PRP. The present study clarifies the basis of treatments involving PRP and provides basic research for improving their practical use. Additional studies are required before regenerative medicine can be widely applied.

The present results may help establish a standard procedure and advance future clinical procedures. Furthermore, determining the time of the greatest increase in growth factors may enable more efficient wound healing.

**Discussion**

When tissue is injured and bleeds, platelets are immediately activated to coagulate at damaged blood vessels and stop the bleeding. When this activation occurs, several growth factors are expressed, and they participate in healing and regeneration. Approximately 100,000–400,000 platelets/mm$^3$ are present in the blood, and á-granules within these platelets contain several growth factors such as PDGF, VEGF, and transforming growth factor-$\alpha$ (TGF-$\alpha$)$^{12}$. When á-granules are destroyed by platelet activity, growth factors are released, and PRP uses these growth factors to heal wounds and regenerate tissue in clinical applications.

In recent years, PRP has been deployed in surgical procedures such as trauma and orthopedic surgery, because it is effective in clinical wound healing$^{13}$. PRP has also recently been used to efficaciously treat Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ). Future use of PRP for BRONJ patients is therefore anticipated$^{14}$.

Since PRP can be used in a variety of surgery scenarios, its shelf-time may also vary. Growth factor abundance changes following PRP preparation are therefore worth understanding.

The present study evaluated how the concentrations of growth factors VEGF and PDGF changed after PRP preparation. These results suggested that the concentrations of VEGF and PDGF in PRP generally increased for up to 24 h following PRP production. Such increases may indicate that VEGF and PDGF exert their effects even at 24 h after PRP preparation.

Su et al.$^{15}$ and Dohan Ehrenfest et al.$^{11}$ reported the amounts of growth factors released over time in platelet-rich fibrin. Su et al.$^{16}$ obtained results similar to those of our study, which indicated a significant increase in the amounts of VEGF and PDGF over time, although these authors used a different production method.

The procedure reported presently yielded uniform results, which may help to establish a standard procedure. However, whether changes in growth factors over time vary based on patient age or gender must be further evaluated.

PDGF contributes to the migration, angiogenesis, and proliferation of mesenchymal cells (fibroblasts and smooth muscle cells); it is also thought to contribute to the development of atherosclerosis and fibroproliferative disease$^{15,16}$. VEGF contributes to angiogenesis; however, it also contributes to malignant changes, i.e., the angiogenesis or metastasis of tumors, and is thought to be closely related to the critical worsening of cancer$^{17,18}$. These growth factors were observed in all PRP produced in this study, and almost no reduction was observed over time; thus, these actions should also be considered in the clinical application of PRP. The present study clarifies the basis of treatments involving PRP and provides basic research for improving their practical use. Additional studies are required before regenerative medicine can be widely applied.

The present results may help establish a standard procedure and advance future clinical procedures. Furthermore, determining the time of the greatest increase in growth factors may enable more efficient wound healing.

**Conflict of interest**

The authors have declared that no COI exists.

**References**

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