Original Article

Efficacy of a New Autologus Platelet Gel: in Vitro Study

Hashemi Tayer A MSc[1], Alizadeh Sh MD[2], Heidari Bateni M MD[2]

1-Department of Hematology, University of Medical Sciences, Arak , Iran
2-Department of Surgery, University of Medical Sciences, Arak , Iran

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Abstract

Background

Autologus platelet gel is easy to prepare and is relatively low cost. The aim of this study was to prepare and evaluate in vitro efficacy of autologus platelet gel.

Materials and Methods

In this experimental study, platelet concentrate and platelet poor plasma were prepared with aphaeresis method. Thrombin was prepared by mixing the plasma with calcium. Thrombin activity level was determined by spectrophotometric method. The platelet gel was obtained by adding thrombin and calcium to the platelet rich plasma. The concentration of growth factors was measured using the enzyme-linked immunosorbent assay technique.

Results

Platelet rich plasma contained a total average of 1×10^{11} platelet in the 100 ml product. The highest activity of prepared thrombin was achieved at a ratio of 5 vol (5ml) platelet poor plasma to 1 volium (1ml) calcium gluconate (p=0.02). Produced thrombin was stable for two hours at 24ºC, six hours at 4ºC, and more than three months at -20ºC (p=0.04). Concentration of platelet derived growth factor and transforming growth factors-β in platelet gel supernatants were generally high (23.2 ± 6 ng/ml, 18.2 ± 5ng/ml respectively), whereas epithelial growth factor and fibroblast growth factor were present at low concentration (870 ± 110 pg/ml, 24.7 ± 3 pg/ml respectively).

Conclusion

With proper production, large numbers of platelets in the platelet rich plasma will be activated, hence high concentration of growth factors will be produced.

Keywords

In Vitro, Platelet-Derived Growth Factor, Platelet-Rich Plasma

Corresponding Author
Hashemi Tayer Akbar. Department of Hematology , University of Medical Sciences, Arak, Iran. Email: hashemiakbar@yahoo.com

Introduction

Autologus Platelet Gel (APG) developed in the early 1990's as a byproduct of Platelet Rich Plasma (PRP) and increasingly used in almost all field of surgery for the treatment of a variety of hard and soft tissue defects, especially in the management of chronic non healing wounds and in accelerating bone formation (1). One of the benefits of APG is that it will accelerate endothelial and epithelial regeneration, also stimulate angiogenesis, enhance collagen synthesis, wound repair, decrease pain, inflammation and provide antimicrobial properties. There are numerous clinical applications of APG, for instance in facial plastic surgery, chronic wounds, general surgery, ophthalmology, urology, orthopedic and burns (2-5).

Also PRP is an autologous concentration of human platelets in a small volume of plasma. These Platelets are attracted by wound or injury site, stimulating the clotting and healing cascades. In addition, degranulation of platelets by proteins such as thrombin causes them to release numerous substances including proteins known as Growth Factors (GFs) (6). Platelet growth factors are responsible for the primary migration of cells to the injured site and the triggering of proliferation of these cells once at the location (7). They are located in the alpha granules of platelets as well as other cells such as endothelium and macrophages and are critical for wound healing. Growth factors isolated from platelet include the Platelet-Derived Growth Factors (PDGFαα, PDGFββ, and PDGFαβ), Transforming Growth Factors-β (TGFβ1 and TGFβ2), Vascular Endothelial Growth Factor (VEGF), Epithelial Growth Factor (EGF), Insulin- Like Growth Factor (IGFI and IGFII) and Fibroblast Growth Factor (FGF). Growth factors send out signals to trigger cell division. For example PDGF initiate angiogenesis in the wound, TGF-β promote cell mitosis and differentiation of fibroblasts and stem cells, VEGF enhance vascular permeability specific for endothelial cells and EGF stimulate epithelial development (7,8).

Platelet granules also contain a variety of active substances, such as catecholamine, serotonin, adenosine triphosphate, albumin, fibrinogen, Von Willeberand Factor (vWF), various clotting factors, calcium ions and proteins to act as cell adhesion molecules and as a matrix for bone, connective tissue, and epithelial migration. These cell adhesion molecules are consist of fibrin, fibronectin and vitronectin. Different investigation revealed that enhance-ment of the wound healing process requires proper production of the platelet gel (1, 7 and 8). Because there are different procedures for platelet gel preparation, the aim of this study was to use simple and safe method for platelet gel preparation, followed by in vitro assessment of its efficacy.

Materials and Methods

In this experimental study, a total of twenty normal healthy unmedicated volunteers (ten men and ten women) were selected. Consent form were read and signed by all volunteers. Further, on screening tests such as Prothrombin Time (PT), Activated Partial Tromboplastin Time (APTT) and platelet count were performed. 200 ml Platelet Poor Plasma (PPP) and 100 ml PRP were collected from the volunteers by an aphaeresis procedure using the haemonetics device (haemonetics corporation, PCS2, USA).

Based on donor Hematocrit (HCT), the ratio of ACD-A (Anticoagulant Citrate Dextrose-A) to blood volume was adjusted in the range of 1/8 to 1/12. Platelet count was performed on both the PPP and PRP using the hematology cell counter (Sysmex KX-21N™ Automated Hematology Analyzer).

Thrombin was prepared by mixing the PPP with calcium (0.2 mol/L calcium gluconate or 25 mM calcium choleride) in various

ratios. Following 30 minutes incubation at 24ºC and centrifugation, the supernatant containing thrombin was collected and thrombin activity level was determined by spectrophotometric method. The produced thrombin was stored at different temperatures (24ºC , 4ºC , -20ºC) and activity was assessed through clot formation in different time intervals.

Platelet gel was then prepared by mixing PRP with thrombin and calcium gluconate (ratio: 5vol. 2vol. 1vol respectively) at 24ºC. This mixture was injected in to a petri dish that activates platelet to form cloth. The products were split in to 1-ml aliquots, and then stored at 37ºC for 30 minute, 6, 24, 48 ad 72 hours.

Platelet gel provides tissue regeneration-inducing GFs. The concentration of GFs were measured in the supernatant using enzyme-linked immunosorbent assay (ELISA, Assaypro Human immunoassay kit), that had been validated for measuring PDGFαβ, TGFβ, EGF and FGF, according to the manufacturer instructions. In addition, release time of the PDGF, TGF, EGF and FGF in the platelet gel clot was recorded.

Statistical Analysis

Collected date**s** were expressed as mean ± standard deviation, and dates were analyzed using Kolmogorov- Smirnov and ANOVA by running SPSS software version 16. *P* value < 0.05 was considered as statistically significant.

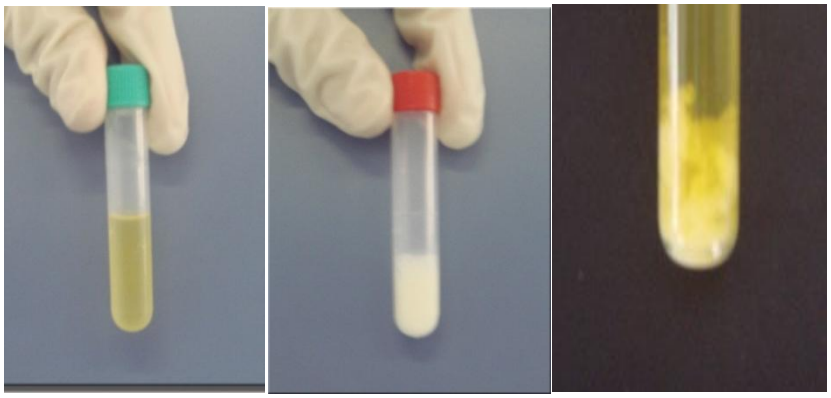
Results

Twenty healthy volunteer donor [mean ± SD (age): 29 ± 5 years] were participated in this study. They have a baseline blood platelet count of 240,000 ± 66,000 per µL. The high number of platelets was achieved with apheresis system. The PRP contained a total avarage of 1×10^{11} platelet in the 100 ml product and the 200 ml PPP contained a total avarage of 1×10^{7} platelet. The results indicate that the highest activity of prepared thrombin was obtained at a ratio of 5 vol PPP to 1 vol calcium gluconate (p=0.02) (Table 1). The mean average activity of thrombin was 42±2.3 NIH unit (National Institute of Health) and has sufficient activity for clot formation. During storage time, it became stable two hours at 24ºC, six hours at 4ºC, and more than three months at -20ºC (p=0.04). Upon the addition of the thrombin and calcium to PRP, a thick, adhesive gel was formed (Figure 1). Concentration of released GFs into platelet gel supernatants varying in time intervals . ELISA assay revealed that concentration of PDGF and TGF in platelet gel supernatant were generally high (ng/ml), whereas EGF and FGF growth factors were present at low concentration (pg/ml). As shown in Figure 2, PDGF level significantly elevated 30 minutes after incubation of platelet gel at 37ºC (23.2 ± 6 ng/ml) but the concentration of FGF, EGF and TGF increased following 24 hours incubation (24.7 ± 3 pg/ml, 870 ± 110 pg/ml and 18.2 ± 5 ng/ml, respectively)($p<0.05$). After this time, the GFs levels were decreased.

Table 1. Thrombin activity level in various ratios of PPP and calcium

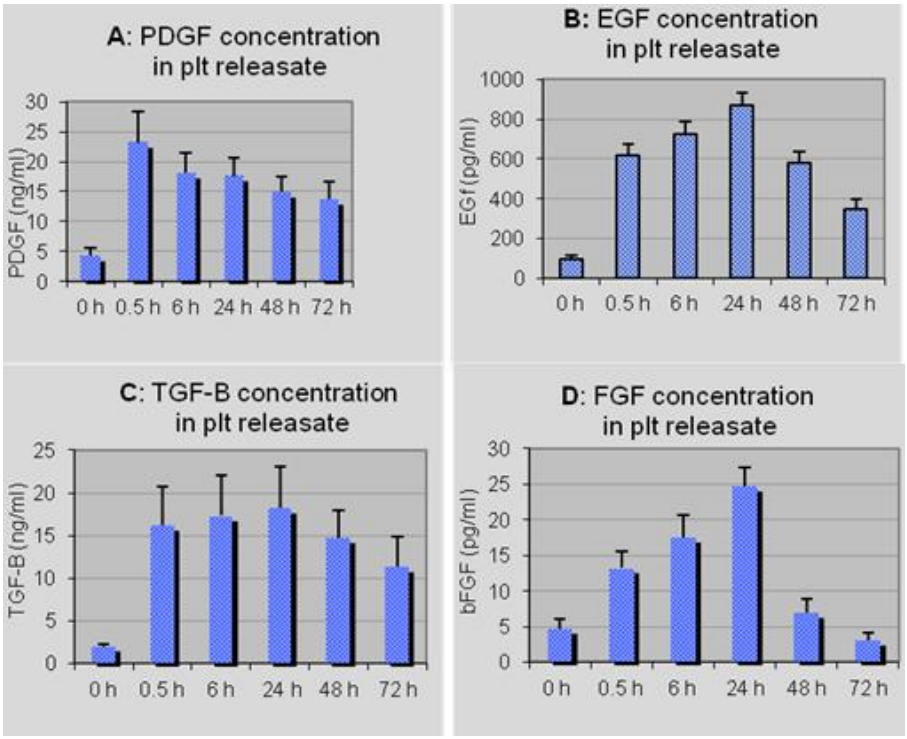
PPP and Ca Ratio	Thrombin activity (NIH unit)	P-value
PPP + Ca gluconate (5:1)	42±2.3	0.02
PPP + Ca gluconate (3:1)	33.5±2.1	
PPP + Ca chloride (5:1)	20±1.5	
PPP + Ca chloride (3:1)	14±1.4	

Figure1. Mixing thrombin and calcium (A) with PRP (B) to prepare platelet gel (C)



A: Thrombin / Calcium B: PRP C: Platelet Gel

Figure 2. Concentration of growth factors in platelet releasate in different time



Discussion

Autologus platelet gel is a safe and cost effective product and well accepted by patients. It is readily available at the point of care, eliminates the risk of disease transmission and immunologic reactions (7). In the present study, APG is made by mixing PRP with autologous thrombin obtained from PPP. Thrombin trigger platelet degranulation , causes them to release complex group of growth factors (9,10). It is well know that platelets contain a large variety of GFs, each having specific function. Several in-vitro and in-vivo models show that all kinds of cells involved in tissue repair are sensitive to GFs. Fibroblasts are reactive to PDGF, FGF, EGF and IGF. Endothelial cells are sensitive to FGF and VEGF. Angiogenesis is particularly enhanced by PDGF, FGF and VEGF. Moreover human mesanchymal cells, which are recruitable during tissue regeneration process, are up-regulated by PDGF as well. All the above mentioned GFs are released by platelets (1, 6, 11).

Platelet gel must be used within 30 minutes of preparation. However the separate components (e.g. PRP [freshly prepared, or stored at room temperature for up to 5 days] and thrombin [stored at room temperature for 2 hours]) can be used to prepare platelet gel within a few hours .

In the present study, we measured significantly different GFs levels in the platelet gel supernatant. This study revealed that concentration of PDGF and TGF in platelet Gel supernatants were generally high (ng/ml), whereas EGF and FGF growth factors were present at low concentration (pg/ml). Many studies demonstrated that for preparation of platelet gel, commercial thrombin with high activity level is necessary to be used (7,12). In the present study, the release rate of FGF at the beginning of the incubation period was low, finally reached to peak after 24 hours. They level of growth factors in the desired time were statistically significant compared to other incubation times. When higher concentrations of thrombin and calcium used for platelet degranulation, release of FGF began immediately and when lower concentrations of thrombin and calcium were used, release of this factor can be delayed. According to our finding, latency in the release of FGF is probably due to the low activity of autologous human thrombin. Kinetic experiments demonstrated that variations in GFs concentration over time differed from one GF to another and there was positive correlation between the platelet concentration and growth factors levels. The results of our investigation are similar to those of previously published data (7, 13).

Conclusion

It can be concluded that with proper production, large numbers of platelets in the PRP are activated producing high concentration of GFs. A variety of growth factors are detected and released from the platelets at significant levels in APG.

Acknowledgement

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Conflict of Interest

None of the authors have any conflicts of interest to declare.

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