Suez Canal University Medical Journal Vol. 22 (1), 2019
Pages 1-8

Review Article

Platelet-rich Plasma: Three Decades and Ongoing, Do We Have a Conclusion?

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Abstract

Blood components for non-transfusion uses have been the focus of researches in various clinical fields for the last thirty years. Platelet-rich plasma (PRP) can expedite healing, with minimal side effects. As a biologic autologous product, it is well-tolerated, and safe choice for many physicians and patients. Yet, no consensus has been reached regarding PRP preparation, needed volume, the use of an activator, number of sessions, and the period between the sessions in different clinical scenarios. Thus, further clinical studies are needed with higher level of evidence taking into consideration the optimization of these aspects. Up to date, there are many PRP preparation systems on the market with FDA clearance. Meanwhile, FDA has not attempted to regulate activated PRP. Determining the main bioactive components which are responsible for the clinical effects of PRP and the inter-individual variability of growth factors and cytokines production and the synergy of platelets count and growth factors will remain as major obstacles to achieve standardization of PRP. PRP is yet to become the standard of care; nevertheless, more clinical studies with longer periods of follow up to understand the best candidate and to determine the best ways to use it to improve healing; it may be an approved practice in the future.

Key words: *platelet-rich plasma, growth factors, platelets, applications, preparation methods*

Introduction

Platelets for non-transfusion purposes have been given many terms; platelet-rich plasma, platelet-leukocyte gel, platelet rich fibrin, platelet gel, platelet rich growth factors and platelets concentrate (PC). Most commonly used is PRP, it is an autologous or allogenic hemoconcentrate with an increased concentration of platelets suspended in a small amount of plasma after centrifugation[1,2]. The first clinical demonstration that platelets growth factors can act locally to promote healing of chronic cutaneous ulcers was back in 1986[3]. Later in 1990s, effectiveness of the PRP in bone regeneration in the field of dental care was elucidated[4]. Since then, PRP has been extensively used in periodontal, oral and maxillofacial surgery, the musculoskeletal field, sports injuries, dermatology (tissue regeneration, wound healing, scar revision, skin rejuvenating effects, and alopecia), aesthetic plastic surgery, treatment of

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chronic skin and soft-tissue ulcers, spinal fusion, heart bypass surgery, gynecology, urology, and ophthalmology[1,5].

Function

Platelets are natural source of various growth factors. Upon activation, platelets release the stored intercellular mediators and cytokines from the cytoplasmic pool

Table 1: Functions of the growth factors and cytokines in the platelet rich plasma[1, 6-9].

Growth factors	Functions
Platelet-derived growth factor (PDGF)	Mitogenetic for mesenchymal cells and osteoblasts and mitogenesis stimulator for fibroblast, glial, or smooth muscle cells Stimulates macrophage and neutrophil chemotaxis and regulates collagenase secretion Chondrocyte chemotaxis and chondrogenesis Angiogenesis stimulator, vascularization Increases hair growth
Transforming growth factor-beta (TGF-β)	Promotes wound healing Stimulates growth and neogenesis of epithelial cells and vascular endothelial cells Stimulates undifferentiated mesenchymal cell proliferation Regulates endothelial, fibroblastic, and osteoblastic mitogenesis Regulates collagen synthesis and collagenase secretion Regulates mitogenic effects of other growth factors Inhibits macrophage and lymphocyte proliferation Promotes hair-cell proliferation and regeneration
Vascular endothelial growth factor (VEGF)	Stimulates growth and new generation of vascular endothelial cells and increases vessel permeability Stimulates mitogenesis of endothelial cells Expressed in dermal papilla cells, increases perifollicular vessel size during the anagen growth phase
Epidermal growth factor (EGF)	Stimulates endothelial chemotaxis Regulates collagenase secretion Stimulates epithelial or mesenchymal mitogenesis Angiogenesis stimulator Stimulates hair-cell proliferation and regeneration
Fibroblast growth factor (FGF)	Endothelial proliferation, angiogenesis, collagen production Promotes tissue repair, cell growth, and hyaluronic acid production Promotes growth and differentiation of chondrocytes and osteoblasts Mitogenetic for mesenchymal cells Increases hair growth by inducing the anagen phase of hair follicles
Insulin-like growth factor 1 (IGF-1)	Cell proliferation, production of proteoglycan and collagen Angiogenesis stimulator Chemotactic for fibroblasts and stimulates protein synthesis Enhances bone formation. Improve early healing in tendon defects Increases hair growth
Connective tissue growth factor (CTGF)	Promotes angiogenesis Cartilage regeneration
Hepatocyte growth factor (HGF)	Angiogenesis stimulator
Keratinocyte growth factor (KGF)	Promote endothelial cell growth, migration, adhesion and survival Angiogenesis stimulator
Interleukins (IL)	Cytokines like IL-1, IL-4, IL-6 help in healing of muscle injury and transdifferentiation of fibroblasts into myoblasts

which have many functions (Table 1). Cell proliferation and angiogenesis are stimulated by hundreds of diverse platelet proteins, resulting in tissue regeneration. These proteins are secreted into the adjacent media, having a paracrine effect on various cell types: myocytes, tendon cells, chondrocytes, osteoblasts, fibroblasts, and mesenchymal stem cells from different origins[10-12]. Moreover, many studies confirmed that platelets secrete antimicrobial peptides, suggesting an antibiotic effect[13-15]. The PRP role in the targeted stimulation of musculoskeletal and mesenchymal stem cells has been extensively investigated and showed promise as a short-term use agent that could promote the simultaneous healing of several musculoskeletal tissues after trauma or elective surgery[7]. PRP plays a role in revascularization of damaged tissue by promoting cell migration, proliferation, differentiation and stabilization of endothelial cells in new blood vessels. PRP preparations are also implicated in reduction of pain perception. In addition, PRP has several cell adhesion molecules including fibrin, fibronectin, vitronectin and thrombospondin that prompt the assimilation of osteoblasts, fibroblasts and epithelial cells[9].

Applications

Many studies indicated that platelets have pro- and anti-inflammatory and analgesic effects and secrete antimicrobial peptides, having antibiotic effects[10, 15-16]. Although not approved by Food and Drug Administration (FDA) as a therapeutic modality, PRP has been extensively used with various preparation methods in many medical specialties.

Orthopedics:

PRP injections have been investigated in hip and knee osteoarthritis, ankle sprains, healing of non-united fractures, tennis elbow, chronic refractory patellar tendinopathy, plantar fasciitis and sports injuries in many clinical studies[17-19]. A prospective randomized controlled study concluded that local application of autologous PRP improved tendon healing in patients undergoing arthroscopic rotator cuff repair and reduced pain in the first postoperative months[20]. In another randomized controlled trial, treatment of patients with chronic lateral epicondylitis with PRP reduced pain and increased function significantly, exceeding the effect of corticosteroid injection even after a follow-up of 2 years[21]. There is high-quality evidence supports the use of leucocyte poor-PRP injections for osteoarthritis of the knee[17]. However, the clinical application of PRP in hip arthritis, rotator cuff tendinopathy, bone repair and high ankle sprains remains controversial with insufficient evidence[7,17]. On the other hand, many randomized trials found no effect of PRP on Achilles tendinopathies[22-24]. A meta-analysis analyzed 23 randomized clinical trials and ten prospective clinical studies did not reveal clear evidence in favor using platelet concentrates in bone or soft tissue lesions in the field of orthopedics[25].

Dentistry

Since 1998, PRP and platelet-rich fibrin (PRF) are increasingly used in dentistry, mainly in dental implants, oral surgery and periodontology. Enhanced wound healing and reduced pain in corticotomy patients has been reported along with variable outcomes in treating secondary alveolar cleft repairs[26]. The use of PRP to augment bone regeneration has been documented in periodontal defects, extraction sockets, during implant placement, and in guided bone regeneration procedures around implants[27].

Dermatology

Although there are many clinical studies which investigated facial rejuvenation, alo-

pecia, scars, and vitiligo, but the lack of randomized control trials and the small sample size could not give evidence of standardized applications in dermatology. In 2017, Gupta and Carviel[28] conducted a meta-analysis to study the efficacy of PRP for androgenetic alopecia and found that it is promising treatment modality, but more researches are required to evaluate protocols of activation and the minimum required frequency of sessions to get effective results. PRP studies within the fields of scars, androgenetic alopecia, infraorbital hyperpigmentation and dermal volume augmentation are consistent with a modest value of PRP application[29].

Surgery

The use of platelet concentrates in diabetic ulcers was found to be the treatment of choice[30]. Moreover, PRP accelerated the process of healing of chronic ulcers and difficult wounds[31]. Platelet-leukocyte rich plasma (PLRP) was applied in an infected high-energy soft tissue injury and the authors indicated that the volume and concentration of platelets and leukocytes were adequate to induce the healing process despite concurrent infection[32]. There was limited data that showed some effect in reducing perioperative pain, which has been attributed most likely to the anti-inflammatory properties of PRP[33]. Nevertheless, a large meta-analysis demonstrated no significant benefit of PRP augmentation of arthroscopically repaired rotator cuffs[34]. PRP has also been used in cardiac surgery, gynecology, urology, plastic surgery, and ophthalmology[35].

Preparation Methods

The process of PRP preparation starts with collection of whole blood using anticoagulant agent, most commonly used is acid citrate dextrose (ACD) or sodium citrate. Then, the blood is centrifuged using variable protocols either one step or two steps, with varying time ranging between 4 and 15 minutes, different speeds (100 - 2000 x g), and a wide range of temperature (12 °C – 26 °C). As a result of these variable protocols, the platelet yield ranges between 3 and 7 folds[2,9]. PRP fall under the prevue of FDA's Center for Biologics Evaluation and Research (CBER). CBER is responsible for regulating human cells, tissues, and cellular and tissue-based products. Biologics may be approved through New Drug Applications (NDAs) or Biologics License Applications (BLA) or cleared via Premarket Notification (510(k)). NDA and BLAs require clinical data to be obtained, while, a 510(k) may not require clinical data. Medical devices for PRP is being approved through a 510(k) clearance as it doesn't require the clinical data. The 510(k) application is for devices that are considered lower risk devices and determined to be "substantially equivalent" to a previously cleared device. There are numerous PRP preparation systems on the market today with FDA clearance. The 510(k) clearance applies only to produce PRP for use with bone graft materials to enhance bone graft handling properties[36]. Standardization of PRP product is impractical. Many argued aspects are indicted in difficulty to reach standardized or optimized PRP. Of them, various methods of preparation either homemade or using commercial devices. The later has three different types: 1- the blood is obtained with a tube containing an anticoagulant, to be used for any type of centrifuge. 2- medical devices with which blood is collected into a tube that contains an anticoagulant; the centrifugation can then be made in any type of centrifuge. 3-medical devices with which the blood is collected into a syringe filled with an anticoagulant; then, the blood is transferred into a secondary device whose shape imposes the use of special centrifuge supplied by the same manufacturer [37]. In addition, homemade products have numerous variables (single- or

double-centrifugation, speed, timing, and temperature) considering that longer centrifugation time would better combine low relative centrifugal force and temperature increase would reduce plasma density[10]. Furthermore, different anticoagulants are used [sodium citrate, ACD, and citrate phosphate dextrose adenine (CPDA)]. Depending on the type of use (application of gel or injection), the volumes required, the exact site of injection/administration and the necessity of freezing of the product, PRP may need to be collected using devices, small volume blood bags, or tubes. Then, may undergo specific processing, division into aliquots and storage in special freezers. In addition, for withdrawal of volumes greater than 200 mL, the patient must meet the criteria for autologous donations[30]. Etiology may direct choose the type of PRP, for instance leukocyte-rich versus leukocyte-poor. Acellular pathologies such as lateral epicondylitis may benefit from leukocyte-rich PRP, which may help induce an inflammatory response to stimulate healing[6]. Magalon and colleagues[38] retrospectively applied their proposed classification (DEPA: Dose of injected platelets, Efficiency of production, Purity of the PRP, Activation of the PRP) on twenty PRP preparations - for which biological characteristics were available in the literature- to characterize the injected PRP. They concluded that the dose of injected platelets varied from 0.21 to 5.43 billion, equivalent to a 25-fold increase. Assessments of the efficiency of production revealed that no device can recover more than 90% of platelets from the blood. Purity of PRP preparations revealed that a majority are contaminated by red blood cells as merely three devices reach more than 90% purity (corresponds to a percentage of platelets compared with red blood cells and leucocytes over). The authors did not compare PRP activation claiming that activation depends on the treatment indications and physician's decision[38]. A common point in all researches is lacking definite therapeutic protocol (amount applied, number of- and intervals- between applications)[2, 39]. Using an activator is another debate with many activators have been implicated either alone or combined (calcium chloride, calcium gluconate, human or bovine thrombin and batroxobin). Some authors apply PRP without previously activating them, arguing that improved results are obtained. Owing to the release of calcium from the cells at the site of injection or the automatic release of growth factors by the platelets at the time of administration[1]. Other authors claimed that, once activated, platelets lose the ability to interact with the environment. For instance, the structural and functional integrity of platelets is required to promote angiogenesis, whereas platelet-derived growth factors such as vascular endothelial growth factor (VEGF) alone are less efficient. Moreover, treatment with high doses of growth factors has shown limited success in some clinical applications[8]. Thus far, FDA has not attempted to regulate activated PRP[36]. Preparation of platelet products is inconsistent both between clinicians and even for the same clinician and the same patient, putting in mind the nature of PRP as an autologous product which could be affected by fluctuation in platelets count, their content of growth factors, and medications taken by the patients. Some other considerations are: aspiration of platelet concentrate (varying needle gauge, and amount of platelet-rich zone to be aspirated), and either leucocyte rich or depleted, time and storage temperature till used, and freezing/thawing[2]. Studies have demonstrated that low platelet concentration is inefficient and that high concentrations have an inhibitory effect on cell growth. Nevertheless, results are contradictory[40].

Complications

There is no evidence that local PRP injections have systemic effects. Due to its autologous nature, there is no reported immune reaction or allergy. To date, minimal reported side effects are pain, heaviness in the site of injection and infection[39].

Conclusion

Even with many reported favorable effects of using PRP with high evidence of safety in managing various clinical settings (especially in the field of dermatology, dentistry, orthopedics, surgery, and ophthalmology), the use of PRP has major limitations; including the paucity of controlled clinical trials and lack of consensus related to PRP preparation techniques. Yet, PRP remains a viable conservative and alternative therapeutic option either alone or in combination with other conventional therapies with low risk of adverse reactions. As many of reported clinical studies do not have adequate statistical power to give conclusive results, further clinical studies are needed with higher level of evidence taking into consideration customizing the preparation method, volume, number of sessions and the period between the sessions for different clinical scenarios. Determining the main bioactive components which are responsible for the clinical effects of PRP and the inter-individual variability of growth factors and cytokines production and the synergy of platelets count, and growth factors will remain as major challenges to achieve standardization of PRP.

Financial support and sponsorship: Nil.

Conflicts of interest: None

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