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Immunological and Antimicrobial Effects of Autologous Platelet Concentrates

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ABSTRACT Antibiotic resistance has remarkable potential in human beings and veterinary medicine. However, to prevent the clinical reflection of this resistance from reaching the feared dimensions, there is a requirement for antimicrobial treatment options supported and improved with new molecular biocursors at the preclinical point. Platelet-rich plasma (PRP) and fibrin (PRF) are biomaterial products that recently used to increase the anti-infective defense system by platelet growth factors to support postoperative wound healing, bone regeneration, graft stabilization, biofilm inhibition, catheter hygiene, and hemostasis. Recently, research has been carried out on antibacterial, antifungal, and prevention of clinical biofilm formation. Autologous platelet concentrates are autogenous and do not cause any immunological reaction or infection. Therefore, the choice and application of regenerative therapies are being favored due to their nominal invasive procedures. In particular, PRP and PRF are of interest because of their influence to stimulate and speed up the injury area healing process. Cytokines and growth factors involved in the formation of PRP are played an important role in the recovery process. This article aims to evaluate the antibacterial, antifungal and antibiofilm properties of PRP and PRF in the field of microbiology. In addition, the act of growth factors in the process of healing and their use in regenerative treatments were also evaluated.

Keywords: *Anti-Infective agents, Immunologic factors, Platelet-Rich fibrin, Platelet-Rich plasma.*

ÖZ

Otolog Trombosit Konsantrelerinin İmmunolojik ve Antimikrobiyal Etkileri

Antibiyotik direnci, insan ve veteriner tıbbında dikkate değer bir potansiyele sahiptir. Ancak bu direncin klinik yansımasının korkulan boyutlara ulaşmasını önlemek için preklinik noktada yeni moleküler biyokürsörlerle desteklenen ve geliştirilen antimikrobiyal tedavi seçeneklerine ihtiyaç vardır. Platelet zengin plazma (PRP) ve platelet zengin fibrin (PRF), postoperatif yara iyileşmesini, kemik rejenerasyonunu, greft stabilizasyonunu, biyofilm inhibisyonunu, kateter hijyenini ve hemostazı desteklemek için trombosit büyüme faktörleri ile anti-enfektif savunma sistemini artırmak için son zamanlarda kullanılan biyomateryal ürünlerdir. Son zamanlarda antibakteriyel, antifungal ve klinik biyofilm oluşumunun önlenmesi üzerine araştırmalar yapılmaktadır. Otolog trombosit konsantreleri otojendir ve herhangi bir immünolojik reaksiyona veya enfeksiyona neden olmaz. Bu nedenle, nominal invaziv prosedürleri nedeniyle rejeneratif tedavilerin seçimi ve uygulanması tercih edilmektedir. Özellikle PRP ve PRF, yara iyileşme sürecini uyarma ve hızlandırma etkileri nedeniyle ilgi görmektedir. PRP oluşumunda rol oynayan sitokinler ve büyüme faktörleri iyileşme sürecinde önemli rol oynamaktadır. Bu makale, mikrobiyoloji alanında PRP ve PRF'nin antibakteriyel, antifungal ve antibiyofilm özelliklerini değerlendirmeyi amaçlamaktadır. Ayrıca büyüme faktörlerinin iyileşme sürecindeki etkisi ve rejeneratif tedavilerde kullanımları da değerlendirilmiştir.

Anahtar Kelimeler: *Anti-infektif ajanlar, İmmünolojik faktörler, Trombosit zengin fibrin, Trombositten zengin plazma.*

INTRODUCTION

Applications and topical use of platelet-rich plasma (PRP), non-transfusional hemocomponents, or autologous platelet concentrates have a potential role in a kind of regenerative medicine treatment. These treatments have been used for over 30 years for various indications and have gained immense popularity in the last 20 years (Everts et al. 2020; Attili et al. 2021).

PRP is an endogenous therapeutic application agent that is used in medicine because of its healing and supportive properties (Chicharro-Alcántara et al. 2018). These applications are followed by in vivo and in vitro PRP applications, which have recently spread to the veterinary

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medicine (Soares et al. 2021). In experimental studies, positive results were found for the simultaneous use of platelet-based products and mesenchymal stem cells or autograft bone for osteogenesis (Raeissadat et al. 2017). This autologous biotechnology provides alternatives that are widely used in the medical fields such as dentistry, veterinary orthopedics, and infection therapy (Anitua et al. 2012). Platelets are rich in cytokines and growth factors that not only form clots and stop local blood but also induce wound healing. In addition, platelet-derived growth factors and TGF-β are released slowly for about 7 days, stimulating collagen production and accelerating the soft tissue and wound healing process (Sharmila et al. 2020). Platelet-rich products are supported angiogenesis and extracellular matrix regeneration. Stem cell addition may also support cellular effects such as chemotaxis, cell proliferation, and involution. The use of thrombocyte-promoted products or growth factors with high concentrations is generally considered, especially in articular cartilage renewal treatment protocols (Sánchez-González et al. 2012).

For this reason, we aimed to share information about the antibacterial, antifungal and antibiofilm properties of platelet-rich plasma and platelet-fich fibrin in the field of microbiology, as well as the effects of growth factors on the healing process and their use in regenerative treatments.

What is Thrombocyte?

The platelets, which are in the blood circulation and produced from megakaryocytes, show an important act in inflammation and clotting in common with the substances secreted by them. They also support vascular development and guide epithelial cell migration (Gurel et al. 2020).

The cytokines as platelet-derived growth factor (PDGF), transforming growth factor β and fibroblast growth factors (FGF) are released from platelets and play an important act in immune activities such as the regenerative, adaptive, and innate immune system and healing process. They can induce cytokines, chemokines, and lipid mediators (Chicharro-Alcántara et al. 2018; Cieślik-Bielecka et al. 2018). The normal platelet count is around 200.000/ milliliter (mL) in total blood, but the desired platelet count in PRP is 1.000.000/mL on average (Theoret and Stashak 2014). In the normal process, the circulating platelets are in a calm condition. They attach to adhesive proteins originating from arachidonic acid and locate at sites of vascular injury (von Willebrand factor (vWf) and collagen) or soluble platelet agonists (thrombin or thromboxane A2) (Wachowicz et al. 2016).

Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF)

PRP is defined as an autologous biological product, a plasma fraction acquired from the ill animal's blood and having an advanced platelet concentration than circulating blood after centrifugation (Chicharro-Alcántara et al. 2018). They have tasks such as modulating the progression of the inflammatory process and supporting healing because of their high concentrations of growth factors contained after PRP injection (Carr et al. 2016). However, platelet amount is not considered as a single important component in the evaluation of PRP. For PRP to be rich in platelets, it is preferred that the platelet value be at least 5 times higher than the initial values of the blood (Carr et al. 2016).

Two different platelet concentrates (L-PRP; Leukocyte and Platelet-Rich Plasma, L-PRF; Leukocyte and Platelet-Rich Fibrin) contain significant quantities of leukocytes. The being of leukocytes has a grand influence on the biology of immune and antibacterial effects. Some antimicrobial peptides produced by the leukocytes such as lactoferrin, defensins, bactericidal/permeability-increasing protein (BPI) have hydrophobic and hydrophilic sides to interact in both the aqueous environment and the lipid-rich membrane. It is known that PRP has an antimicrobial effect, and in recent years, *in vitro* antibacterial activity of the platelet cell contents (lysate) against different bacterial strains (oxygen derivative such as hydrogen peroxide, etc.) has been evaluated (Farghali et al. 2019). PRF is a multiplex mixture of cells containing thrombocytes and diverse granulocytes, with a third-dimensional fibrin structure, diversity of growth factors, and plasma proteins. The platelets have a different molecular content that can interact with different microorganisms (Mariani et al. 2014). PRF is the secondary origination platelet concentration and contains biologically active proteins that promote curative healing and tissue repair but induce a moderate anti-inflammatory immune response. PRF is a true biomaterial that contains fibrin clots, platelets, leukocytes, and growth factors and it can clear pathogens from the bloodstream via binding and removing microorganisms (Kazemi et al. 2014; Nagaraja et al. 2019). PRF has recently been used to promote healing, bone recovery, graft stabilization, biofilm inhibition, and catheter hygiene. Because the well-organized fibrin matrix is better and can more efficiently run stem cell migration and recovery programs (Balaram et al. 2013). Enriched bioactive substances in i-PRF also accelerate the healing process, too (Sharmila et al. 2020). The release of these growth factors to damaged tissue individually or synergistically can reduce cell multiplication, angiogenesis, fibroblast, collagen, osteoblast production, and inflammatory reaction (Perego et al. 2020).

Preparation of Platelet-Rich Plasma (PRP) and Platelet-Rich Fibrin (PRF)

In general, the sequential steps of a PRP preparation include bloodletting, centrifugation to separate, collecting of platelets, and platelet activation stage are shown in Figure 1 (TGA 2019; Zhang et al. 2019). PRP is prepared in a tube containing an anticoagulant. To isolate cell-free plasma, red blood cells (RBC; erythrocytes) are differentiated by centrifugation, while thrombocytes and leukocytes accumulate in the upper layers of the plasma. The platelet-free plasma layer (PPP; platelet-poor plasma) is discarded and the PRP layer is separated. PRF, on the other hand, is a fibrin matrix that provides localization and increases the concentration of growth factors of platelets and WBC and is a second-generation PRP. For PRF, no anticoagulants are added after blood collection and the blood is quickly centrifuged after collection to isolate the fibrin matrix. After centrifugation, there is platelet-poor plasma in the upper layer, PRF in the middle layer, and RBC in the lower layer (Piao et al. 2017; TGA 2019).

Immunological Perspective of PRP

Platelets act as protector cells to detect the presence of potentially harmful microbes. Besides platelets, leukocytes in a clot are an important part of the immune system. They degranulate their contents and discharge them into phagosomes to kill ingested microorganisms. They also produce bioactive peptides that greatly contribute to healing, such as inflammatory cytokines and growth factors (Cieślik-Bielecka et al. 2019). They can also secrete platelet-derived growth factors BB, TGFβ1, and PDGF AB at the site of injury. TGFβ1 stimulates chemotaxis in monocytes, macrophages, and fibroblasts for tissue repair

(Serafini et al. 2020). PDGF has the potential to trigger growth factors and fibronectin gene expression *in vitro* fibroblasts. It is noted that increasing the RBC concentration of PRP may increase the amount of undesirable inflammatory mediators, especially IL-1 and TGF-a. It has been shown that monocytes provide an increase in collagen production in fibroblasts in parallel with a rising in cellular metabolism, but a decrease in interferon-y and IL-12 secretion. It has also been shown that platelets have particularly stimulating effects on collagen production by increasing IL-6 by activating mononuclear cells (lymphocytes, monocytes, etc.) in the peripheral blood. In addition, leukocyte-poor PRP is thought to be more beneficial than leukocyte-rich PRP in maintaining the balance of tendon integrity and preventing inflammation that may occur due to osteoarthritis (Carr et al. 2016).

PRP causes an 8-fold increase in growth factor concentrations compared to total blood. With increasing platelet count, the concentration of growth factors also increases, and with its use, patients can be treated with their derivatives and growth factors, thus preventing infection and immunological reactions (Mariani et al. 2014). For example, PRP has been widely used in orthopedic applications for the topical treatment of wounds and soft tissue injuries. It is also a proper alternative to fetal calf serum for a buildup of mesenchymal root cells from fatty tissue (Sánchez-González et al. 2012). Platelet activation is an important step that can be revealed by the action of various events such as calcium chloride, collagen, trauma, and thrombin (Zhang et al. 2019). Platelets have intracellular compartments include granules (dense and alpha) and lysosomes. Platelet dense granule components like polyphosphates, contribute to hemostasis and clotting. Alpha granules contain different types of cytokines, pro-inflammatory and anti-inflammatory factors. Other bioactive molecules are present as key regulators in the microhabitat of the growing thrombus (Golebiewska and Poole 2014; Yadav and Storrie 2017). Activation of platelets induces α-granules to release cytokines, growth, coagulation, and activating factors, as well as inflammatory and adhesion molecules (Davis et al. 2014).

Platelets contain a type of platelet microbicidal peptides (PMPs), also known as platelets, in their α-granules. A-granules accommodate large adhesive and curative proteins and are also host to many different types of big proteins, such as coagulation factors and protease inhibitors. They also take part in antibody-mediated cytotoxicity tasks to eliminate pathogens, and at the end of the immunological process, platelets secrete a kind of potent antimicrobial peptide (Kazemi et al. 2014; Nagaraja et al. 2019). The inflammatory stage may be accelerated by coagulation and platelet degranulation. This stage is characterized by the release of serotonin and histamine, which support neutrophil, leukocyte, and macrophage cells to reach the damaged area. The highest neutrophil presence is seen within 1-2 days after injury, and macrophages play the main role in the prevention of bacterial infections in this process. They also activate fibroblasts, keratinocytes, and immune cells. At the end of the inflammatory phase of lesion healing, macrophages secrete TGF-β, interleukins, and TNFs (Chicharro-Alcántara et al. 2018). Along with the modulation of interleukin-1 production by macrophages, PRP can also prevent acute early inflammation that can result in dense scar tissue (Sánchez-González et al. 2012).

Antibacterial and Antifungal Properties of PRP

Platelets can be clarified as immune cells with a wide variety of antimicrobial tasks (Speth et al. 2013). They can also increase neutrophil oxidative burst activity (hydrogen peroxide and superoxide anion etc.) in reply to Gram-positive and Gram-negative bacteria and some yeast. Antimicrobial effects of PRP against pathogenic factors are shown in Figure 2 (Zhang et al. 2019). Drago et al. (2014) showed that the antimicrobial activity of platelet concentrates against bacteria species such as *Streptococcus agalactiae* (*S. agalactiae*)*, Streptococcus oralis* (*S. oralis*)*, Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*) is promoted by a synergy of plasma components and platelet-derived factors.

Platelets are generally not stimulated by bacteria (*S. aureus*, *Streptococcus pneumoniae* (*S. pneumoniae*), *Streptococcus pyogenes* (*S. pyogenes*), and *Porphyromonas gingivalis* (*P. gingivalis*), etc.) as a result of adhesion. In particular, antibacterial peptides are produced by macrophages, neutrophils, and platelets and have direct effects on several cytokines/chemokines. *In vitro* tests have demonstrated the antimicrobial activity of peptides against *Escherichia coli* (*E. coli*), *S. aureus*, *Candida albicans* (*C. albicans*), and *Cryptococcus neoformans* (*C. neoformans*). It has also been evaluated that peptides are generally more powerful against bacteria compared with fungi (Speth et al. 2013). In addition, platelet polypeptides exhibited relatively potent activities against pathogens that tend to enter the bloodstream, including *S. aureus*, *Streptococcus sanguis* (*S. sanguis*), *E. coli*, *C. albicans*, and *C. neoformans* (Mussano et al. 2016; Zhang et al. 2019). Thrombocidins (TC-1, TC-2, etc.), which are included in the antimicrobial capacity and effector mechanisms of platelets, have the potential to keep from the growth of bacteria such as *S. aureus*, *E. coli*, *Bacillus subtilis*, and fungal agents such as *C. albicans*. They also increase the fungicidal effect of antimycotic drugs (Speth et al. 2013). In addition, it has been shown that platelet cell concentrates can inhibit the growth of S. *aureus*, *E. coli*, *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Klebsiella pneumonia* (*K. pneumonia*), and platelet gel supernatants show bactericidal effects against *S. aureus* (Feng et al. 2020). The leukocytes in different PRP preparations have been the subject of various debates in the last decade. Delivery of leukocytes to an injury site can increase the release of anabolic and pro-inflammatory mediators and trigger the release of growth factors and anti-inflammatory mediators. Therefore, they could play an important role in antibacterial activity (Mariani et al. 2015). In vitro studies have shown that leukocyte and PRP gel is active against many bacterial strains (Cieslik-Bielecka et al. 2018). It has been reported that PRP has a microbicidal effect against methicillin-resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), *E. faecalis*, and *P. aeruginosa*, but shows very weak activity against *E. coli* and *K. pneumoniae* (Cieslik-Bielecka et al. 2018).

It has been reported that the antibacterial effects of leukocytes and platelet-rich plasma and pure platelet-rich plasma preparations last for most of 18 hours after administration (Mariani et al. 2020). TGF-β1 in PRP has been found to have bacteriostatic effects against *S. aureus* (Lopez et al. 2014). Wu et al. (2013) reported a reduction in the number of E. *coli*, *P. aeruginosa*, and *K. pneumoniae* within the first 8 to 12 hours of PRP administration, but the greatest reduction was noted at 0 to 4 hours. They also observed that bacterial counts increased again after the 4-hour time point. Drago et al. (2014) reported that PRP has no effect against *P. aeruginosa*, but has a growth restrictive

effect on Gram-positive bacteria and yeasts such as *E. faecalis*, *S. agalactiae, S. oralis*, and *C. albicans*. It has been reported that platelet lysate-based materials show bacteriostatic activity against methicillin-resistant *S. aureus* and have a potent activity comparable to gentamicin and oxacillin, especially against MSSA (Cieslik-Bielecka et al. 2018). PRP promotes the release of many antibacterial proteins, including connective tissue activating peptide 3, chemokines and normal denoted T cell, fibrinopeptide A and B, IL6, neutrophil-activating protein 2. Upon activation, chemokine's relation with the bacterial cell wall, resulting in increased membrane permeability and inhibition of protein synthesis (Mussano et al. 2016; Zhang et al. 2019).

Antibacterial activity of platelet PRF concentrates against *S. aureus* and *E. coli* has been reported (Kour et al. 2018; Feng et al. 2020). It has been reported that L-PRF has antibacterial effects against *P. aeruginosa*, *S. aureus*, *E. faecalis*, *E. coli*, *P. gingivalis, Fusobacterium nucleatum* (*F. nucleatum*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), and *Prevotella intermedia* (*P. intermedia*). It has also been stated that the gel supernatant of platelets can inactivate opportunistic pathogens such as *S. aureus* and *Acinetobacter baumannii* on the epidermal surface (Castro et al. 2019). It is claimed that biofilms play a role in 80% of infections affecting animals and humans today Bacteria that can form a biofilm and are in a biofilm can become 100-1000 times more resistant to antimicrobial agents (Abdullahi et al. 2016). In addition, bacteria in the platelet-containing biofilm also show resistance to antibiotic treatment. It has also been assumed that biofilm-producing bacterial infections are common in wounds developing after surgery and are a factor that causes the wound to become chronic (Bjarnsholt T. 2013). In addition, various pathogens, which are also important for public health, such as *E. coli*, *S. aureus*, *Staphylococcus epidermidis* (*S. epidermidis*), *P. aeruginosa*, *Candida parapsilosis* and *C. albicans* have also been isolated from biofilms (Abdullahi et al. 2016). Platelets act as a host factor for circulating commensal streptococci to mature biofilm development and somewhat defend the bacteria against antibiotics. Streptococci such as *S. mutans, S. gordonii* in the biological structure of biofilms can also induce platelet aggregation, which accelerates the development of multilayered biofilms (Jung et al. 2012).

PRP Application in Animals

The bacterial infection is a serious complication that impairs wound healing and the tissue regeneration. Using platelet-derived products in veterinary treatments are includes injury defects, articular lesions, mainly in equine, canine and feline patients (Soares et al. 2021). It has been reported that treatment with PRP in MRSA-infected dermatological diseases in dogs causes rapid epithelialization and granulation tissue formation, a rapid healing process, reduction of inflammation, and bacterial decline (Attili et al. 2021). In dogs, clinical studies have centered on orthopedic uses in infected skin wounds with antimicrobial effects of PRP, and in dermatology for the treatment of dermal ulcers with wide tissue misplacement surgically induced wounds (Perego et al. 2021). It has been stated that PRF promotes greater bone formation compared to PRP after tooth extraction in beagle dogs (Hatakeyama et al. 2014). Platelet lysate is rich in bioactive proteins that play a very important role in tissue healing, has been used intra-articularly for the treatment of osteoarthritis for temporarily managing in horses (Tyrnenopoulou et al. 2016). In horses, PPP and PRP have been reported to inhibit the growth of *E. coli* in vitro and the addition of thrombin significantly enhances the restrictor effect of PRP (Aktan et al. 2013). In another study conducted in horses, it has been stated that PRP is caused more GF release than PRF, and TGFβ1 is showed a greater increase in PRF compared to PRP (McLellan and Plevin 2014). It has been reported that treatment with PRP or in the combination of PRP with antibiotics in acute mastitis does not show significant differences, but it has been emphasized that it is successful compared to the use of a single antibiotic in chronic mastitis (Marini et al. 2016). Platelet-rich plasma products obtained from rabbits have been reported to be effective at the antimicrobial point against agents such as MSSA or MRSA, and Group A *Streptococcus* (Li et al. 2013). In the presence of lipopolysaccharide, a bacterial endotoxin, PRP has been reported to increase IL1β, IL8 and ameliorate lipopolysaccharide induced changes in bovine endometrial cells. It has also been reported that PRP has strong effects on endometrial cells, which play an important act in reproduction (Marini et al. 2016).

CONCLUSION

Regenerative therapies are currently the preferred abortive or non-invasive procedures to accelerate healing in both humans and different animal species. Bioactive molecules derived from PRP are preferred because of their antimicrobial properties, reducing effect on necrotic tissue, and promoting wound healing. However, the antibacterial activity of PRP may be short-acting or weak compared to commercial preparations. In addition, due to the synergistic effect of PRP with antibiotics, its use in the treatment of bacterial infections is considered. Bioactive molecules secreted from PRP can enhance the ability of leukocytes to destroy pathogens and promote necrotic tissue healing. The ability of leukocytes in PRP to increase inflammatory cytokine production, support phagocytic activity, increase in vitro antimicrobial effects and treat selected infections in the veterinary clinical field should be investigated further.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: VÖ
Supervision / Consultancy: ŞK
Data Collection and / or Processing: VÖ
Analysis and / or Interpretation: VÖ, ŞK
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