

Antibacterial effect of fresh and liquid PRP on three nosocomial bacteria including methicilline resistant coagulase negative staphylococcus (MRCONS) ,E.coli (ESBL) and pseudomonas aeruginosa (ATCC 27853)

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Received January 30,2022; Accepted February 10,2022; Published online April 10,2022

Abstract

Recent product and formulation of PRP (plasma rich platelet) has been opening new avenues in the field of regenerative medicine (Anitua et al., 2012). Because of its antimicrobial activities PRP is a new inductive therapy which is being increasingly used for the treatment of infected wounds and soft and hard tissue repair (D'asta et al., 2018). It is new technology and a promising treatment method for infections, which is currently being used by a variety of surgical specialties (Bielecki et al., 2007). The aim of this study was to evaluate the potential antimicrobial effects of platelet rich plasma on three different bacteria strain *in vitro*. Although the mechanism is not clear yet, platelets have largely demonstrated their implication in anti-infectious immunity (Li & Li, 2013). This effect is ensured by the secreted molecules stored mostly in platelet alpha granules (Burnouf et al., 2013). Subsequent evidence suggests that platelets have multiple functional attributes in antimicrobial host defense (including the capacity to generate antimicrobial oxygen metabolites (Badade et al., 2016) and the antimicrobial peptides) and interact directly with microorganisms, contribute to clearance of pathogens from the blood (D'asta et al., 2018). The increased quantity of platelets makes this formulation of considerable value for their role in tissue healing and microbicidal activity (Chen et al., 2013). In recent years the discussion was enriched by a few studies focusing on platelet and leukocyte concentrates and their antimicrobial properties related to the presence of natural antibacterial peptides (Çetinkaya et al., 2019). Antibacterial peptides (HDP: Host Defense Peptides) are produced by macrophages and epithelial cells as well as neutrophils and thrombocytes and are one of the most important elements shaping the natural human immunity (Intravia et al., 2014). These proteins are synthesized with the use of

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lipopolysaccharides from the cellular membrane of gram-negative bacteria and display a wide spectrum of activity against gram-positive and gram-negative bacteria as well as viruses and fungi (Yang et al., 2010).

In vitro laboratory susceptibility to liquid PRP was determined by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Several of the same colonies of bacteria from fresh, 18-hour bacterial cultures were suspended in sterile physiological NaCl solution (0.85%) to obtain a suspension with a density of 0.5 on the McFarland scale, which approximately corresponds to a number from $1-2 \times 10^8$ to 8 c.f.u./ml . Agar plates were inoculated with one of the following bacterial strains: methicillin-resistant *coagulase negative staphylococcus* (MRCONS), *Escherichia coli* (Extended Spectrum Beta Lactamase, ESBL), *Pseudomonas aeruginosa* (ATCC 27853). After drying (up to 15 minutes), sterile paper blank discs with a diameter of 6 mm was placed on agar and inoculated by $100 \mu\text{l}$ of fresh liquid PRP. After 24 hours of incubation in 35 C° the results were checked and analyzed.

We observed an inhibition zone for *Escherichia coli* but not for two other bacteria. This antimicrobial susceptibility testing revealed that the liquid PRP inhibited the growth of *Escherichia coli* and no antibacterial effect on *Staphylococcus* and *pseudomonas*. Antimicrobial activity was assessed after 24 hours by measuring zone of inhibition across the center of the embedded disc. The diameter of the zone of inhibition was measured. Inhibition zone against *Escherichia coli* was 15 mm whereas no inhibition zone observed for *pseudomonas* and *staphylococcus*.

In recent years, plasma and its derivatives have been used extensively in wound healing and its antimicrobial effects against different bacterial strains verified (Fabbro et al., 2016). In 2019 Rıza Aytaç Çetinkaya did an *in vitro* antibacterial study of platelet-rich plasma as an additional therapeutic option for infected wounds with multi-drug resistant bacteria. The results showed that PRP (platelet rich plasma) and PPP (platelet poor plasma) significantly suppressed bacterial growth of MRSA, *K. pneumoniae*, and *P. aeruginosa* at 1, 2, 5, and 10 hours after incubation. VRE (Vancomycin resistant Enterococcus) was the only bacteria that PRP and PPP showed limited activity against (Çetinkaya et al., 2019). In another study in 2018, Agata Cieślík-Bielecka noted the *in vitro* microbicidal effect of L-PRP (Leukocyte and platelet rich plasma) against methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. No activity of L-PRP was noted against *Klebsiella pneumoniae* and both studied strains of *Escherichia coli* (Cieślík-Bielecka et al., 2018). Also in 2021, Laurence Camoin-Jau explained the beneficial effect of statins on *Staphylococcus aureus* infective endocarditis and throughout their study about the Statins checked out the antibacterial effect of platelets on *Staphylococcus aureus* (Hannachi et al., 2021). The antibacterial activity of APG (autologous platelet-rich gel) against *Staphylococcus aureus* was further confirmed, and the effect may be attributed to the activation of platelets in APG. The effect of APG against *Escherichia coli* and *Pseudomonas aeruginosa* comes probably from previously used antibiotics. Therefore, APG itself may have no antibacterial activity against these two bacteria (Chen et al., 2013). Yang studied the antibacterial effect of autologous platelet-rich gel derived from health volunteers *in vitro* and noted that APG may have potential bacteriostatic effect against *Staphylococcus aureus* by platelet mediating. Either APG or APG-

APO (APG combined with apocynin) has no obvious bacteriostatic effect against *Escherichia coli* or *Pseudomonas aeruginosa*. PRP has no antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* or *Pseudomonas aeruginosa* bacteria (Yang et al., 2010). Dalip Sethi in 2021 released a review article on evidence and mechanisms of action for platelet-rich plasma as an antibacterial agent against both gram positive and gram negative bacteria (Sethi et al., 2021).

In this study fresh PRP was used in liquid form without any additive but based on other studies it seems that PRP in APG form with calcium and thrombin is much more effective in wound healing and antibacterial activities (Smith et al., 2021). Although the mechanism is not completely clarified but multiple roles of native platelets in host defense against infection include: 1. generate antimicrobial oxygen metabolites; 2. facilitate complement fixation on bacteria; 3. internalize and clear pathogens from the blood stream; 4. execute antibody-dependent cell cytotoxicity; 5. potentiate antimicrobial mechanisms of leukocytes (Aktan et al., 2013); and 6. degranulation and release a variety of cationic antimicrobial peptides such as VEGF (vascular endothelial growth factor), PDGF-BB (platelet-derived growth factor with two B subunit), IGF-1 (insulin-like growth factor 1) and TGF- β 1 (transforming growth factor beta 1) (Wang et al., 2019).

The use of platelet concentrates technologies for the collection and use of natural antimicrobial agents of the body is a very interesting approach which needs further investigation to be clarified.

Keywords: PRP, Antibacterial effect, *Pseudomonas aeruginosa*, *Staphylococcus*, *E.coli*, Infection, Wound

Acknowledgments

The author would like to thank the Microbiology Department of Islamic Azad University, Qom branch, Qom, Iran.

Conflict of interests

The authors declare that there are no competing interests.

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