Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/b36vme15

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF PLASMA RICH PROTEIN AND PLASMA RICH PROTEIN LOADED WITH THREE DIFFERENT ANTIMICROBIAL AGENTS AGAINST ENTEROCOCCUS FAECALIS AN IN VITRO STUDY.

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ABSTRACT

This study aimed to evaluate and compare the antimicrobial activity of plasma-rich protein (PRP) alone and in combination with three antimicrobial agents—Clindamycin, Metronidazole, and Cefaclor—against Enterococcus faecalis, a common and resilient endodontic pathogen. PRP is known for its regenerative properties, and its role as a potential antimicrobial carrier was explored in this context. Blood samples were obtained from healthy participants and processed using a standardized two-step centrifugation method to isolate PRP. Clinical isolates of E. faecalis were collected from patients with oral and dental infections. The study included five experimental groups: PRP alone, PRP combined with each of the three antibiotics, and phosphate-buffered saline (PBS) as a control. The antibiotic concentrations within PRP were standardized before application. Antimicrobial susceptibility was assessed using the agar disc diffusion technique by measuring the zones of inhibition.

The results revealed that PRP alone did not exhibit any significant antibacterial effect against E. faecalis. However, when PRP was loaded with antibiotics, a marked increase in antimicrobial activity was observed compared to the control. Among the antibiotics tested, Clindamycin demonstrated the greatest efficacy when used individually, while PRP combined with Cefaclor produced the largest zone of inhibition among the PRP-antibiotic combinations.

In conclusion, this study demonstrates the potential of PRP as a bioactive carrier for antimicrobial agents. The combination of PRP with antibiotics may enhance localized drug delivery and infection control, especially in cases involving resistant organisms like E. faecalis, while also supporting tissue

regeneration in endodontic therapy.

Keywords: Antibiotics; Antimicrobial agents; Antimicrobial efficacy; Enterococcus faecalis; Plasma rich protein.

INTRODUCTION

Endodontic regenerative procedures are biologically driven processes that involve the replacement of damaged components of the dentin-pulp complex and the regeneration of root resorption in cervical, middle, or apical regions.[1]

Recently, plasma rich protein [PRP] has been described as potentially ideal scaffold for regenerative endodontic procedures that promotes cell growth, maintain vitality of pulp tissue.[2] Plasma rich protein is a part of the plasma fraction of autologous blood with platelet concentration above the baseline.[3] Over the past two decades, a deeper understanding of the physiological healing role of platelets has resulted in the use of platelet concentrates as therapeutic tools in regenerative treatments. [1]

Platelets play multiple roles in antimicrobial defense, including the release of potent antimicrobial peptides from their alpha granules. These peptides have demonstrated broad-spectrum activity against gram-negative, gram-positive, and fungal pathogens.[3] PRP promotes tissue regeneration, enhance collagen synthesis, and trigger angiogenesis and immune responses by releasing growth factors and cytokines. [4] It has a low cost and has represented a potentially suitable system for the delivery of both bioactive molecules and drugs. [5]

Microorganisms responsible for endodontic infections are typically of low virulence. Their pathogenesis and persistence are driven by the release of lipopolysaccharides, toxins, and enzyme production. Pulpal and periapical lesions are linked to a diverse microbiota, including aerobic, anaerobic, Gram-positive, and Gram-negative microorganisms. [1] PRP is active against microorganisms colonizing the oral cavity including Enterococcus faecalis indicating that PRP is a potentially useful substance in the fight against postoperative infections. [6]

In this study, we added commonly used antibiotics to the PRP formulation to create the platelet-rich plasma antibiotic delivery system. The aim of this study was to evaluate and compare antimicrobial activity of plasma rich protein and plasma rich protein loaded with three different antimicrobial agents, Clindamycin, Metronidazole and Cefaclor, against Enterococcus faecalis.

MATERIALS AND METHODS

The present study protocol was reviewed and approved by the Institutional Ethics Committee and Institutional Review Board, approval no. 544/SMBT/04/PG/IEC/143/2023. Written informed consent was taken from the participants.

PRP Preparation

Fresh human whole blood from donors was processed to create a platelet concentrate. Patients of age group from 18-45 was included for blood samples. The exclusion criteria included patients with any systemic illness, a current or recent history of cancer, or those who had received anticoagulant, antibacterial, or immunosuppressive therapy within the past 6 months. About 13.5ml blood on an average was drawn from each adult and the peripheral blood was collected into a pre-filled syringe with 1.5ml trisodium citrate solution [3.8%]. [Figure 1A]. The syringe was inverted several times to ensure complete mixing of the blood with the anticoagulant, preventing clotting in the syringe. The blood was then transferred into a 15 ml sterile centrifuge tube and centrifuged at 2500 rpm for 10 minutes using a Remi R8C Laboratory Centrifuge. Erythrocytes [RBCs] were carefully removed from the bottom of the tube with a PRP kit and discarded [see Figure 1B].

The remainder was further centrifuged at 3000 rpm for 10 minutes to precipitate the platelets. The platelets, along with 2-5% RBCs, were collected from the bottom of the tube using the PRP kit to yield approximately 2 ml of PRP. The PRP was then shaken by hand for 30 seconds to suspend the platelets. Platelet and RBC counts were measured in both the whole blood and the PRP to assess the efficiency of the PRP separation.

Bacterial strains

Bacterial strain, Enterococcus faecalis ATCC 29212, was obtained from the microbial tissue collection center and were used for the study. The obtained pure culture was preserved on nutrient agar medium at 37°C and was periodically transferred to fresh media to maintain its viability in the microbiology laboratory [Department of Microbiology, SMBT Institute, Nashik, India] during the study period.

Experimental Groups

Four different experimental groups were included using plasma rich protein compared with three different antimicrobial agents-

- Group A: Plasma rich protein
- Group B1: Plasma rich protein + Clindamycin
- Group B2: Plasma rich protein + Metronidazole
- Group B3: Plasma rich protein + Cefaclor
- Group C1: Clindamycin
- Group C2: Metronidazole
- Group C3: Cefaclor
- Group D: Phosphate buffered saline [positive control]

These groups were then treated with E. faecalis. Total 32 tubes in which each tube contained a different combination of experimental group to E. faecalis.

At five different time points [0, 1, 4, 8, and 24 hours], each tube was plated in duplicate at two different dilutions for each group.

Determination of antimicrobial activity

Agar diffusion test

In vitro susceptibility tests were performed with oral microbes according to the Kirby Bauer disc diffusion method. The inoculum of oral microbes was plated on brain heart infusion media. The inoculum was uniformly distributed across the agar plate using a cotton swab [Figure 2A]. A 6mm PRP disc was placed on each inoculated plate.

Concurrently, an agar disc diffusion test was conducted using an antimicrobial susceptibility test disc containing Clindamycin, Metronidazole, and Cefaclor. The agar plates were incubated at 37°C for 24 hours in ambient air [Figure 2B]. After incubation, the inhibition zones were measured in millimeters.

RESULTS

In this study, disc-diffusion method was used to assess the antimicrobial activity of PRP, antibiotics, PRP loaded with antimicrobial agents against E. faecalis, which is common in root canal infections. The inhibition diameter produced by each disc was measured as shown in figure 3. The inhibition diameter of the discs coated with Clindamycin was 29.5. Clindamycin was more effective against E faecalis when compared with Cefaclor and Metronidazole. PRP loaded with Cefaclor significantly inhibited the growth of E. faecalis with mean zone diameter of 35.25 mm with a clear bacteriostatic zone. PRP loaded with Cefaclor showed higher activity against E. faecalis when compared with PRP loaded with Clindamycin and Metronidazole. There was no significant difference [$p>0.05$] in antimicrobial inhibitory zone diameters of Metronidazole and PRP loaded with Metronidazole. Using PRP alone had no inhibitory effect on E. faecalis.

DISCUSSION

The antimicrobial efficacy of plasma-rich protein (PRP) and platelet-rich fibrin (PRF) has been widely recognized in literature, aligning with the outcomes of our study. These platelet-derived preparations demonstrate significant antibacterial and antifungal properties, contributing to their clinical relevance in managing infections. Nagaraja et al. [1] emphasized the effectiveness of PRF and PRF matrices in reducing microbial load, which supports our findings of PRP's baseline antimicrobial properties against E. faecalis. The regenerative potential of PRP and its inherent antimicrobial activity have also been established by Alagl et al. [2], who showed its utility in treating infected immature non-vital permanent teeth. This dual functionality is critical for applications in endodontic infections, corroborating our observation of enhanced antimicrobial efficacy when PRP was combined with antimicrobial agents.

Intravia et al. [3] compared PRP's bacteriostatic effects with antibiotics, reporting notable inhibition of bacterial growth. Our findings extend this understanding by demonstrating that PRP, particularly when loaded with antimicrobials, offers a synergistic effect that enhances its efficacy against E. faecalis. Similarly, Çetinkaya et al. [4] demonstrated PRP's activity against multi-drug-resistant bacteria, validating its potential in combatting resilient pathogens. The addition of antibiotics to PRP has been shown to enhance both antimicrobial efficacy and wound healing, as reported by Wang et al. [5]. In our study, PRP's role as a carrier for antimicrobial agents, providing sustained release and localized action, further supports its use in managing infections like those caused by E. faecalis.
Studies by Drago et al. [6] and Yang et al. [7] demonstrated PRP's antimicrobial activity against periodontal pathogens, highlighting its broad-spectrum potential. Bhamjee [8] also reported the antibacterial properties of advanced PRF (A-PRF), indicating its role in oral and endodontic infections, which resonates with our findings of PRP's efficacy.

The innovative bifacial PRF matrix by Lucarelli et al. [9] offers a novel approach to optimizing platelet concentrates for therapeutic applications. Its enhanced properties could inform the preparation of PRP and PRF for clinical use. Additionally, Fouad and Verma [10] explored PRP's healing potential in infected and non-infected sites, further supporting its clinical relevance.
Understanding the microbial landscape of endodontic infections, as highlighted by de Mendonça Cavalcante et al. [11], is crucial for developing targeted therapies. The superior antimicrobial activity of PRP compared to other platelet concentrates, as noted by Karde et al. [12], underscores its potential in treating infections caused by pathogens like E. faecalis.

The mechanism underlying PRP's antimicrobial properties is attributed to platelet-derived antimicrobial peptides, as discussed by Tang et al. [13], and growth factors, as described by Toffler et al. [14] and Dohan et al. [15]. These molecules enhance both bacterial inhibition and tissue regeneration, explaining PRP's multifaceted action.

Badade et al. [16] and Bielecki et al. [17] have further confirmed the antimicrobial effects of PRP and PRF, emphasizing their role in reducing bacterial load while promoting immune responses. These findings align with our results, which demonstrated significant microbial inhibition with PRP, especially when loaded with antimicrobial agents.
A systematic review by Fabbro et al. [18] consolidated evidence on the antimicrobial properties of platelet rich preparations, reinforcing their potential in infection control. Bielecki et al. [19] and Cieslik-Bielecka et al. [20] highlighted the antibacterial activity of autologous platelet gels, supporting the therapeutic role of PRP in clinical infections.

The variability in PRP preparation techniques, as noted by Mazzocca et al. [21], and the role of human factors in determining its efficacy, as described by Yeaman [22], are critical considerations. Mariani et al. [23] demonstrated PRP's inhibitory effects on bacterial growth, while Dhurat and Sukesh [24]

provided standardized preparation protocols, ensuring consistent results in antimicrobial efficacy.Our findings align with this body of literature, demonstrating that PRP, alone and in combination with antimicrobial agents, offers a robust approach to managing infections like those caused by E. faecalis. This study was conducted in vitro, meaning the results may not fully reflect the complexities of human biology, where factors like tissue interaction, immune response, and blood circulation can affect the outcomes. Additionally, the study focused on Enterococcus faecalis as a single microbial strain, and the antimicrobial efficacy of PRP and its loaded agents may differ for other pathogens. Variability in PRP preparation methods, as noted in previous research, may also influence its antimicrobial properties, and further studies with standardized preparation protocols and diverse bacterial strains are needed to confirm the generalizability of the findings. Future research should aim to optimize PRP preparation techniques, explore its broader antimicrobial applications, and evaluate its clinical effectiveness in endodontic therapies**.**

CONCLUSION

This study provides the potential use and viability of combining plasma rich protein and antibiotics as a drug delivery system for both bacterial elimination and the acceleration of bone regeneration. When using PRP in regenerative endodontic procedures, its antibacterial properties could serve as a valuable addition. To fully assess its potential, PRP should be analyzed for antimicrobial effects over extended periods and tested against a broader range of clinical strains with a larger sample size. Moreover, the molecular mechanisms responsible for the antimicrobial activity of these bio-scaffolds should be thoroughly investigated.

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FIGURE LEGENDS

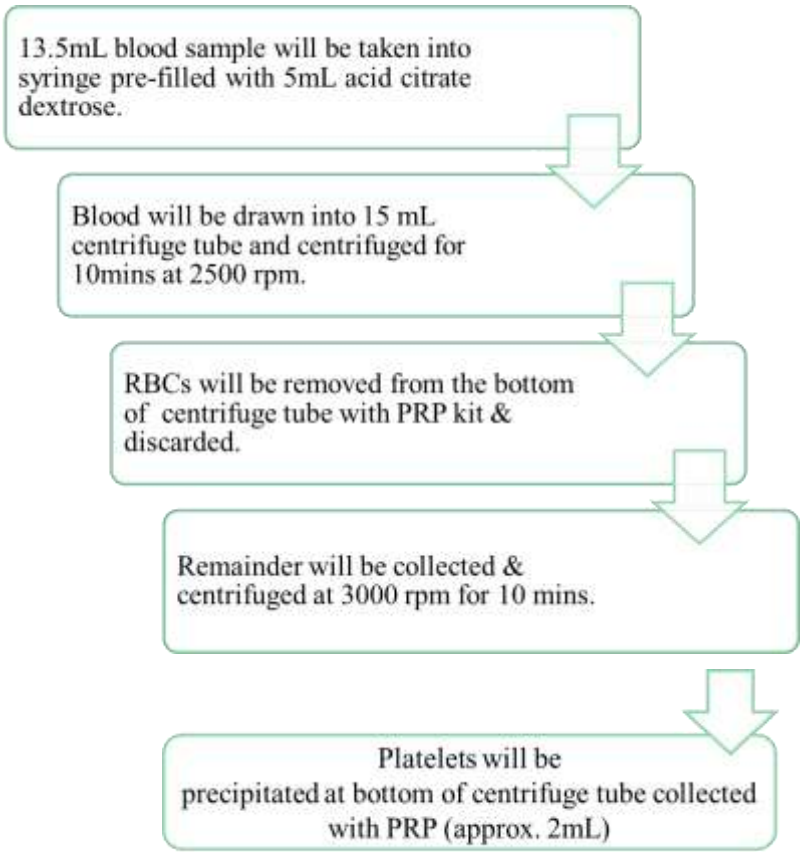

Chart 1. PRP Preparation

Table 1. Inter Group Comparison Of Values

	Group	N	Mean	Std. Deviation	Std. Error Mean	T value	p value of t test
PRP + Clindamycin	A	8	29.25	4.773	1.688	-3.810	.002**
	B	8	36.13	1.808	.639		
PRP + Cefaclor	A	8	34.00	1.512	.535	-1.852	.085#
	B	8	35.25	1.165	.412		

There was a statistically highly significant difference seen for the values between the groups [p<0.01] for Clindamycin with higher values in group B.
There was a statistically non-significant difference seen for the values between the groups [p>0.05] for Cefaclor.

Table 2. Inter Group Comparison Of Frequencies

	Group Metronidazole		
	A	B	Total
	8	8	16
Total	8	8	16

No statistics are computed because Metronidazole is a constant.
Metronidazole * Group

Table 3. Inhibition Diameter Produced by PRP Against E. Faecalis.

Group C	PRP
1.	10
2.	11
3.	11
4.	10
5.	10
6.	10
7.	8
8.	8

Table 4. Inhibition Diameter Produced By Different Antibiotics Against E. Faecalis.

Sample B	Clindamycin	Cefaclor	Metronidazole
1.	37	34	10
2.	34	35	10
3.	27	34	10
4.	30	35	10
5.	22	36	10
6.	31	31	10
7.	26	33	10
8.	27	34	10

Table 5. Inhibition Diameter Produced By Different Antibiotics Combined With PRP Against E. Faecalis.

Sample C	Clindamycin + PRP	Cefaclor + PRP	Metronidazole + PRP
1.	35	37	10
2.	35	36	10
3.	37	36	10
4.	37	34	10
5.	35	36	10
6.	35	34	10
7.	35	34	10
8.	40	35	10

Table 6. Shows the inhibition diameter produced by each disc was measured.

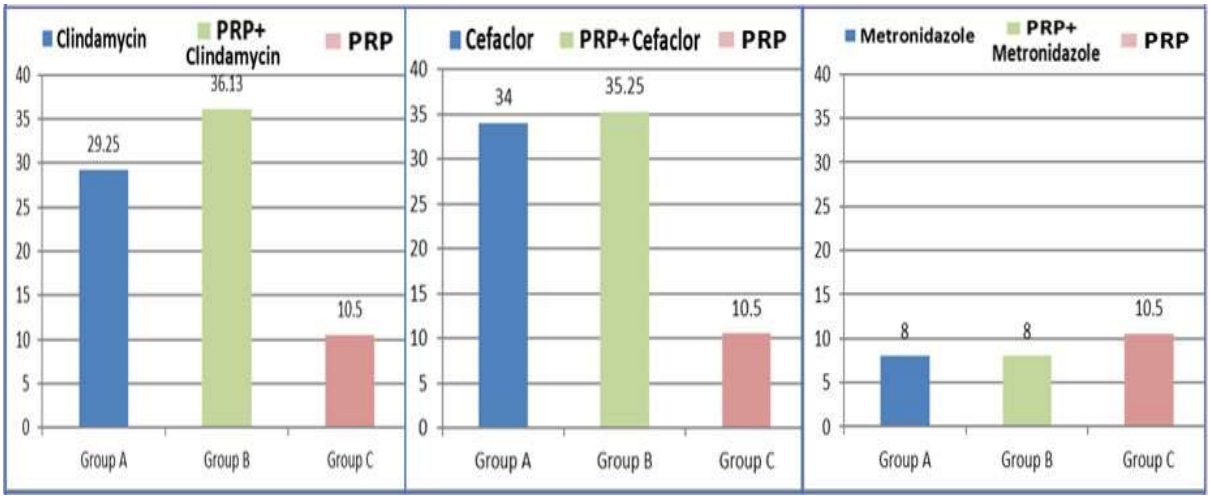

Figure 1A. Fresh human whole blood collected from donor.

Figure 1B. Erythrocytes separated from the samples and PRP was obtained.

Figure 2A. Inoculum spread over the entire surface of the agar plate using a cotton swab.

Figure 2B. Agar disc diffusion test performed using an antimicrobial susceptibility test disc loaded with Clindamycin, Metronidazole and Cefaclor.

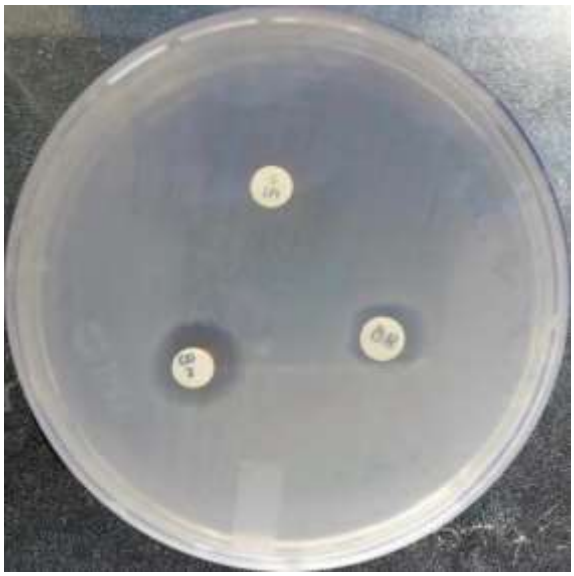

Figure 3. Image showing antibacterial activity of PRP loaded with Clindamycin, Cefaclor and Metronidazole inhibition zones.