IJBC 2016; 8(1): 5-8

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

In Vitro Evaluation of the Anti-bacterial Effect of Human Platelet Concentrate

Elnaz Jafarzadeh[1], Mojgan Pourmokhtar[2*], Setareh Tavili[1]

1. Department of Pharmaceutics, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

ARTICLE INFO

Article History:
Received: 15.08.2015
Accepted: 7.01.2016

Keywords:
Human platelet concentrate
Antibacterial effect
Disc diffusion method
Infections

**Corresponding author:*
Mojgan Pourmokhtar,
Address: Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, IBTO bldg., Hemmat Exp. Way, Next to the Milad Tower, P.O. Box: 14665-1157, Tehran, Iran
Tel: +98 21 82052185
Fax: +98 21 88628741
Email: mpourmokhtar@gmail.com

ABSTRACT

Background: Recently the role of platelets in the tissue regeneration, wound healing and prevention and control of infections has been reported. We aimed to assess the antimicrobial effect of human platelet concentrate against six bacteria, commonly found in wound and hospital-acquired infections.
Methods: In vitro susceptibility to samples of 10 random human platelet concentrates was determined by disc diffusion method against Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, and Proteus vulgaris. The assay was performed in triplicate for each strain and the antibacterial activities were assessed by measuring the zones of inhibition at 20, 24 and 48 hours after incubation at 37 °C.
Results: Human platelet concentrate showed antibacterial activity against Staphylococcus aureus and Staphylococcus epidermidis with the mean diameter zone of inhibition of 11.4±1.1 and 10.2±1.1 mm, respectively. Whereas, no activity was observed against Micrococcus luteus, Pseudomonas aeruginosa, Escherichia coli, and Proteus vulgaris. Also, there was no significant difference in antibacterial effect of human platelet concentrate after 20, 24, and 48 hours.
Conclusion: Human platelet concentrate which is a biocompatible and safe product could be potentially useful in wound healing and hospital-acquired infections.

Please cite this article as: Jafarzadeh E, Pourmokhtar M , Tavili S. In Vitro Evaluation of the Anti-bacterial Effect of Human Platelet Concentrate. IJBC 2016; 8(1): 5-8.

Introduction

Platelets are mainly known for their crucial role in the hemostasis.[1] Various recent studies have also indicated the important role of platelets in tissue regeneration, wound healing, and prevention and control of infection.[2-7] Therefore, nowadays platelet products are used in various fields of medicine, including dermatology, cosmetic and plastic surgery, ophthalmology, orthopedics, rheumatology, sports medicine and dentistry. Undoubtedly, such a widespread use of platelet products is largely related to the anti-inflammatory properties of platelets and the presence of multiple growth factors in these natural products along with their potential antibacterial activity.[1,8,9]

Bacterial infections are among the most serious complications that provoke many social health concerns. Although the use of antibiotics is recommended for certain infectious situations, they can cause various adverse reactions. Improper usage of antibiotics may contribute to the increasing emergence of antibiotic resistance which has been referred to as one of the world's most pressing health problems.[10]

Considering the need for new effective, biocompatible and safe antimicrobial compounds, and since the antibacterial effect of platelet concentrate (PC) against some bacteria has been reported in a few in vitro

studies,[2,8,11-14] we aimed to investigate the antibacterial effect of PC against three gram negative and three gram-positive bacteria which are mainly responsible for wound and hospital-acquired infections.

Methods

Bacteria and Preparation of Inoculums

Three gram-negative bacteria including Escherichia coli (PTCC 1399), Pseudomonas aeruginosa (PTCC 1430), and Proteus vulgaris (PTCC 1079) and three gram-positive bacteria including Staphylococcus epidermidis (PTCC 1435), Staphylococcus aureus (PTCC 1431) and Micrococcus luteus (PTCC 1408) were selected for the study. The bacterial strains were obtained from Pasteur Institute (Tehran, Iran) and maintained on Nutrient agar at 4 °C at Islamic Azad University laboratory. To prepare inoculums of bacteria culture, the stock culture from Nutrient agar was subcultured on Muller-Hinton agar (Merck, Germany) and incubated overnight at 37 °C, then a suspension of freshly grown bacteria in sterile distilled water was prepared for each strain with an optical density equal to 0.5 McFarland (1×10^8 CFU/mL).

Platelet Concentrate Preparation

Each of 10 random PCs was obtained from Tehran Blood Transfusion Center on the day of experiment. It should be noted that PCs prepared from whole blood of healthy blood donors using platelet-rich plasma method[15] were stored and shipped at 20 to 24ºC along with continuous agitation during storage.

Determination of Antibacterial Activity

In vitro laboratory susceptibility to PC was determined by disc diffusion method[16] on Mueller–Hinton agar (MHA). For this purpose, agar plates were coated with one of the following bacterial strains: Staphylococcus epidermidis, Staphylococcus aureus and Micrococcus luteus as Gram-positive bacteria and Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris as Gram-negative bacteria. Then standard 6 mm discs soaked with PC and positive or negative control were placed on the coated agar media. The inoculated agar plates were then incubated at 37 °C for 48 hours. The baseline antimicrobial activity was assessed by measuring the diameter zones of inhibition after 20, 24, and 48 hours after incubation at 37 °C and results were expressed as mean ± SD. It should be noted that the assay was performed in triplicate for each strain and Penicillin and Gentamicin were used in all assays as positive controls for Gram-positive and Gram-negative bacteria, respectively. Mueller-Hinton Broth was used as a negative control.

Results

The mean values for zone of inhibition produced by PC, positive control and negative control against six bacteria are shown in table 1. PCs showed antibacterial activity against Staphylococcus aureus (figure 1) and Staphylococcus epidermidis (figure 2) with the mean diameter zone of inhibition of 11.4±1.1 and 10.2±1.1 mm, respectively. There was no activity against Micrococcus luteus, Pseudomonas aeruginosa, Escherichia coli and Proteus vulgaris. Moreover, there was no significant difference in antibacterial effect of PCs after 20, 24, and

Table 1: Zones of inhibition, exerted by platelet concentrate, positive control and negative control against six bacteria after 24 hours of incubation

Sample \ Bacteria	Staphylococcus epidermidis	Staphylococcus aureus	Micrococcus luteus	Escherichia coli	Pseudomonas aeruginosa	Proteus vulgaris
Platelet Concentrate	10.2±1.1 mm	11.4±1.1 mm	-	-	-	-
Positive control	19±2.0 mm	40.9±1.8 mm	59±3.7 mm	21±2.1 mm	19.5±0.7 mm	19.6±0.7 mm
Negative control	-	-	-	-	-	-

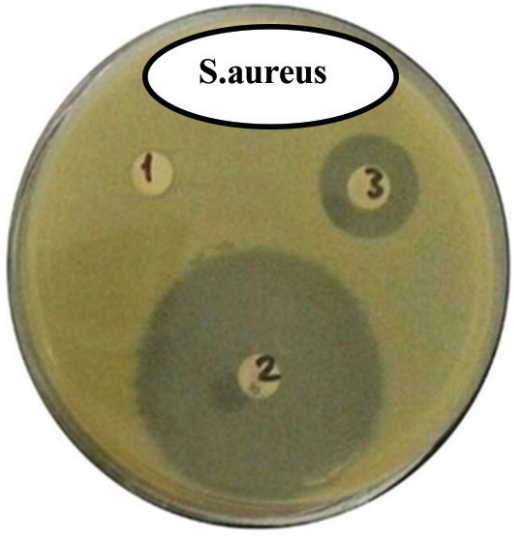

Figure 1: The zone of inhibition exerted by 1-Mueller Hinton Broth (negative control), 2-Penicillin (positive control) and 3-Platelet concentrate against Staphylococcus aureus after 24 hours of incubation

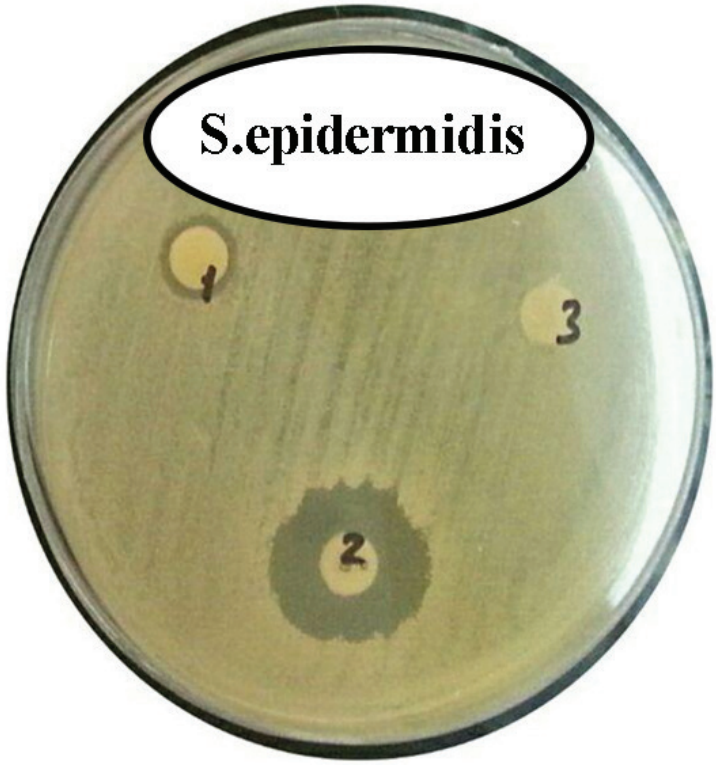

Figure 2: The zone of inhibition exerted by 1- Platelet concentrate, 2-Penicillin (positive control) and 3-Muller Hinton Broth (negative control) against Staphylococcus epidermidis after 24 hours of incubation.

48 hours.

Discussion

Despite a wide spectrum of available potent antimicrobials, bacterial infection remains a major problem. This is largely due to the emergence of bacterial resistance, caused by the inappropriate or inadequate use of antibiotics.[10] Therefore research for finding an alternative treatment and a solution for antibiotic resistance is crucial.

In the case of wound infections and hospital-acquired infections, it seems that platelet products could be appropriate adjuncts to antibiotics. Platelets can interact with microbial pathogens directly and indirectly through multiple molecular and cellular mechanisms. It has been suggested that platelets not only reduce incidence of bacterial infections but also promote wound healing.[1,5,6] Therefore, platelet products have recently attracted interest in this regard. But it seems that research in this field is still limited and insufficient. This study was designed to determine the in vitro antibacterial activity of human platelet concentrates against 6 common causes of wound and hospital-acquired bacterial infections.

The results of this study confirmed the previously reported antibacterial effects of human platelet concentrates against S. aureus.[4,8,11,13,14,17] The observed antibacterial activities of PCs against S. epidermis were similar to findings of Anitua et al., while Burnouf et al. found different results in their research.[3,8] On the other hand, PCs were not effective against four other bacteria in our study. It should be noted that previous studies conducted on P. aeruginosa and E.coli yielded contradictory results.[3,9,11] PCs have not been tested against M. luteus and P. vulgaris yet.

It seems that donor's variability along with differences in the quality, viability, activation and degradation rate of platelets could cause variation in the susceptibility pattern of the gram-positive and gram-negative bacteria in comparison to other studies. Our study used PTCC bacterial strains which may behave in a way different from clinical isolates or ATCC bacterial strains. Therefore, further studies (both in-vitro and in-vivo) are needed to investigate the antimicrobial effect of platelet concentrates against viruses, fungi and other bacterial strains along with similar studies using clinical isolates.

Conclusion

The findings of this study regarding the antibacterial effect of PCs against S. aureus and S. epidermidis were consistent with some other studies supporting the clinical use of platelets as a biocompatible and safe product in wound healing and hospital-acquired infections. Further research on PCs should be employed to determine exact antibacterial spectrum, their antimicrobial capacity along with antibiotics and their efficacy in in-vivo conditions.

Acknowledgments

The authors acknowledge with grateful appreciation the kind assistance and technical support provided by Dr. Bagheri, Ms. Mashhouri (Faculty of Pharmacy, Islamic Azad University, Tehran, Iran) And Mrs. Abbasi (Tehran Blood Transfusion Center, Tehran, Iran).

Conflict of Interest: None declared.

References

1. Michelson AD: Platelets. Third ed. San Diego, CA,

Academic Press, 2013.
2. Drago L., Bortolin M., Vassena C., et al. Antimicrobial activity of pure platelet-rich plasma against microorganism isolated from oral cavity. BMC Microbiology. 2013;13: 47-51. DOI: 10.1186/1471-2180-13-47**.**
3. Burnouf T, Chou ML, Wu YW, Su CY, Lee LW. Antimicrobial activity of platelet (PLT)-poor plasma, PLT-rich plasma, PLT gel, and solvent/ detergent-treated PLT lysate biomaterials against wound bacteria. Transfusion.2013;53(1):138-46.doi: 10.1111/j.1537-2995.2012.03668.x.PubMed PMID: 22563709.
4. Li, H., Li, B. PRP as a New Approach to Prevent Infection: Preparation and In vitro Antimicrobial Properties of PRP. J. Vis. Exp. 2013;74, e50351, doi:10.3791/50351.
5. YuanT, Zhang CQ, Tang MJ, Guo SC, Zeng BF. Autologous Platelet-rich Plasma Enhances Healing of Chronic Wounds. WOUNDS 2009;21(10):280–285.
6. Hom DB, Linzie BM, Huang TC. The Healing Effects of Autologous Platelet Gel on Acute Human Skin Wounds. Arch Facial Plast Surg. 2007; 9 (3):174-183. doi: 10.1001/archfaci.9.3.174. PubMed PMID: 17519207.
7. Geremicca W, Fonte C, Vecchio S. Blood components for topical use in tissue regeneration: evaluation of corneal lesions treated with platelet lysate and considerations on repair mechanisms. Blood Transfus. 2010;8(2):107–112. doi: 10.2450/2009.0091-09. PubMed PMID:20383304.PMCID: PMC2851214.
8. Anitua E, Alonso R, Girbau C, Aguirre JJ, Murozabal F, Orive G. Antibacterial effect of plasma rich in growth factors (PRGF-Endoret) against Staphylococcus aureus and Staphylococcus epidermidis strains. Clin Exp Dermatol. 2012;37(6):652-7.doi: 10.1111/j.1365-2230.2011.04303.x. PubMed PMID: 22329713.
9. Cieslik-Bielecka A, Dohan Ehrenfest DM, Lubkowska A, Bielecki T. Microbicidal properties of Leukocyte- and Platelet-Rich Plasma/Fibrin (L-PRP/L-PRF): new perspectives. J Biol Regul Homeost Agents. 2012;26(2 Suppl 1):43S-52S. PMID: 23648198.
10. Andersson DI, Hughes D. Persistence ofantibiotic resistance in bacterial populations. FEMS Microbiol Rev. 2011; 35(5): 901–911. DOI:10.1111/j.1574-6976.2011.00289.x. PubMed PMID:21707669.
11. Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Krol W, Wielkoszynski T. Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances: an in vitro study. J Bone Joint Surg Br. 2007;89:417–420. doi: 10.1302/0301-620X.89B3.18491.
12. Moojen DJ, Everts PA, Schure RM, Overdevest EP, van Zundert A, Knape JT, et al. Antimicrobial activity of platelet-leukocyte gel against Staphylococcus aureus. J Orthop Res. 2008;26:404–410. doi: 10.1002/ jor.20519. PubMed PMID: 17960651.
13. Zabidi MA, Yusoff NM, Abdul Kader ZS. Preliminary comparative analysis of antibacterial effects of activated and non-activated of expired platelet concentrate by disc diffusion method. Indian J Pathol Microbiol. 2012;55(1):47-51. doi: 10.4103/0377-4929.94855.
14. Intravia J, Allen DA, Durant TJ, McCarthy MB, Russell R, Beitzel K, et al. In vitro evaluation of the anti-bacterial effect of two preparations of platelet rich plasma compared with cefazolin and whole blood. Muscles Ligaments Tendons J. 2014;4(1):79-84. eCollection 2014. PubMed PMID: 24932452; PMCID: PMC4049655.
15. Fung MK, Grossman BJ, Hillyer CD, Westhoff CM, Technical Manual, 18TH ed. Bethesda, Maryland. AABB, 2014.
16. Black JG. Microbiology Principles and Exploration textbook. 5th ed. USA: John Wiley and Sons Inc.; 2002. p. 137-52.
17. Alvarez ME, Lopez C, Giraldo CE, Samudio I, Carmona JU. In vitro bactericidal activity of equine platelet concentrates, platelet poor plasma, and plasma against methicillin-resistant Staphylococcus aureus. Arch Med Vet. 2011;43:155-61. DOI: 10.4067/ S0301-732X2011000200008.