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Preliminary comparative analysis of antibacterial effects of activated and non-activated of expired platelet concentrate by disc diffusion method

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Abstract

Background: Platelets release more than 30 cytokines to provide primary hemostatic function. In addition, platelets are also known to release antimicrobial peptides upon activation by thrombin. **Materials and Methods:** In this study, comparative analysis of antibacterial activity of activated and non-activated expired platelet concentrate was determined against Gram-positive and Gram-negative bacteria by Kirby-Bauer disk diffusion method. Thrombin was used to prepare activated platelet concentrate. Gram-positive bacteria tested in this study were *S.aureus* and *S.pyogenes* and Gram-negative bacteria were *E.coli* and *K.oxytoca*. All the bacteria used in this study were sensitive strains from clinical isolates. Activated and non-activated platelet showed no zone of inhibition against *S.pyogenes* and *E.coli*. **Results:** Activated platelet showed antibacterial activity against *S.aureus* and *K.oxytoca* with the zone of inhibition of 8.3 ± 0.6 mm and 7.7 ± 0.2 mm, respectively. Zone of inhibition observed in non-activated platelet against *S.aureus* and *K.oxytoca* were 7.8 ± 0.4 mm and 7.5 ± 0.3 mm, respectively. **Conclusions:** These findings showed that no significant differences in antibacterial activity produced by activated and non-activated platelet. However, zone of inhibition observed in activated and non-activated platelet indicate the presence of antibacterial property in expired platelet.

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Full Text

Introduction

The principal function of platelets is to prevent bleeding. Thus, platelets are commonly known to play a fundamental role in hemostasis and are a natural source of growth factors. There are several granules in platelets contain clotting factors, calcium, serotonin, adenosine diphosphate (ADP) or adenosine triphosphate (ATP), and growth mediators. When activated, platelets release the contents of these granules in order to initiate clotting cascade to prevent bleeding and assist in healing. [1],[2] However, it has been known and recognized for many years that platelets are also able to secrete potential antibacterial proteins upon activation to kill the invading pathogens. A number of studies on the role of platelet to clump bacteria and the sequestration of bacteria within platelet aggregates have been reported. [3],[4],[5],[6] Findings from several investigations to elucidate the antimicrobial role of platelet revealed the presence of platelet microbicidal peptides (PMP) and other releasates from aggregation and activation of platelets which possess antimicrobial properties. [6],[7],[8],[9],[10] In another study, Ivanov and Gritsenko, [11] investigated the antibacterial activity of PMP against Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus lysodeikticus, and Escherichia coli. They noted that all the tested bacteria showed different level of susceptibility at different concentration of PMP except E.coli that was found to be relatively resistant to the lethal action of PMP.

In another study, normal human platelets were stimulated with human thrombin in vitro to produce releasates for further characterization. This study showed presence of at least seven identifiable thrombin-releasable antimicrobial peptides from human platelets which are platelet factor 4 (PF-4), RANTES, connective tissue activating peptide 3 (CTAP-3), platelet basic protein, thymosin-4 (T-4), fibrinopeptide B (FP-B), and fibrinopeptide A (FP-A). With the exception of FP-A and FP-B, all peptides were also purified from acid extracts of non-stimulated platelets. [12] Further investigations to characterize the microbicidal peptides have been reported in the literature. The findings from those investigations showed that the peptides were more potent against bacteria than fungi, activity was greater at acidic pH, and antimicrobial activities were dose-dependent. However, the mechanism of destruction of invading bacteria by antimicrobial peptides from platelet is not identified.

Nevertheless, it comes into realization now-a-days that platelet has been actively involved in host defence mechanism. In several studies reported in the literature, platelet rich plasma and fresh platelet were used to prepared platelet releasate in which broth dilution method, radial diffusion assay, and agar-well diffusion method were utilized to determine the antibacterial effects against selected microorganism. [13],[14],[15] However, in this study, expired platelets concentrate were used to determine and compare the antibacterial activity of activated and non-activated platelets. Agar disc diffusion procedure according to Kirby Bauer method was used to determine antibacterial activity of the platelet releasate. The antibacterial effects of activated and non-activated of expired platelet concentrate were determined against two in the genus of Gram-positive bacteria that include Staphylococcus aureus and Streptococcus pyogenes and two in the genus of Gram-negative bacteria which are Escherichia coli and Klebsiellae oxytoca.

Materials and Methods

Maintenance of Bacteria

All the four bacteria used in this study were obtained from stock culture collection section, Infectious Disease Cluster, Advanced Medical and Dental Institute (AMDI), Universiti Sains

Malaysia. The selected bacteria were Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, and Klebsiellae oxytoca. These bacteria were maintained on nutrient agar slant (NAS) (Oxoid) at 4 0 C.

Preparation of Inoculums

To prepare inoculums of bacteria culture, the stock culture from NAS was subcultured on Muller-Hinton agar (MHA) (Bio-Media Lab) and incubated overnight at 37 o C to produce pure culture with single isolated colony. A single pure and isolated colony from the MHA plate was inoculated in nutrient broth (NB) (Bio-Media Lab) by aseptic technique and incubated overnight at 37 o C. An overnight culture in NB was used as the inoculums for all the experiment performed in this study. To prepare the inoculums for the experiment, the overnight culture of each bacteria in Muller-Hinton Broth (MHB) (Bio-Media Lab) was diluted in ten-fold dilutions to give final concentration 10 7 CFU per ml which is equivalent to McFarland 0.5 Turbidity Standard.

Expired Platelet Concentrate Sample

Eleven bags of expired platelet concentrate were taken from Blood Bank, Hospital Pulau Pinang, Malaysia. The collected platelet concentrate bags were within day 1 to day 5 after the expiry date and total platelet count was equal or more than 0.5 x 10 6 /µl. Platelet bags that showed sign of leakage and presence of red blood cells were not used in this study.

Ethical Approval

The ethical approval was obtained for specimen collection, culture collection, and for the study design from Clinical Research Committee (NMRR-09-689-4462) from Ministry of Health Malaysia and Research Ethic Committee of AMDI (USM/IPPT/2000/G-5/i), Universiti Sains Malaysia.

Preparation of Platelet

A total of 1.5 ml of expired platelet concentrate was transferred into sterile 5 ml test tube and centrifuged at 3000 rpm for 3 minutes. After centrifugation, the supernatant was discarded and the pellet was washed by adding 3 ml of sterile saline. The saline suspension of platelet was centrifuged at 3000 rpm for 3 minutes and the supernatant was discarded. This process was repeated three times to complete the washing procedure. After the final washing, the platelet was suspended in normal saline and the count was monitored by full blood count analyzer (Sysmex) and the final count was adjusted to 0.5-1.5 x 10 5 platelet per µl.

Thrombin Activation

Thrombin activation was performed in 1.5 ml sterile microtube. To carry out the activation, 1.5 ml of the washed platelet was transferred into the microtube and subsequently 1.5 µl of thrombin (1NIH unit per µl) (Nacalai Tesque) at 37 o C for 1 hour. The activation of platelet was monitor by observing changes in the shape and aggregation of the platelet under light microscope. Shortly after end of incubation, the platelet was subjected to gentle mixing at 200 rpm by thermo mixer at 37 o C. This mixing process resulted to a floating clump of aggregated platelet indicated a successful activation by thrombin. The activated platelet was centrifuged at 13,000 rpm for 15 minutes at 37 o C (Eppendoff high speed refrigerated centrifuge). After centrifugation, the supernatant was saved as releasate. The non-activated platelet was prepared as above but without addition of thrombin. The positive control antibiotic discs used in Kirby Bauer disc diffusion method include Penicillin (10 units) (Oxoid) for Gram-positive bacteria and Gentamicin (12 µg/µl) (Oxoid) for Gram-negative bacteria. For negative control, the negative control was 30 µl of sterile distilled water pipetted on to blank disc.

Screening of Antibacterial Activity

Screening of antibacterial activity was performed by standard disc diffusion method. [16] Thirty microliters of sample was pipetted on to blank sterilized disc of filter paper (6 mm of diameter). The prepared inoculums were lawned evenly on the surface of the agar media by sterile cotton swab. Muller-Hinton Blood Agar (MHBA) (Bio-Media Lab) was used for S.pyogenes and MHA (Bio-Media Lab) for S.aureus, E.coli, and K.oxytoca. The filter disc containing the releasate was placed accordingly within the zone assigned on the lawned media [Figure 1]. This step was repeated with the non-activated platelet, positive and negative controls, respectively. The inoculated plates were incubated at 37 o C for 24 hours. The zone of inhibition was measured to the nearest millimetre (mm) after 24 of incubation. The assay was done in triplicate to get the statistical average of the result.{Figure 1}{Figure 2}

Statistical Analysis

Mean value for the zone of inhibition was calculated.

Results

The mean of platelet counts for each of the 11 platelet concentrate bags is 10.84 x 10 5 ± 2.68/µl. The measured zones of inhibition produced by the activated and non-activated platelet against S.aureus are illustrated in [Table 1]. The mean values for zone of inhibition produced by activated, non-activated, and positive controls are 8.3 mm, 7.8 mm, and 36.7mm, respectively. [Table 2] shows the measured zone of inhibition produced by activated and non-activated against K.oxytoca. The mean values for zone of inhibition produced by activated, non-activated, and positive controls are 7.7 mm, 7.5 mm, and 25.9 mm, respectively. There was no measurable zone of inhibition produced by the activated and the non-activated platelet against S.pyogenes and E.coli. [Figure 2] exemplify the zone of inhibition produced by the activated platelet against S.aureus.{Table 1}{Table 2}

The releasates obtained from four different bags of expired platelet concentrate showed greater zone of inhibition against S.aureus compared with zone of inhibition produced by the non-activated platelets. Although the differences in the measurement of the zone of inhibition varies in millimeter, but the difference in antibacterial effect by the activated and non-activated expired platelet concentrate were demonstrated. S.aureus has been repeatedly proven to be sensitive or susceptible to the PMP releasate of thrombin activated platelet. Reported study by [12] showed the significant level of antimicrobial activity of the PMPs exerted its activity against at least two bacteria that include S.aureus and E.coli and on two fungi that are Candida albicans and Cryptococcus neoformans.

In subsequent assay, greater zones of inhibitions were also observed in the releasate obtained from the subsequent four bags of expired platelet concentrate against K.oxytoca compared with the zone of inhibition produced by non-activated expired platelet. However, slightly lower zones of inhibitions were also observed on the non-activated expired platelet compared with the zones of inhibitions produced by the releasate from activated expired platelet.

Discussion and Conclusion

Emergence of antibiotic resistance is a major concern in the treatment of infectious diseases. Research for alternative treatment and solution for antibiotic resistance have become crucial in infectious disease. Primary function of platelet is to maintain hemostasis. Many findings reported in literature indicated that platelet produces several cationic peptides with microbicidal function that known as platelet microbicidal peptide (PMP).

In this study, the disk diffusion method was used to determine the antibacterial activity of the activated and non-activated expired platelets The disk diffusion method or also better known as Moist Disc Absorption Kirby Bauer Technique 1959 is the agar-based technique that has been used extensively by a few laboratories because they are simple, economical, and easy to perform simultaneously on large numbers of organisms. [17] This conventional method is a convenient technique to perform and validate the antibacterial effect of the platelet.

Most of the studies in the literature focused on antimicrobial activity presence in the fresh platelet concentrate and platelet rich plasma (PRP). In this study, expired platelet was used to determine its antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria. Platelets are discarded after five days of storage at 22 o C. Because of its short expiry date, frequently large numbers of platelet bags are discarded in routine blood bank practice. Therefore, this study was designed to explore the potential benefit and presence of residual microbicidal activity in the expired platelet bags. The thrombin activated platelet releasates were produced from 11 bags expired platelet concentrates. The bactericidal activities of the releasate were tested against Gram-positive bacteria and Gram-negative bacteria.

The inhibition zone produced by releasate showed that antibacterial activity was present in the activated expired platelet concentrate. The antibacterial activity could be produced due to the cationic peptides presence in the releasate. However, zones of inhibitions observed in the non-activated platelet also show the presence of antibacterial components. The components could be the PMPs released from the non-activated platelet. Reported study in the literature [12] showed that some of PMP that include thymosin-4 (T-4) and CTAP-3 can be released without thrombin activation. In addition, the fragile membrane structure of the expired platelet concentrates may also caused the release of more PMP without activation by thrombin.

On the other hand, activated and non-activated expired platelet showed no zone of inhibition when tested against S.pyogenes and E.coli. These finding showed that PMPs do not have selective susceptibility against Gram-positive bacteria or Gram-negative bacteria. To our knowledge, the mechanism of the antibacterial action of PMPs is not clearly defined. Therefore, the variation in the susceptibility pattern of the Gram-positive bacteria and Gram-negative bacteria against the PMPs require further investigation. Similar studies also reported in literatures [10],[11],[18] showed wide range of variation in susceptibility and resistant pattern of Gram-positive bacteria and Gram-negative bacteria when tested against PMPs. The study by Dankert [10] also observed the variation in the susceptibility against PMPs when tested against 10 strains of Streptococcus viridans.

The concentration of the platelets in all the 11 platelet concentrate bags varied ranging from 6.75 x 10 5 /ul to 15.25 x 10 5 /ul. The activated susceptibility of the activated and non-activated expired platelet prepared from the platelet bags were varies and no correlation of the platelet count and zone of inhibition in the antibacterial susceptibility testing was observed.

The platelet bags with low platelet concentrate bags with low platelet counts showed greater inhibition effects compared with platelet concentrate with high platelet and vice versa. These findings showed the variation in the quality, viability, and degradation rate of the platelet.

Overall, the antibacterial activity observed in the activated and non-activated expired platelet concentrate was very significant although the zones of inhibitions were lower than the positive control. The results obtained in this study show that after further processing, [19],[20],[21],[22] the expired platelet concentrate has a potential in medical application for the treatments in wound infection and to enhance healing.

On the other hand, a greater antibacterial effect could be seen if the releasate was further enriched by analytical procedures. Further study is required to determine the significance of antibacterial activity by the expired platelet concentrate against other bacteria and fungus. It is also useful to determine the therapeutic alternative of the activated concentrate in the treatment of microbial infection.

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References

1 Yeaman MR. The role of platelets in antimicrobial defense. Infect Immun 1997a;65:1137-41.
2 Ribatti D, Crivellato GB. The discovery of platelets. Leuk Res 2007;31:1339-41.
3 Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. Nat Rev Microbiol 2006;4:445-57.
4 O'Brien L, Kerrigan SW, Kaw G, Hogan M, Penades J, Litt D, *et al*. Multiple mechanisms for the activation of human platelet aggregation by Staphylococcus aureus: Roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. Mol Microbiol 2002;44:1033-44.
5 Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res 2002;22:913-22.
6 Clawson JA. Platelet interaction with bacteria I: Reaction phases and effects of inhibitors. Am J Pathol 1971;65:367.
7 Anderson RC, Rehders M, Yu PL. Antimicrobial fragments of the pro-region of cathelicidins and other immune peptides, Biotechnol Lett 2008;30:813-8.
8 Bayer AS, Kupferwasser LI, Brown MH, Skurray RA, Grkovic S, Jones T, *et al*. Low-level resistance of staphylococcus aureus to thrombin-induced platelet microbicidal protein 1 in vitro associated with qacA gene carriage is independent of multidrug efflux pump activity. Antimicrob Agents Chemother 2006;50:2448-54.
9 Yeaman MR, Yi-Quan Tang, Shen AJ, Bayer AS, Selsted ME. Purification and in vitro activities of rabbit platelet microbicidal proteins. Infect Immun 1997b;65:1023-31.
10 Dankert J, Van Der Werff J, Zaat SA, Joldersma W, Klein D, Hess J. Involvement of Bactericidal Factors from Thrombin-Stimulated Platelets in clearance of Adherent Viridans Streptococci in Experimental Infective Endocarditis. Infect Immun 1994;63:663-71.
11 Ivanov IB, Gritsenko VA. Comparative activities of cattle and swine platelet microbicidal protein. Probiotics Antimicrob Proteins 2009;10:1-5.
12 Yi-Quan Tang, Yeaman MR, Selsted ME. Antimicrobial peptides from human Platelets. Infect Immun 2002;70:6524-33.
13 Su-Pin Koo, Bayer AS, Georgsahl H, Proctor RA, Yeaman MR. Staphylocidal action of thrombin-induced platelet microbicidal protein is not solely dependent on trans-membrane potential. Infect Immun 1996;64:1070-4.
14 Yeaman MR, Waring AJ, Yount NY, Gank KD, Wiese R, Bayer AS, *et al*. Modular determinants of antimicrobial activity in platelet factor-4 family kinocidins. Biochem Biophys Acta 2007;1768:609-19.
15 Kahn RA, Flinton LJ. The relationship between platelets and bacteria. Blood 1974;44:715-21.
16 Black JG. Microbiology Principles and Exploration textbook. 5 th ed. USA: John Wiley and Sons Inc.; 2002. p. 137-52.
17 Rex JH, Pfaller MA, Rinaldi MG, Polak A, Galgiani JN. Anti-fungal Susceptibility Testing. J Clin Microbiol 1993;6:367-81.
18 Lee SY, Choe SJ. Inflammation: Basic principles and clinical correlates, Penicillin-induced killing and postantibiotic effect in oral streptococci are enhanced by platelet microbicidal proteins. Int J Antimicrob Agents 2003;23:457-61.
19 Kazakos K, Lyras N, Tryfonidis C, Tilkeridis C, Verettas D. The use of autologous platelet-rich plasma gel as an aid in the management of acute trauma wounds. Injury Extra 2008;39:185-6.
20 Bielecki TM, Gazdzik TS, Arendt J, Szczepanski T, Król W, Wielkoszynski T. Antibacterial effect of autologous platelet gel enriched with growth factors and other substances. J Bone Joint Surg Am 2007;89-B:417-20.
21 Gardner MJ, Demetrakopoulos D, Klepchick PR, Mooar PA. The efficacy of autologous platelet gel in pain control and blood loss in total knee arthroplasty: An analysis of the haemoglobin, narcotic requirement and range of motion. Int Orthop 2007;31:309-13.
22 Krasna M, Domanovic D, Tomsic A, Svajger U, Jeras M. Platelet gel stimulates proliferation of human dermal fibroblasts *in vitro*. Acta Dermatovenerol Alp Panonica Adriat 2007;16:105-10.