IJPSR (2012), Vol. 3, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 August, 2012; received in revised form 01 October, 2012; accepted 28 November, 2012

INFUSIBLE PLATELET MEMBRANES IMPROVE HEMOSTASIS IN THROMBOCYTOPENIC RABBITS: STUDIES WITH TWO DIFFERENT INJECTION DOSES

Saleh Nasiri*[1, 2], Majid Heidari [2] and Saeid Rivandi [2]

Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine [1], Hemmat Exp. Way, Next to the Milad Tower, Tehran, Iran

Iranian Blood Research and Fractionation Company, Quality Control Department [2], Tehran, Iran

Keywords:
Infusible platelet membrane, Platelet substitute, Bleeding time, Thrombocytopenic rabbits

Correspondence to Author:

Saleh Nasiri

Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Hemmat Exp. Way, Next to the Milad Tower, Tehran, Iran

E-mail: tamildeiva@gmail.com

QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

Due to the limited life span of platelets during regular storage in blood banks, we were interested to investigate the both potential hemostatic effect and dose-dependent response properties of non-viable platelet preparations with injection of two high and low doses in experimental animal model. Infusible platelet membrane (IPM) was prepared from outdated platelet concentrates. Platelet concentrates were pooled, disrupted by freeze-thaw procedure, pasteurized for 20 hours to inactivate possible viral or bacterial contaminants and finally formulated with sucrose and human serum albumin. Rabbits were made thrombocytopenic. Administration of IPM at two doses of 0.5 and 2.0 mg per kg results significant reduction in the bleeding time. The values of bleeding time in the 80 data sets were obtained. Reduction in the percentage of bleeding time elevation during 2, 4, 6 and 24 hours after injections of 0.5 mg/kg were found 56.8, 66.0, 73.7 ,96.8 and after 2.0 mg/kg injections were observed 24.8, 39.0, 52.4 and 95.6 respectively. We concluded that IPM can significantly reduce bleeding time in thrombocytopenic rabbits with two high and low doses of IPM that confirms potential hemostatic efffect and also shows dose-dependent response property and finally may support its clinical potential utility as a transfusion substitute for platelets.

INTRODUCTION: Platelets play a vital role in the maintenance of normal hemostatic activity. For patients with thrombocytopenia or impaired platelet function, platelet transfusion can have an important value in preventing or treating hemorrhage. Unfortunately, blood platelet units are generally stored in blood banks for 3-5 days; thereafter they are discarded [1-3].

For preserving hemostatic effects of platelets for a long period, many efforts have been made to develop substitutes for platelets.

A number of studies have confirmed that platelet preparations with impaired metabolic or functional integrity still retain a certain degree of hemostatic property [4-7]. Infusible platelet membrane (IPM) as a potential transfusion substitute for platelets prepared from outdated human platelets has been shown to correct prolonged bleeding times in thrombocytopenic rabbits with the additional advantage of long shelf life and increased viral safety [8-9]. Platelet microparticles are microvesicles of platelet membranes that form during the activation or mechanical disruption of platelets [10].

They form spontaneously during platelet storage and can be detected in platelet concentrates [11-13], fresh frozen plasma and cryoprecipitate [14]. They have properties of procoagulant activity [15]. Due to these microparticles have similar hemostatic properties as intact platelets, they can be considered as a strategy for the development of a platelet substitute. However, the earliest efforts of these preparations were not successful in vivo and produced considerable distress in experimental animals [16].

With regard to this problem, the investigations postponed for nearly three decades until experiments in thrombocytopenic rabbits provided preclinical evidence of their hemostatic efficacy without significant morbidity [17].

In this study we want to show that IPM is effective at both high and low injection doses to shortening the bleeding times in thrombocytopenic rabbits with dose-dependent response property and may be clinically used as a potential platelet substitute in the treatment of bleeding due to thrombocytopenia.

MATERIALS AND METHODS:

Preparation of IPM: IPM is prepared from 8 outdated platelet units of Tehran Blood Transfusion Center. The units were pooled and centrifuged for 15 min at 1000 RPM to remove contaminating red cells and white cells. The supernatant was centrifuged for 30 min at 2500 RPM to remove plasma. The precipitate was resuspended in 25 ml physiological saline solution (0.9 g%). For lysis and disruption of platelets, freeze-thaw procedure was repeated three times at -80°C and room temperature for 6 and 2 h respectively. The solution was washed twice with physiological saline solution for removing of intracellular components by centrifugation (30 min at 2500 RPM). The precipitate was resuspended in 45 ml of the same solution.

Pasteurization of Sample: The sample of IPM with 0.4 M sodium caprylate concentration was prepared and heated at 60°C for 20 h to inactivate possible viral or bacterial contaminants and formulated with sucrose 1 M and human serum albumin 0.1%.

***In vivo* Haemostasis Assay:** The hemostatic activity of IPM was measured by bleeding time assay to correct prolonged bleeding time in thrombocytopenic rabbits. White New Zealand rabbits 3 to 3.3 Kg in weight were made thrombocytopenic by subcutaneous administration of busulfan dissolved in polyethylene glycol 400 (15 mg/kg on Days 0, 2, 4 and 6). We measured platelet count and bleeding time on Day 7, 9, 11. Animals with 50-70 × 10^3 per µL with prolonged bleeding time (≥7 min) were used in this assay.

For bleeding time assay, a standard device (ITC Surgicut Bleeding time, Fisher Scientific Inc.) was used to provide uniform standardized incision depth in the ear (Incision D×L: 1.0 x 5.0 mm) with improved test standardization and reproducibility of bleeding time test results. We applied a Whattman filter paper to the flow of blood from the wound.

A stopwatch was started immediately and every 30 seconds filter paper was used to draw off the blood. The time from when the incision was made until all bleeding has stopped was called the bleeding time.

We determined the preinjection bleeding time in one ear and administrated IPM at a dose of 0.5 mg per kg by injection into the marginal vein of the other ear at a rate of 2 mL per minute. The bleeding times was then measured at various times after injection, 2, 4, 6 and 24 hours. After 48 hours of the first injection, the second injection at a dose of 2.0 mg per kg was performed with the same protocol on the same rabbits. All bleeding time assays were performed in duplicate.

RESULTS AND DISCUSSION: In previous studies bleeding time has been used to determine the hemostatic effectiveness of various agents in the rabbit model [18-20].

In this study, we administered IPM at two different doses of 0.5 and 2.0 mg per kg, to thrombocytopenic rabbits with platelet count of 50 to 70 ×10^3 per µL, and we performed 80 sets of bleeding time measurements before and 2, 4, 6 and 24 hours after IPM injection.

The values of bleeding time in these 80 data sets are summarized in **Table 1**.

TABLE 1: RESULTS OF BLEEDING TIME MEASUREMENTS DURING BEFORE AND AFTER IPM INJECTIONS AT TWO DOSES OF 0.5 AND 2.0 MG/KG IN RABBITS

Bleeding* time (min)	Before IPM injection	Time after IPM injection (hours)							
		2		4		6		24	
Rabbit No 1	7.0	4.4[1]	1.9[2]	4.9	3.6	5.2	4.1	6.7	6.8
Rabbit No 2	7.3	3.9	1.6	4.5	2.2	4.7	3.5	7.1	7.3
Rabbit No 3	7.2	4.1	1.6	5.3	2.4	5.8	4.1	6.8	6.8
Rabbit No 4	10.0	5.5	2.7	6.1	4.1	7.5	4.8	9.5	9.6
Mean	7.9	4.5	2.0	5.2	3.1	5.8	4.1	7.5	7.6

* Bleeding time was performed in duplicate and the mean was calculated; [1] At a dose of 0.5 mg/kg, [2] At a dose of 2.0 mg/kg

Because all the platelet counts in these animals are much lower than the normal mean value of 430 × 10^3 per µL, the bleeding times were considerably longer than the normal value of 1.7 minutes. In this study for reliability of the results, the bleeding time was measured in duplicate and the mean was calculated.

The 20 sets of percentages of bleeding time elevations, and the corresponding data from 2 administrations of excipient (sucrose and human serum albumin in 0.9% siodium chloride) to another group of rabbits, are summarized in Fig-1. For comparison of results, we expressed decrease in the percentage of bleeding time elevation after the administration of IPM to thrombocytopenic rabbits (**Fig. 1**). As one can see from top curve in Fig. 1, there is no significant change in the bleeding times of animals given excipient used in IPM formulation. Reduction in the percentage of bleeding time elevation during 2, 4, 6 and 24 hours after injections of 0.5 mg/kg were found 56.8, 66.0, 73.7 ,96.8 and after 2.0 mg/kg injections were observed 24.8, 39.0, 52.4 and 95.6 respectively (Fig. 1).

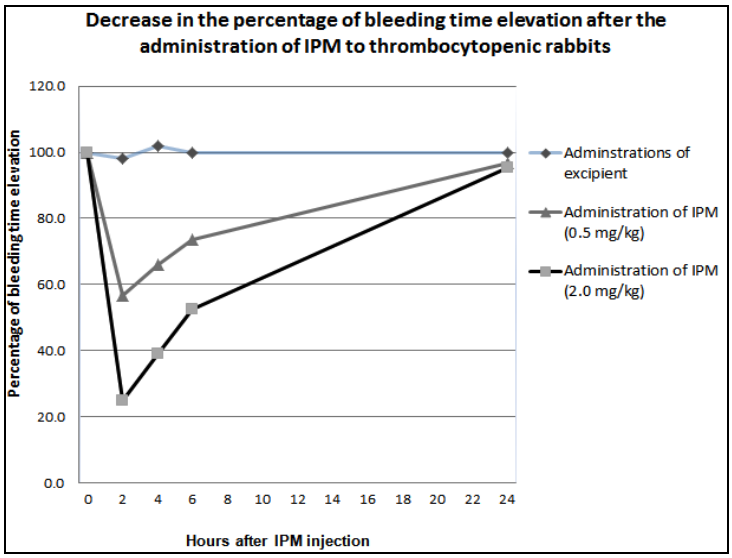

FIG. 1: DECREASE IN THE PERCENTAGE OF BLEEDING TIME ELEVATION AFTER THE ADMINISTRATION OF IPM TO THROMBOCYTOPENIC RABBITS. The bleeding time was measured to a maximum of 15 minutes after 8 administrations of IPM at two doses of 0.5 and 2.0 mg per kg and 2 administrations of excipient (sucrose and human serum albumin in 0.9% sodium chloride).

The results of previous study was nearly similar to our study and has shown that administration of IPM (2 mg/kg) can shorten the prolonged bleeding time in thrombocytopenic rabbits at least 4 h after infusion [8]; however, in our study the maximum decrease in the percentage of bleeding time was observed at 2 h after infusion instead of 4 h. In addition, its dose-dependent response was the most important finding in this research that reveals more hemostatic effectiveness as a potential transfusion substitute for platelets. In both studies this hemostatic effect was no longer detectable after 24 h of IPM administrations.

The reduction of bleeding time in experimental animals in our study confirms the potential utility of IPM as a substitute for platelets in the treatment of thrombocytopenia in humans. The clinical utility of the bleeding time in clinical aspects is controversial. Many experts regard the bleeding time as useless, in that it does not predict surgical bleeding. Articles supporting this view are often presented by pathologists. Despite such articles, the bleeding time continues to be used by many clinicians, primarily surgeons [21-25].

However, in this research, the bleeding time was used to determine the response in a group of rabbits, almost identical in age and weight, that are relatively healthy, though thrombocytopenic. An important finding of this study was the change in the bleeding time during the early hours after the injection of IPM. An acceptable number of animals has been used, and the each assay was performed in duplicate.

Our results indicate hemostatic effectiveness of IPM and its dose-dependent response in this experimental setting.

The possible efficacy of IPM in the treatment of bleeding in a large population of thrombocytopenic patients with various clinically significant consequences is not resolved and can be determined only by human clinical trials which will be performed in future.

CONCLUSION: Data obtained in our experimental setting indicate that the injection of IPM at the two high and low doses:

1) can significantly reduce bleeding time in thrombocytopenic rabbits;

2) confirms its dose-dependent response property;

3) shows maximum decrease in the percentage of bleeding time elevation after two hours of injections;

4) may support its clinical potential utility as a transfusion substitute for platelets.

REFERENCES:

1. FDA memo dated June 2, 1986 to all registered blood establishments entitled, "Reduction of the Maximum Platelet Storage Period to 5 Days in an Approved Container". United States Food and Drug Administration, Washington, D.C.
2. Blajchman MA, Slichter SJ, Heddle NM, Murphy MF: New strategies for the optimal use of platelet transfusions. Hematology Am Soc Hematol Educ Program 2008; 198-204.
3. Hess JR: Conventional blood banking and blood component storage regulation: opportunities for improvement. Blood Transfusion 2010; 8(Suppl 3):s9-s15.
4. Graham SS, Gonchoroff NJ, Miller JL: Infusible platelet membranes retain partial functionality of the platelet GPIb/IX/V receptor complex. Am J Clin Pathol. 2001; 115(1):144-7.
5. Hawiger J: Mechanisms involved in platelet vessel wall interaction. Thromb Haemost 1995; 74:369-72.
6. B S Coller, K T Springer, J H Beer, N Mohandas, L E Scudder, K J Norton, S M West: Thromboerythrocytes. In vitro studies of a potential autologous, semi-artificial alternative to platelet transfusions. J Clin Invest. 1992; 89(2):546–555.
7. Read MS, Reddick RL, Bode AP, Bellinger DA, Nichols TC, Taylor K, Smith SV, McMahon DK, Griggs TR, Brinkhous KM: Preservation of hemostatic and structural properties of rehydrated lyophilized platelets: potential for long-term storage of dried platelets for transfusion. Proc Natl Acad Sci USA. 1995; 92(2):397-401.
8. Chao FC, Kim BK, Houranieh AM, Liang FH, Konrad MW, Swisher SN, Tullis JL: Infusible platelet membrane microvesicles: a potential transfusion substitute for platelets. Transfusion 1996; 36(6):536-42.
9. Nasiri S, Heidari M: Application of sodium caprylate as a stabilizer during pasteurization of infusible platelet membrane and evaluation of effectiveness by turbidity assay. International Journal of Analytical, Pharmaceutical and Biomedical Sciences 2012; 1(2):34-36.
10. Owens M: The role of platelet microparticles in haemostasis. Transfusion Medicine Reviews 1994; 8:37-44.
11. Solberg C, Holme S, Little C: Morphological changes associated with pH changes during storage of platelet concentrates in first-generation 3-day container. Vox Sanguinis 1986; 50:71-77.
12. George JN, Pickett EB, Heinz R: Platelet membrane microparticles in blood bank fresh frozen plasma and cryoprecipitate. Blood 1986; 68:307-309.
13. Bode AP, Orton SM, Frye MJ, Udis, BJ: Vesiculation of platelets during in vitro aging. Blood 1991; 77:887-895.
14. George JN, Pickett EB, Heinz R: Platelet membrane microparticles in blood bank fresh frozen plasma and cryoprecipitate. Blood 1986; 68:307-309.
15. Owens M.R, Holme S, Cardinali S: Platelet microvesicles adhere to subendothelium and promote adhesion of platelets. Thrombosis Research 1992; 66:247-258.
16. Hjort PF, Perman V, Cronkite EP: Fresh, disintegrated platelets in radiation thrombocytopenia: Correction of prothrombin consumption without correction of bleeding. Proceedings of the Society of Experimental Biology and Medicine 1959; 102:31-35.
17. McGill M, Fugman DA, Vittorio N, Darrow C: Platelet membrane vesicles reduced microvascular bleeding times in thrombocytopenic rabbits. Journal of Laboratory and Clinical Medicine 1987; 109:127-133.
18. Wagner SJ, Bardossy L, Moroff G, Dodd RY, Blajchman MA**:** Assessment of the hemostatic effectiveness of human platelets treated with aminomethyltrimethyl psoralen and UVA light using a rabbit ear bleeding time technique. Blood 1993; 82:3489-3492.
19. Levi M, Friederich PW, Middleton S, de Groot PG, Wu YP, Harris R, Biemond BJ, Heijnen HF, Levin J, ten Cate JW: Fibrinogen-coated albumin microcapsules reduce bleeding in severely thrombocytopenic rabbits. Nat Med. 1999; 5(1):107-11.
20. Wong PC, Crain EJ, Watson CA, Jiang X, Hua J, Bostwick JS, Ogletree ML, Schumacher WA, Rehfuss R: Platelet aggregometry and receptor binding to predict the magnitude of antithrombotic and bleeding time effects of clopidogrel in rabbits. J Cardiovasc Pharmacol. 2007; 49(5):316-24.
21. Lind SE: Prolonged bleeding time. Am J Med. 1984; 77(2):305-12.
22. Rodgers RP, Levin J: A critical reappraisal of the bleeding time. Semin Thromb Hemost. 1990; 16(1):1-20.
23. Lind SE: The bleeding time does not predict surgical bleeding. Blood. 1991; 77(12):2547-52.
24. Lehman CM, Blaylock RC, Alexander DP, Rodgers GM: Discontinuation of the bleeding time test without detectable adverse clinical impact. Clin Chem. 2001; 47(7):1204-11.
25. Peterson P, Hayes TE, Arkin CF, Bovill EG, Fairweather RB, Rock WA, Triplet DA, Brandt JT: The preoperative bleeding time test lacks clinical benefit [Review]. Arch Surg. 1998; 133:134-139.

How to cite this article:
Nasiri S, Heidari M and Rivandi S: Infusible Platelet Membranes improve Hemostasis in Thrombocytopenic Rabbits: Studies with two different Injection Doses. *Int J Pharm Sci Res*. 3(12); 4895-4898.