Case Report

J Vet Sci 2015, 16(1), 127-130 · http://dx.doi.org/10.4142/jvs.2015.16.1.127

JVS

Application of vincristine-loaded platelet therapy in three dogs with refractory immune-mediated thrombocytopenia

Hyung-Jin Park, Ja-Won Kim, Kun-Ho Song, Kyoung-Won Seo*

College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Korea

Three dogs presented with refractory immune-mediated thrombocytopenia (IMT). All patients failed to respond to prednisone, which is considered a mainstay of immunosuppressive therapy. Vincristine-loaded platelets (VLPs), which act selectively on mononuclear phagocytes,were introduced. After the VLPs were transfused, two dogs responded quickly withimproved clinical signs while the third dogwith recurrent IMT was euthanized due to its deteriorating condition. This case report describesthe efficacy of VLP therapy in refractory IMT patients.

Keywords: refractory immune-mediated thrombocytopenia, vincristine-loaded platelets

Immune-mediated thrombocytopenia (IMT) is an autoimmune disorder in which antibodies are formed against platelets, leading to phagocytosis and destruction by macrophages [1]. Idiopathic IMT accounts for approximately 5% to 15% of patients with overrepresentation among females and certain breeds such as cocker spaniels, poodles, and English sheepdogs [1,2,4]. Clinical signs of IMT are similar to those of primary hemostatic disorders including petechiae, ecchymosis, epistaxis, and gastrointestinal bleeding. Short-term survival rates range from 74% to 97% with recurrence rates between 26% and 58% [2,4,5]. The standard choice of treatment is corticosteroids. Other therapies, such as the immunosuppressive drugs azathioprine, cyclosporine, and leflunomide, may also be considered [6]. In most IMT patients, platelet count recovery is observed within 1～15 days after glucocorticoid treatment. However, additional therapeutic approaches are certainly needed for refractory IMT patients that failed to respond to first-line therapy and experienced sustained thrombocytopenia [6]. In cases of refractory IMT, splenectomy, vincristine, and human intravenous immunoglobulin can be considered.

Vincristine is thought to increase platelet counts in patients with IMT through several mechanisms [1]. In addition to inhibiting phagocytosis of platelets, interfering with antiplatelet antibody formation, and preventing the binding of antiplatelet antibodies to platelets, vincristine also accelerates megakaryocytic breakdown and stimulates thrombopoiesis [1]. Compared to prednisone, administration of vincristine rapidly increases platelet numbers and shortens the duration of hospitalization for IMT dogs [1]. However, vincristine injected intravenouslyis cleared from the circulation so rapidly that the effectiveness must be considered along with adverse effects of this compound as an anti-neoplastic agent. To minimize the adverse effects and maximize the effectiveness of vincristine as an IMT therapy, we focused on the traditional method of delivering vincristine-loaded platelets (VLPs) or the so-called "Trojan horse." VLPs react with antiplatelet antibodies, thus reinforcing the selective delivery of the drugs to cells inthe mononuclear phagocyte system that destroy platelets in IMT patients [8]. Previous studies describedthe usefulness of this treatment modality for refractory IMT [8,9]. The aim of this case report was to describe our experience using VLPs to treat three dogs with refractory IMT and severe clinical signs to demonstrate the efficacy of this technique.

A 7-year-old female miniature schnauzer weighing 12.6 kg (case 1), a 4-year-old neutered male Shih Tzu weighing 6.2 kg (case 2), and an 11-year-old neutered male Shih Tzu weighing 5.15 kg (case 3) were referred with a history of ocular hyphema (case 1), anorexia, melena, and hematemesis (cases 2 and 3). One dog (case 3) had undergone IMT treatment 2 years prior to the study and had been treated for recurred IMT in a local hospital prior to the investigation. For case 1, petechiae on the oral mucosa was identified during physical examination.

Received 7 May 2014, Revised 30 Jul. 2014, Accepted 27 Sep. 2014

*Corresponding author: Tel: +82-42-821-6756; Fax: +82-42-821-8903; E-mail: kwseo@cnu.ac.kr

pISSN 1229-845X
eISSN 1976-555X

Results of the complete blood cell count (CBC) revealed that the dog was suffering from severe thrombocytopenia (41 × 10^3/L) and leukocytosis (18 × 10^3/L). Platelets were not found in the blood smear evaluation. For case 2, pale mucous membranes, conjunctival hemorrhage, and numerous petechial and large ecchymotic lesions on the ventral abdomen were observed. The dog had been previously treated with prednisone (2 mg/kg, PO, twice daily) for 2 days at a local animal hospital. Blood examination revealed that the animal had normocytic, normochromic, non-regenerative anemia (hematocrit, 19.6%; reference range: 37～55%) and severe thrombocytopenia (< 1 × 10^3/L, reference range: 200～500 × 10^3/L; Table 1). Biochemistry profiles indicated increased blood urea nitrogen (BUN, 43 mg/dL; reference range: 8～31 mg/dL), hypoalbuminemia (2.6 g/dL, reference range: 2.9～4.2 g/dL), and hypoproteinemia (3.5 g/dL, reference range: 5.4～7.4 g/dL). For case 3, blood examination revealed that the dog had severe anemia (hematocrit, 7.8%) and thrombocytopenia (0 × 10^3/L, Table 1). Biochemistry profiles indicated increased creatinine (1.9 mg/dL, reference range: 0.8～1.6 mg/dL) and increased BUN (> 160 mg/dL; reference range: 8～31 mg/dL). Increased BUN and hypoproteinemia were associated with gastrointestinal bleeding. Thoracic and abdominal radiographic examinations for all cases were conducted, and no specific abnormalities were found. Testing for *Ehrlichia canis*, *Babesia canis*, *Rickettsia rickettsii*, and *Anaplasma phagocytophilum* using a commercially available 4Dx kit (SNAP 4Dx; IDEXX Laboratories, USA) produced negative results.

All dogs were diagnosed as having primary IMT. The three animals were given prednisone (2 mg/kg, PO, twice daily, Solondo; Yuhan, Korea) and mycophenolate mofetil ([MMF], 15 mg/kg, PO, twice daily, CellCept; Roche, Italy). Two weeks later, clinical signs for case 1 had not improved and the dog still had severe thrombocytopenia. Human IV immunoglobulin (1 g/kg constant rate infusion for 6 h, IV-globulin 2.5G; Green Cross, Korea) was added to her treatment protocol. However, the thrombocytopenia did not resolve.

Case 2 received a transfusion of 70 mL of whole blood. On day 4, petechial and ecchymotic lesions were still noted, and melena was detected several times. CBC results revealed the presence of severe anemia (hematocrit, 13%; Table 1). Platelet-rich plasma (PRP; 120 mL) was administered along with human IV immunoglobulin at a dosage of 1 g/kg over 6 h. Over the next 4 days, a second transfusion with packed red blood cells (RBCs) was delivered. On day 14, evaluation of CBC results indicated that the dog still suffered from leukocytosis (20.3 × 10^3/L, reference range: 6～17 × 10^3/L) and mild anemia (hematocrit, 26.6%). After a packed RBC transfusion, the platelet count remained low while petechial and ecchymotic lesions were still present. Even with the application of second-line therapy such as human IV immunoglobulin and MMF, thrombocytopenia and clinical signs such as petechial and ecchymotic lesions persisted.

Case 3 received a transfusion of whole blood (20 mg/kg). On day 2, the patient was still anemic (hematocrit, 11.5%; reference range: 37～55%). Therefore, 90 mL of packed RBCs were transfused along with human IV immunoglobulin at a dosage of 1 g/kg over 6 h. On day 3, the hematocrit results were still low (15.7%, reference range: 37～55%) and the platelet count was 61 × 10^3/L. The patient showed an increased amount of melena.

We decided to initiate therapy with VLPs for all three dogs in a manner analogous to what has been conducted in previous human studies [8,10]. The patients were transfused with treated platelets over a 30-min period using standard blood administration sets. After VLP transfusion, the clinical signs in case 1 were dramatically improved and the platelet count measured on day 17 had increased (48.9 × 10^3/L, reference range: 200～500 × 10^3/L; Table 1). For case 2, the follow-up platelet count on day 17 was 11.6 × 10^3/L and the hematocrit was 32% (Table 1). Over the next 2 days, the platelet count increased (28.5 × 10^3/L) while the petechial and ecchymotic lesions disappeared. The dog was clinically normal with a good appetite when the patient dischared. Seven weeks after initial presentation, no evidence of IMT was detected and the prognosis was excellent. For case 3, the melena worsened while the severe anemia (hematocrit, 5.9%) and thrombocytopenia (49× 10^3/L) persisted. A second whole blood transfusion was needed but the patient was euthanized due to economic

Table 1. Complete blood cell count data for the three dogs included in the present study

		Case 1			Case 2			Case 3			
	Reference range	Day 0	Day 14	Day 17	Day 0	Day 14	Day 17	Day 0	Day 2	Day 3	Day 4
WBC	6.0～17.0 × 10^3/mL	18.7	28.3	42.5	12.5	20.3	30.5	7.6	5.6	5.3	4.5
RBC	5.5～8.5 × 10^3/μL	8.69	6.84	6.01	2.75	3.61	2.75	1.12	1.72	2.37	0.89
HCT	37～55 (%)	57.3	46.4	41.6	19.6	26.6	32	7.8	11.5	15.7	5.9
Hb	12～18 g/dL	21	16.7	14.3	6.7	7.5	7.4	2.6	3.9	5.4	4.5
Platelets	200～500 × 10^3/μL	41	34	489	0	0	116	0	1	61	49

WBC: white blood cell, RBC: red blood cell, HCT: hematocrit, Hb: hemoglobin.

constraints of the owner.

In this report, the efficacy of VLPs in three refractory IMT patients is described. The two dogs with refractory IMT dogs survived while the remaining animal with severe melena was euthanized. All patients had a bleeding tendency and failed to respond to conventional therapies including prednisone and mycophenolate mofetil with or without human IV immunoglobulin. Therefore, these animals received VLPs, and two of the three dogs showed a prompt response.

Vincristine is a vinca alkaloid that has both mild myelosuppressive and thrombopoietic properties [9]. Vinca alkaloids have a high affinity for tubulin, a major component of platelet microtubules [11]. Antibody-coated platelets with vinca alkaloids are phagocytized by macrophages, thus selectively delivering the alkaloids to macrophages expressing several Fc receptors [12]. The superiority of VLPs over a single vincristine injection has never been demonstrated [13]. However, vincristine delivered via an intravenous injection is cleared from circulation so rapidly that binding cannot be optimized. Platelets pre-incubated with vincristine were designed to maximize vincristine delivery and platelet binding. In the present study, vincristine-loaded platelets were prepared using a protocol similar to that reported for previous human studies [7,8]. A total of 200 mL of normal, cross-matched canine PRP was isolated by centrifugation (1,000 × g). One mg of vincristine sulfate was added to the PRP, the mixture was divided into four tubes, and incubated for 1 hat 37°C on a rotor in the dark because vincristine is degraded by light. Next, the solution was centrifuged at 2,000 × g for 20 min to pack the platelets. The supernatant was discarded, leaving 35 mL of PRP. These prepared platelets were transfused into the three dogs. All transfusions were well tolerated and effective without any side effects. A prospective clinical trial evaluating the efficacy of human IV immunoglobulin versus vincristine in dogs with IMT did not uncover differences in platelet recovery time or duration of hospitalization [2]. However, the patients in our study did not respond to human IV immunoglobulin while two out of three canines had a positive response to VLP therapy. Vincristine and VLPs have different mechanisms of action and therefore cannot be directly compared [14]. We presume that VLP transfusions may be more efficient than intravenous vincristine therapy because of superior drug delivery to target cells, such as macrophages. In this case series, responsiveness to VLP therapy was different for each patient. Cases 1 and 2 survived to discharge while case 3 was euthanized due to a poor response to therapy and deteriorating health. Both cases 2 and 3 had profound thrombocytopenia with a high BUN concentration and melena at the time of initial presentation, so several blood transfusions were needed. Because a high BUN concentration is associated with the risk of gastrointestinal mucosal bleeding secondary to thrombocytopenia, it can be considered a negative prognostic indicator for IMT patients [3]. Case 3 that was euthanized had a much higher BUN concentration (> 160 mg/dL) than that of case 2 (43 mg/dL).

These case reports demonstrate the therapeutic efficacy of VLP transfusions for treating refractory IMT patients. The platelets served as a carrier for directly delivering a large quantity of vincristine to targeted macrophages that engulf the antibody-coated vincristine-loaded thrombocytes, leading to macrophage dysfunction and death. Macrophage phagocytic dysfunction due to VLP administration lasts longer than that caused by intravenous vincristine injection. We believe that it is worthwhile to utilize VLPs as a salvage treatment for refractory IMT patients who fail to respond to other conventional immunosuppressive therapies.

In conclusion, we used VLP therapy to treat three canines with refractory IMT that did not respond to conventional immunosuppressive therapies. Two of the three dogs had a positive response while the remaining one was euthanized due to lack of a response and worsening clinical signs. Based on these case reports, VLPs therapy should be considered for patients with refractory IMT.

Acknowledgments

This work was supported by Chungnam National University and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF 2014007297).

Conflict of Interest

There is no conflict of interest.

References

1. **Ahn YS, Byrnes JJ, Harrington WJ, Cayer ML, Smith DS, Brunskill DE, Pall LM.** The treatment of idiopathic thrombocytopenia with vinblastine-loaded platelets. N Engl J Med 1978, **298**, 1101-1107.
2. **Balog K, Huang AA, Sum SO, Moore GE, Thompson C, Scott-Moncrieff JC.** A prospective randomized clinical trial of vincristine versus human intravenous immunoglobulin for acute adjunctive management of presumptive primary immune-mediated thrombocytopenia in dogs. J Vet Intern Med 2013, **27**, 536-541.
3. **Bianco D, Armstrong PJ, Washabau RJ.** Treatment of severe immune-mediated thrombocytopenia with human IV immunoglobulin in 5 dogs. J Vet Intern Med 2007, **21**, 694-699.
4. **Helfand SC, Jain NC, Paul M.** Vincristine-loaded platelet therapy for idiopathic thrombocytopenia in a dog. J Am Vet Med Assoc 1984, **185**, 224-226.
5. **Jans HE, Armstrong PJ, Price GS.** Therapy of immune mediated thrombocytopenia. A retrospective study of 15 dogs. J Vet Intern Med 1990, **4**, 4-7.

6. **Lewis DC, Meyers KM.** Canine idiopathic thrombocytopenic purpura. J Vet Intern Med 1996, **10**, 207-218.
7. **Nenci GG, Agnelli G, De Cunto M, Gresele P.** Infusion of vincristine-loaded platelets in acute ITP refractory to steroids: an alternative to splenectomy. Acta haematol 1981, **66**, 117-121.
8. **O'Marra SK, Delaforcade AM, Shaw SP.** Treatment and predictors of outcome in dogs with immune-mediated thrombocytopenia. J Am Vet Med Assoc 2011, **238**, 346-352.
9. **Putsche JC, Kohn B.** Primary immune-mediated thrombocytopenia in 30 dogs (1997-2003). J Am Anim Hosp Assoc 2008, **44**, 250-257.
10. **Rosse WF.** Whatever happened to vinca-loaded platelets? N Engl J Med 1984, **310**, 1051-1052.
11. **Shvidel L, Sigler E, Shtalrid M, Berrebi A.** Vincristine-loaded platelet infusion for treatment of refractory autoimmune hemolytic anemia and chronic immune thrombocytopenia: rethinking old cures. Am J Hematol 2006, **81**, 423-425.
12. **Stasi R, Evangelista ML, Stipa E, Buccisano F, Venditti A, Amadori S.** Idiopathic thrombocytopenic purpura: current concepts in pathophysiology and management. Thromb Haemost 2008, **99**, 4-13
13. **Weigert O, Wittmann G, Gruetzner S, Cnossen J, Becker MC, Dreyling M, Ostermann H.** Successful salvage treatment of chronic refractory immune thrombocytopenia using a simplified method to generate vincristine-loaded platelets. Thromb Haemost 2008, **100**, 705-707.
14. **Williams DA, Maggio-Price L.** Canine idiopathic thrombocytopenia: clinical observations and long-term follow-up in 54 cases. J Am Vet Med Assoc 1984, **185**, 660-663.