J Anim Reprod Biotechnol 2024;39:58-61
pISSN: 2671-4639 • eISSN: 2671-4663
https://doi.org/10.12750/JARB.39.1.58

Clinical Report

Stem cell therapy for dogs with immune-mediated hemolytic anemia

Yeonmi Lee[1], Eunju Kang[1] and Junguk Ju[2,3,*]

[1]Department of Biomedical Science, College of Life Science, CHA University, Seongnam 13488, Korea
[2]AniCom Therapeutics Inc., Seoul 04599, Korea
[3]AniCom Medical Center, Animal Hospital, Seoul 04599, Korea

Received November 7, 2023
Revised November 17, 2023
Accepted November 19, 2023

***Correspondence**
Junguk Ju
E-mail: fire0809@hanmail.net

Author's Position and Orcid no.
Lee Y, Senior research scientist,
https://orcid.org/0000-0003-4407-7731
Kang E, Associate professor,
https://orcid.org/0000-0002-7240-7868
Ju J, DVM, MS, President,
https://orcid.org/0000-0002-9442-1548

ABSTRACT Three different dogs who had immune-mediated hemolytic anemia (IMHA) were treated for more than two weeks with blood transfusion in an animal clinic. Despite this treatment and hospitalization, there was no clinical improvement in clinical signs as well as complete blood cell count (CBC) including hematocrit (HCT) and C-reactive protein (CRP). All cases were then injected two or three times with allogeneic stem cells through an intravenous route for treatment. Upon administrating stem cells to the IMHA dogs, clinical conditions and the indexes of HCT and CRP were clinically improved within or close to normal ranges.

Keywords: hemolysis, immune-mediated hemolytic anemia, stem cells

INTRODUCTION

Immune-mediated hemolytic anemia (IMHA) is a condition characterized by the destruction of red blood cells (hemolysis) due to an abnormal immune response. In this condition, the immune system mistakenly identifies the body's red blood cells as foreign and attacks them, leading to their destruction. The clinical signs of IMHA are severe anemia and inflammatory response (Balch and Mackin, 2007) including lethargy, anorexia, pale mucous membranes, collapse, and vomiting (Assenmacher et al., 2019). The classic treatment for IMHA is to use immunosuppressive and antithrombotic drugs to prevent clinical signs associated with anemia (Swann et al., 2019). However, these traditional methods have a lot of adverse effects and limitations from a fundamental point of view. In recent studies, stem cells have been suggested as a new effective treatment option for IMHA. Stem cells have a therapeutic potential by modulating immunomodulatory actions and it could be an additional option or only treatment (Garner, 2022). In this study, we described the therapeutic effects of amnionic membrane-derived mesenchymal stem cells in dogs with IMHA.

CASE

The first case, an unknown dog (case 1), was diagnosed with IMHA with anemia (hematocrit, HCT; 19.13%) and inflammation (C-reactive protein, CRP; 69 mg/dL). A 5-year-old female Maltese dog (case 2) was 2.5 kg and diagnosed IMHA with anemia (HCT; 18.8%) and inflammation (CRP; 84 mg/dL). A 10-year-old neutered male maltipoo dog (case 3) was 7.2 kg and diagnosed IMHA with anemia (HCT; 19.8%) and inflammation (CRP; 59 mg/dL). There were no clinical improvements although continuous blood transfusions and hospitalization before

the stem cell injection. Actually, all patients showed lethargy and anorexia (Table 1).

All dogs were treated at the Anicom Medical Center (Seoul, Korea) for stem cell therapy. Physical examination, complete blood count (CBC), and serum biochemistry should be obtained prior to stem cell injection. The dogs were premedicated with ampicillin and antihistamines. Allogeneic mesenchymal stem cells were infused intravenously for the treatment of IMHA. 1×10^6 cells/kg were resuspended in normal saline with 200 IU heparin sulfate and administered intravenously with a syringe pump operating 10 mL/kg/hr. The dose was based on previous studies and proved not to be associated with adverse events as well (Kim et al., 2021; Noh et al., 2021). The patient appeared clinically normal and stable during the infusions and immediately after.

Patients received stem cell therapy two or three times through the same procedure (case 1: 3 times injections on days 0, 10, and 21; case 2: 3 times injections on days 0, 2, and 7; case 3: 2 times injections at day 0 and 3). All dogs had better conditions and vital signs, and no side effects were found during the second treatment. After three times of stem cell therapy, the patient's body condition almost returned to normal.

Table 1. Information list of patients before stem cell transplantation

Case	Age	Breed	Weight (kg)	Symptom	Hematologic levels	
					HCT (%)	CRP (mg/dL)
1	–	–	–	Lethargy, Anorexia	19.13	69.00
2	5	Maltese	2.50	Lethargy, Anorexia, Pale Mucous Membranes	18.80	84.00
3	10	Maltipoo	7.20	Lethargy, Anorexia, Vomiting	19.80	59.00

HCT, hematocrit; CRP, C-reactive protein.

The HCT level of Case 1 was 19.13% 17 days before cell transplantation, and it decreased to 14.51% before the first cell treatment on day 0 (Fig. 1A and Table 2). After the first stem cell treatment (day 10), it increased to 35.30%. However, after the second (D21) and third stem cell treatment (D26), it decreased from 23.79% to 17.03%. Finally, it rose again to 47.00% and reached the normal range on day 85 after cell transplantation. The CRP level was 69.00 mg/dL 17 days before cell transplantation, however, the CRP level was not measured after stem cell treatment in this case (Fig. 1B and Table 2).

The HCT level of Case 2 was 18.80% 18 days before cell transplantation (Fig. 1A and Table 2). Before the first cell treatment (day 0), the HCT level was 22.52%, it increased from 27.91% to 28.22% after the first and second stem cell treatment. After the third stem cell treatment, it dramatically elevated to 42.10%, being similar to the normal range. The CRP level was 84.00 mg/dL 18 days before cell transplantation and it decreased to 6.00 mg/dL within the normal range before the first cell treatment on day 0 (Fig. 1B and Table 2). After the first and second stem cell treatments, CRP level was maintained within the normal range (3.00 mg/dL).

The HCT levels of Case 3 were 19.80% and 25.21% 9 days before cell transplantation and on the day of the first cell treatment (day 0), respectively (Fig. 1A and Table 2). After the first and second stem cell treatments, the level increased from 31.29% to 44.13%, approaching the normal

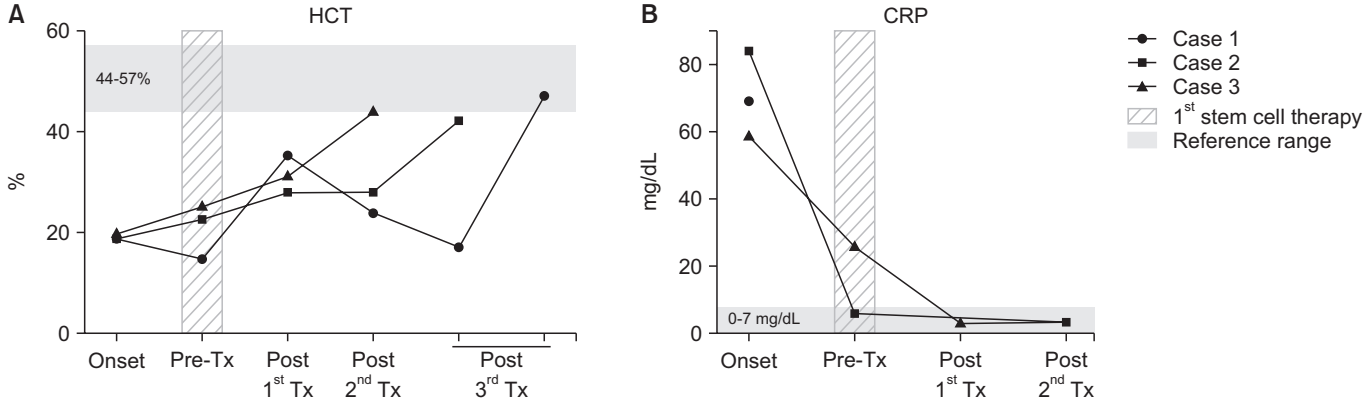

Fig. 1. HCT (A) and CRP (B) levels at pre and post cell transplantation in 3 dogs with IMHA. HCT and CRP levels were within or similar to normal ranges following cell transplantation. Tx, transplantation.

Table 2. Hematologic levels associated in 3 dogs with IMHA

Index	HCT	WBC	Globulin	GOT	GPT	ALP	CRP
Case1							
Onset (-D17)	19.13	14.49	-	70.00	96.00	1029.00	69.00
Pre-Tx (D0)	14.51	8.60	-	130.00	405.00	3500.00	-
Post 1st Tx (D10)	35.30	18.48	-	211.00	1000.00	3500.00	-
Post 2nd Tx (D21)	23.79	39.19	-	-	-	-	-
Post 3rd Tx (D26)	17.03	18.59	-	206.00	1000.00	3500.00	-
Post 3rd Tx (D85)	47.00	-	-	-	155.00	1382.00	-
Case2							
Onset (-D18)	18.80	16.37	-	18.00	183.00	308.00	84.00
Pre-Tx (D0)	22.52	17.65	-	26.00	409.00	784.00	6.00
Post 1st Tx (D2)	27.91	15.84	-	25.00	307.00	630.00	-
Post 2nd Tx (D7)	28.22	13.74	-	29.00	202.00	634.00	3.00
Post 3rd Tx (D42)	42.10	16.96	-	-	-	-	-
Case3							
Onset (-D9)	19.80	41.42	-	-	-	-	59.00
Pre-Tx (D0)	25.21	45.30	-	-	-	-	26.00
Post 1st Tx (D3)	31.29	36.68	2.50	-	-	-	3.00
Post 2nd Tx (D15)	44.13	26.05	-	-	-	-	3.00

Tx, transplantation; HCT, hematocrit; WBC, white blood cells; GOT, glutamic oxaloacetic transaminase; GPT, glutamine pyruvic transaminase; ALP, alkaline phosphatase; CRP, C-reactive protein. Onset indicates 9 to 17 days prior to the first cell transplantation. Pre-Tx means the day of the first stem cell treatment, occurring before the actual cell transplantation takes place.

range. Further, the CRP level was 59.00 mg/dL 9 days before cell transplantation and it decreased to 26.00 mg/dL before the first cell treatment on day 0 (Fig. 1B and Table 2). After the first and second cell treatment, the CRP level decreased to 3 mg/dL, reaching the normal range.

Based on the results, stem cell therapy in dogs with IMHA could improve the hematological level, such as HCT and CRP, and the clinical signs.

DISCUSSION

IMHA is a common type of anemia in dogs and is more common in middle-aged and females. Clinical symptoms include severe anemia and inflammation (Balch and Mackin, 2007). In addition, the history of dogs affected by IMHA is lethargy, anorexia, pallor, jaundice, vomiting, collapse, and weakness. Also, physical examination findings include systolic heart murmur, fever, tachycardia, rapid breathing, hepatomegaly, and abdominal pain (Sharp and Kerl, 2008).

Although treatment with a variety of immunosuppressive and antithrombotic agents is traditionally used, there is still no complete consensus among veterinarians on the optimal regimen for treatment and maintenance after diagnosis of the disease (Swann et al., 2019). Blood transfusions, corticosteroids, azathioprine, cyclosporine, meclofenoxate, and leflunomide are frequently used treatment for IMHA currently. Unfortunately, only about one-third of these treatments appear to be curative, while another one-third do not lead to recovery or result in death from uncontrolled and rapid hemolysis (Garner, 2022).

Recently, many studies have researched the efficacy of stem cells in the treatment of IMHA. Among them, mesenchymal stem cells show great promise for the treatment of these diseases. Effective immunomodulation can aid in the treatment and prevention of autoimmune diseases. It has been described that IMHA can be treated with allogeneic mesenchymal stem cells. These stem cell treatments can be an addition to traditional treatment for IMHA and can be the only treatment available when current therapies can not be applied (Garner, 2022).

The amniotic membrane serves as a valuable source for obtaining mesenchymal stem cells. Mesenchymal stem cells derived from the amniotic membrane exhibit comparable characteristics to those isolated from different sources, which expressed specific CD markers associated with mesenchymal stem cells and differentiated into mesenchymal lineages, including adipogenesis, osteogenesis,

and chondrogenesis (Dizaji et al., 2017; Ma el al., 2019). Further, the mesenchymal stem cells derived from the amniotic membrane could possess the ability to robustly suppress immune responses and exhibit immunomodulatory effects in preclinical animal models (Insausti et al., 2014). Finally, the amniotic membrane has the advantage of being easily obtained through a non-invasive method, enabling the isolation of a large number due to abundant tissue from a single pregnant animal.

In this case, intravenous injection of allogeneic amniotic membrane-derived mesenchymal stem cells was used for improvement of IMHA, especially, clinical signs and blood tests for CBC including HCT and CRP. The absence of consecutive blood tests immediately especially for CRP and applications of different stem cell treatments are the limitations of this case. Despite the limitations of this case report, it suggested that allogeneic stem cell therapy was not associated with adverse effects and may benefit patients already undergoing conservative medical treatment for IMHA.

CONCLUSION

In conclusion, stem cell therapy in dogs with IMHA could help to increase HCT decrease CRP, and improve clinical signs. It is thought that the potential application of IMHA treatment as a stem cell therapy might be promising, helping to maintain health from IMHA. However, large-scale clinical trials are required to be accepted as innovative treatments for IMHA.

Author Contributions: Conceptualization, E.K., J.J.; methodology, J.J.; investigation, J.J.; data curation, J.J.; writing—original draft preparation, J.J.; writing—review and editing, Y.L., E.K., J.J.; supervision, E.K., J.J.; project administration, J.J.; funding acquisition, J.J.

Funding: None.

Ethical Approval: Not applicable.

Consent to Participate: Not applicable.

Consent to Publish: Not applicable.

Availability of Data and Materials: Not applicable.

Acknowledgements: None.

Conflicts of Interest: No potential conflict of interest relevant to this article was reported.

REFERENCES

Assenmacher TD, Jutkowitz LA, Koenigshof AM, de A Lucidi C, Scott MA. 2019. Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases (2004-2013). J. Am. Vet. Med. Assoc. 255:366-376.

Balch A and Mackin A. 2007. Canine immune-mediated hemolytic anemia: pathophysiology, clinical signs, and diagnosis. Compend. Contin. Educ. Vet. 29:217-225.

Dizaji Asl K, Shafaei H, Soleimani Rad J, Nozad HO. 2017. Comparison of characteristics of human amniotic membrane and human adipose tissue derived mesenchymal stem cells. World J. Plast. Surg. 6:33-39.

Garner SD. 2022. Allogenic mesenchymal stem cell therapy for immune mediated cytopenias – a 22 case series. Safari Veterinary Care Centers. https://stemcellsafari.com/clinical-reports/22-cases-of-imha-review

Insausti CL, Blanquer M, García-Hernández AM, Castellanos G, Moraleda JM. 2014. Amniotic membrane-derived stem cells: immunomodulatory properties and potential clinical application. Stem Cells Cloning 7:53-63.

Kim T, Noh SA, Ju J. 2021. Case report of amniotic membrane derived-stem cells treatment for feline chronic obstructive pulmonary disease. J. Anim. Reprod. Biotechnol. 36:323-326.

Ma J, Wu J, Han L, Jiang X, Yan L, Hao J, Wang H. 2019. Comparative analysis of mesenchymal stem cells derived from amniotic membrane, umbilical cord, and chorionic plate under serum-free condition. Stem Cell Res. Ther. 10:19.

Noh SA, Kim T, Ju J. 2021. Case reports of amniotic membrane derived-cell treatment for feline chronic renal failure. J. Anim. Reprod. Biotechnol. 36:116-120.

Sharp C and Kerl ME. 2008. Immune-mediated hemolytic anemia. Stand. Care Emerg. Crit. Care Med. 10:1-6.

Swann JW, Garden OA, Fellman CL, Glanemann B, Goggs R, LeVine DN, Mackin AJ, Whitley NT. 2019. ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs. J. Vet. Intern. Med. 33:1141-1172.