DOI:10.22059/IJVM.2024.375273.1005555 **Iranian Journal of Veterinary Medicine** **Original Article**

Online ISSN: 2252-0554

Successful Treatment of Feline Infectious Peritonitis (FIP) with Mesenchymal Stem Cells

Running title: **Treatment of FIP with Stem Cells**

Samira Mohamadian[1,4] ,Pejman Kazerooni[1,2] ,Ali Taheri Mirghaed[3] ,Mahsa Kia[4] ,Minoo Soltani [5] ,Ali Akbaripazouki[6] ,Saman Ahani[7] ,Pouria Asgari[8]

[1] Department of Internal Medicine, Pouya Pet Clinic, Tehran, Iran

[2]Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ahwaz, Khuzestan, Iran

[3] Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[4] Stem Cell Research Technical Department, Treata Caspian Parsian Company, Tehran, Iran

[5] Rastegar Reference Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[6] Department of Cell and Molecular Biology, Faculty of Biology, University of Tehran, Tehran, Iran

[7] Department of Diagnostic Imaging, Faculty of Veterinary Medicine, Islamic Azad University, Tehran, Iran

[8] Department of Clinical Science, Faculty of Veterinary Medicine, Islamic Azad University, Urmia, Iran

Abstract

Feline infectious peritonitis caused by Feline Coronavirus is a common disease leading to a cytokine storm and causes organs failure with high mortality rate in feline patients. This first case report is gained by treatment of three cats with feline infectious peritonitis by using allogeneic bone marrow mesenchymal stem cells.

Our aim was to evaluate the effectiveness of cell therapy in the mentioned disease in a shorter period of time with higher efficiency.

Infected cats received five doses of bone marrow stem cells through intravenous infusion. During the treatment period, the patients were kept in an isolated place and their clinical conditions were evaluated under the supervision of an internal specialist.

This treatment resulted in full recovery of all cats within 21 days of treatment. One cat re-infected two months later and two cats remained in remission at the time of writing this report.

This case report suggests the effectiveness of using bone marrow mesenchymal stem cells therapy in treatment of feline infectious peritonitis.

Keywords: Allogeneic, Bone morrow, Cytokine storm, Mesenchymal, Stem cells

Case history

Clinical Presentation History

Three female domestic shorthaired cats with the average age of 9 months brought to Pouya Pet Clinic with a complaint of lethargy. Other history was recurrent fever, weight loss and anorexia. All cats were born in the street and had contact with feral cats. The condition of the animals at the time of presentation is shown in table1.

Neurological and ocular signs including decreased postural reflexes and ocular opacities were seen in one cat (Table 1).

History of Feline Infectious Peritonitis

In 1963, feline infectious peritonitis (FIP) was identified as a specific disease in cat which is caused by mutant feline coronavirus (FCoV) (Holzworth, 1963; Poland *et al*., 1996; Vennema *et al*., 1998; Pedersen *et al*., 2008). FIP can be categorized in two forms including effusive (wet), with accumulation of inflammatory fluid in the abdominal and thoracic cavities, and non-effusive (dry) which is characterized by necrotizing vasculitis and multifocal pyogranulomatous inflammation in various organs-such as the abdominal viscera, eyes, central nervous system, lungs and also virus-induced lymphopenia (Kennedy *et al*., 2020; Tasker *et al*., 2023). One of the outstanding features of FIP, is lymphopenia along with apoptosis of T cells which is associated with cytokines secretion by the virus-infected macrophages and other immune cells. Continuous replication of the virus causes an inflammatory response in FIP which case multiorgans failure which currently, there is no effective licensed treatment for this disease (Dean *et al*., 2003; Takano *et al*., 2007; Tasker *et al*., 2023).

According to the recent studies, there are similarities and differences between the Covid-19 and FIP. These two diseases are different in terms of some pathogenic, pathological and clinical features. However, some similar pathogenic and immunogenic events have been found in both of them. In addition, it has been shown that the drug used such as GS-441524, which is the active form of Remdesivir and is recommended in the control of the Covid-19, has also shown good responses in the FIP treatment through controlling the viruses replication (Bearden *et al*., 2017; Decaro and Lorusso, 2020; Jiang *et al*., 2020;

Mavian *et al*., 2020; Sironi *et al*., 2020; Tiwari *et al*., 2020; Wong *et al*., 2020; Malaiyan *et al*., 2021). Since cytokine storm are found in FIP, it is essential to consider a combination of imunmodulatory agent for treating cytokine storm and antiviral in inhibiting the virus replication. Mesenchymal stem cells (MSCs) have more advantages than many other anti-inflammatory agents because in addition to regenerative properties, they have immunomodulatory effects in the host cells of both the innate and the adaptive immune system. MSCs by secreting cytokines and modulating the immune response regulate cell function and down regulate the inflammatory cytokines (Iyer *et al*., 2020; Senegaglia *et al*., 2021). We hypothesized that stem cells in the body can simultaneously control the inflammation caused by the virus, prevent its proliferation and repair the damaged tissues.

To the author's knowledge, there have been no reports using of BW-MSCs in successful treatment of FIP.

Diagnostic Testing

Feline infectious peritonitis was diagnosed by a combination of clinical signs (weight loss, recurrent fever, ascites, decrease appetite and anemia) as well as laboratory tests including blood analysis, Rivalta test (effusive), and RT-PCR. Radiologic and ultrasonographic studies of the thoracic and abdominal cavity were also performed to exclude other factors leading to ascites, such as cardiac failure, incarcerated hernias, or neoplastic masses. According to the results of radiology and ultrasound, free fluid was seen

around the abdomen in two cases (effusive) which interpretation ascites (figure1). No free fluid was seen in one case (none-effusive case) and finding indicated hepatomegaly (figure2).

In all cases, the A/G ratio was low (with mean of 0.46) and total protein was high (with mean of 9.6 g/dl). Two cats had severe anemia considering Hgb and HCT levels (Table 2).

Treatments Performed

Paracentesis was performed in cases with ascites, during which a needle was inserted into the peritoneal cavity to obtain ascitic fluid by surgeon on the first day.

Frozen Bone morrow stem cells were provided from Treatacaspian Co stem cells bank with number of 10^6 stem cells. For each dose administration, one vial was removed from the azoth tank. The outside of vials was wiped with 70% ethanol, then the cells were quickly thawed in 37°C water bath by gently swirling the vials. 20 µL aliquot of cells was mixed with 20 µL of the medium and then stained by Trypan Blue for cell counting. The remaining cell suspension were transferred to conical tube using a pipette and rinsed with 1 ml medium (DEMEM high glucose) by centrifuging the cell suspension at 300 x *g* for 10 minutes at room temperature. The cells pellet resuspended by gently flicking the tube and then 5 ml of medium was added to conical tube (Bearden *et al*., 2017; Zhang *et al*., 2018). All patients received BM-MSCs at days 1, 4, 7, 14 and 21 with dosage of $1x10^6$ in 15 ml of normal saline and injected

intravenously during one hour (Webb *et al*., 2013). Any reaction following the medical administration, such as skin rash or loss of appetite, were considered and noted in table 3.

All treated cats underwent physical examination, blood work consisting of CBC and serum biochemistry each week. (Table 2). Three days after the first treatment, the appetite was regained. Also, the lethargy and fever were regressed in all cats. The blood sample and radiological studies were done to follow the condition of the cats after recovery. The blood factors in all cats showed normal level.

After the period of 21 days of treatment, no detectable ascites and abnormality in organs were seen on sonography and radiography pictures (figure1, 2).

At the follow-up, two months after the treatment, two cats had remained healthy, showing no signs of relapse but one cat was reinfected. It had come in contact with a severely diseased cat. In this stage, the continuation of the treatment with Remdesivir with the dose of with dose of 30mg/kg went ahead, but was not successful and recovery was not gained. The outcomes of this report are summarized in Tables1, 2 and 3.

The results clearly revealed that BM-MSCs showed a striking efficacy and treated cats responded to the treatment with rapid improvement of clinical signs. In this report, all cats

achieved remission after 21 days of treatment. The stem cells with Follow-up of the cats in our report is currently ongoing and more cats with FIP are being treated using this method.

Assessments

The main reason of the progression of FIP in patients are the immune abnormalities. When Corona viruses invading the body, it will rapidly replicate and triggering the immune system for releasing inflammatory cells and antibodies (Akkoc, 2020; Addie *et al*., 2020). It leads the immune regulatory network to unbalance, which resulted the release of a large number of inflammatory cytokines and cytokine storm syndrome. MSCs have immunoregulatory abilities which can regulate both the adaptive and innate immune system (Pinky *et al*., 2021; Guy *et al*., 2021; Chen *et al*., 2022). This ability may make them the most promising cell-based therapy for FIP.

In addition to immunomodulatory and anti-inflammatory effects of MSCs, these cells also can aid to repair the damaged tissues, and in this way can significantly reduce the number of damaged organs associated with FIP (Salari *et al*., 2020). Liver is one of the organ which is affected during FIP. Since Remdesivir and GS-441524 are used as a choice of FIP treatment, will be metabolized in the liver and could worsen the liver function or even it may not be possible to

continue the treatment (Grein *et al*., 2020; Wang *et al*., 2020). So it is necessary to use a component to repair the damaged tissues during the treatment. Regenerative properties of stem cells along with their immunomodulatory property would make it as the best option in FIP treatment with the minimum side effects.

In our cell therapy results, the duration of treatment is shorter (21 days) compared to the duration of treatment with Remdesivir (84 days) (Murphy *et al*., 2018; Pedersen *et al*., 2019). Since there is no access to oral Remdesivir/GS-441524 for animals in the country under study, the need to visit the patient's case daily to receive injectable medicine is eliminated. And since the majority of the infected cases are found among the street cats, if the shortest treatment period can be introduced, more cases will have a chance to be cured. On the other hand, the costs related to treatment in this way are much lower compared to the aforementioned treatment, which will be considered a positive factor of cell therapy.

The small number of studies of using MSCs products in cats, reveal that this treatment is mostly still in the discovery phase. Cell sources, administration routes and dosages can have different results. In current report, bone marrow stem cells were used for the first time in the treatment of FIP disease. Despite the failure of adipose stem cells in FIP treatment in some reports, this type

of stem cells with the recommended dose and method of administration has been able us to achieve he positive results in treatment of FIP in cats.

Conclusion

The results showed that the stem cells had the ability to cure the disease by controlling the inflammation and proliferation of the virus, as well as repairing the damaged tissues caused by the virus in the animal's body. This makes this treatment especially superior to other routine treatments. In order to check the long-term effectiveness of stem cells, more cases are needed.

REFERENCES

1. Addie, DD., Curran, S., Bellini, F. (2020) Oral Mutian®X stopped faecal feline coronavirus shedding by naturally infected cats. Research in *Veterinary Science*., 130, 222–229. https://doi.org/10.1016/j.rvsc.2020.02.012. PMID: 32220667.

2. Akkoc, T. (2020) COVID-19 and mesenchymal stem cell treatment; mystery or no. *Advances in Experimental Medicine and Biology*., 1298, 1–10. https://doi.org/10.1007/5584_2020_557. PMID: 34158414.

3. Bearden, RN., Huggins, SS., Cummings, KJ., Smith, R., Gregory, CA. (2017) Saunders WB. In-vitro characterization of canine multipotent stromal cells isolated from synovium, bone marrow, and adipose tissue: a donor-matched comparative study. *Stem cell research and therapy*., 8, 218. https://doi.org/10.1186/s13287-017-0639-6. PMID: 28974260.

4. Chen, L., Qu, J., Kalyani, FS., Zhang, Q., Fan, L., Fang, Y., Li, Y., Xiang, C. (2022) Mesenchymal stem cell-based treatments for COVID-19: status and future perspectives for clinical applications. *Cellular and Molecular Life Sciences*., 79(3), 142. https://doi.org/10.1007/s00018-021-04096-y. PMID: 35187617.

5. Decaro, N., Lorusso, A. (2020) Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. *Veterinary Microbiology*., 244, 108693. https://doi.org/10.1016/j.vetmic.2020.108693. PMID: 32402329.

6. Grein, J., Ohmagari, N., Shin. (2020) Compassionate use of remdesivir for patients with severe Covid-19. *The New England Journal of Medicine*. 382(24):2327–2336. doi: 10.1056/NEJMoa2007016.

7. Guy, L., Yuan, Z., Jia-Xian, W., Yat-Ping, T. (2021) Targeting mesenchymal stem cell therapy for severe pneumonia patients. *World Journal Stem Cells*., 13(2), 139–154. https://doi.org/10.4252/wjsc.v13.i2.139. PMID: 33708343.

8. Holzworth, J. (1963) Some important disorders of cats. Cornell Veterinary. 53, 157–160. PMID: 13961523.

9. Iyer, M., Jayaramayya, K., Subramaniam, MD. (2020) COVID-19: an update on diagnostic and therapeutic approach. *Journal of Biochemistry and Molecular Biology*., 53(4), 191–205. https://doi.org/ 10.5483/BMBRep.

10. Kennedy, MA. (2020) Feline Infectious Peritonitis: Update on Pathogenesis, Diagnostics, and Treatment. The Veterinary Clinic of North America. *Small Animal Practice*., 50(5), 1001-1011. https://doi.org/10.1016/j.cvsm.2020.05.002. PMID: 32563530.

11. Liu, P., Jiang, J Z., Wan, X F., Hua, Y., Li, L., Zhou, J., Wang, X., Hou, F., Chen, J., Zou, J., Chen, J. (2020) Are pangolins the intermediate host of the 2019 novel coronavirus (SARS-CoV-2) *PLoS Pathology*., 16(5), e1008421. https://doi.org/10.1371/journal.ppat.1008421. PMID: 32407364.

12. Malaiyan, J., Arumugam, S., Mohan, K., Gomathi, RG. (2021) An update on the origin of SARS-CoV-2: Despite closest identity, bat (RaTG13) and pangolin derived coronaviruses varied in the critical binding site and O-linked glycan residues. *Journal of Medical Virology*., 93(1), 499-505. https://doi.org/10.1002/jmv.26261. PMID: 32633815.

13. Mavian, C., Pond, SK., Marini, S., Magalis, BR., Vandamme, AM., Dellicour, S., Scarpino, SV., Houldcroft, C., Villabona-Arenas, J., Paisie, TK., Trovão, NS., Boucher, C., Zhang, Y., Scheuermann, RH., Gascuel, O., Lam, TT., Suchard, MA., Abecasis, A., Wilkinson, E., Salemi, M. (2020) Sampling bias and incorrect rooting make phylogenetic network tracing of SARS-COV-2 infections unreliable. *Proceedings of the National Academy of Science of the United States of America*., 117, 12522–12523. https://doi.org/10.1073/pnas.2007295117. PMID: 32381734.

14.Murphy, BG., Perron, M., Murakami, E. (2018) The nucleoside analog GS-441524 strongly inhibits feline infectious peritonitis (FIP) virus in tissue culture and experimental cat infection studies. Vetetirany Microbiol., 219: 226–233.

15. Pedersen, NC., Allen, CE., Lyons, LA. (2008) Pathogenesis of feline enteric coronavirus infection. *Journal of Feline Medicine and Surgery*., 10, 529–541. https://doi.org/10.1016/j.jfms.2008.02.006. PMID: 18538604.

16.Pedersen, NC., Perron, M., Bannasch, M. (2019) Efficacy and safety of the nucleoside analog GS-441524 for treatment of cats with naturally occurring feline infectious peritonitis. J Feline Med Surg., 21: 271–281.

17. Pinky, A., Gupta, S., Krishnakumar, V. (2021). Mesenchymal stem cell derived exosomes: a nano platform for therapeutics and drug delivery in combating COVID-1. *Stem Cell Reviews and Reports*., 17(1), 33-43. https://doi.org/10.1007/s12015-020-10002-z. PMID: 32661867.

18. Salari, V., Mengoni, F., Del Gallo, F., Bertini, G., Fabene, PF. (2020) The Anti-Inflammatory Properties of Mesenchymal Stem Cells in Epilepsy: Possible Treatments and Future Perspectives. *International Journal of Molecular Sciences*., 21(24), 9683. https://doi.org/10.3390/ijms21249683. PMID: 33353235.

19. Senegaglia, AC., Rebelatto, CLK., Franck, CL., Lima, JS., Boldrini-Leite, LM., Daga, DR. (2021) Combined use of tocilizumab and mesenchymal stromal cells in the treatment of severe covid-19: case report. *Cell Transplant*., 30, 1-11. https://doi.org/10.1177/09636897211021008.

20. Sironi, M., Hasnain, S. E., Rosenthal, B., Phan, T., Luciani, F., Shaw, MA., Sallum, MA., Mirhashemi, ME., Morand, S., González-Candelas, F. (2020). SARS-CoV-2 and COVID-19: A genetic, epidemiological, and evolutionary perspective. *Infection, Genetics and Evolution*., 84, 104384. https://doi.org/10.1016/j.meegid.2020.104384. PMID: 32473976.

21. Takano, T., Hohdatsu, T., Hashida, Y. (2007) A "possible" involvement of TNF-alpha in apoptosis induction in peripheral blood lymphocytes of cats with feline infectious peritonitis. *Veterinary Microbiology*., 119, 121–31. https://doi.org/10.1016/j.vetmic.2006.08.033. PMID: 31118053.

22. Tasker, S., Addie, DD., Egberink, H., Hofmann-Lehmann, R., Hosie, MJ., Truyen, U., Belák, S., Boucraut-Baralon, C., Frymus, T., Lloret, A., Marsilio, F., Pennisi, MG., Thiry, E., Möstl, K., Hartmann K. (2023) Feline Infectious Peritonitis: European Advisory Board on Cat Diseases Guidelines. *Viruses*., 15(9), 1847. https://doi.org/10.3390/v15091847. PMID: 37766254.

23. Tiwari, M., Mishra, D. (2020) Investigating the genomic landscape of novel coronavirus (2019-nCoV) to identify non-synonymous mutations for use in diagnosis and drug design. *Journal of Clinical Virology*., 128, 10444. https://doi.org/10.1016/j.jcv.2020.104441. PMID: 32425659.

23. Wang, Y., Zhang, D., Du, G. (2020) Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 395(10236):1569–1578. doi: 10.1016/S0140-6736(20)31022-9.

24. Wong, G., Bi, Y H., Wang, QH., Chen, XW., Zhang, ZG., Yao, YG. (2020) Zoonotic origins of human coronavirus 2019 (HCoV-19/SARS-CoV-2): Why is this work important. *Zoological Research*., 18, 213–219. https://doi.org/10.24272/j.issn.2095-8137.2020.031. PMID: 32314559.

درمان موفقیت‌آمیز پریتونیت عفونی گربه‌سانان (FIP) با استفاده از سلول‌های بنیادی مزانشیمی

سمیرا محمدیان[4،1] پژمان کازرونی[2،1] علی طاهری میرقائد[3] مهسا کیا[4] مینو سلطانی[5] علی اکبری‌پازوکی[6] سامان آهنی[7] پوریا عسگری[8]

[1]بخش داخلی، کلینیک دامپزشکی پویا، تهران، ایران
[2] گروه داخلی، دانشکده دامپزشکی، دانشگاه اهواز، خوزستان، ایران
[3]گروه آبزیان، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران
[4] بخش فنی تحقیقات سلول های بنیادی، شرکت تریتا کاسپین پارسیان، تهران، ایران
[5] بخش آزمایشگاه مرجع رستگار، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران
[6]گروه سلولی و مولکولی، دانشکده زیست شناسی، دانشگاه تهران، تهران، ایران
[7]گروه تصویربرداری تشخیصی، دانشکده دامپزشکی، دانشگاه آزاد اسلامی، تهران، ایران
[8]گروه علوم بالینی، دانشکده دامپزشکی، دانشگاه آزاد اسلامی، ارومیه، ایران

چکیده

پریتونیت عفونی گربه‌سانان ناشی از کروناویروس گربه و یک بیماری شایع است که منجر به طوفان سیتوکین و نارسایی اندام‌ها با میزان مرگ و میر بالا در گربه‌های بیمار می‌شود. مطالعه حاضر اولین گزارش موردی در درمان سه گربه با بیماری پریتونیت عفونی گربه‌سانان با استفاده از سلول‌های بنیادی مزانشیمی آلوژنیک مغز استخوان می‌باشد.

هدف این مطالعه ارزیابی اثربخشی سلول درمانی در بیماری مذکور در بازه زمانی کوتاه تر و راندمان بالاتر بوده‌است.

گربه‌های آلوده 5 دوز سلول‌های بنیادی مغز استخوان را از طریق انفوزیون داخل وریدی دریافت کردند. در طول دوره درمان بیماران در یک مکان ایزوله نگهداری شدند و شرایط بالینی آنها تحت نظر متخصص داخلی مورد بررسی قرار گرفت.

این درمان منجر به بهبودی کامل همه گربه‌ها در مدت زمان 21 روز شد. در بین گربه‌های تحت درمان، یک گربه دو ماه بعد از اتمام درمان دوباره آلوده شد و دو گربه دیگر در زمان نوشتن این گزارش در سلامت کامل به سر می‌برند.

این گزارش موردی نشان‌دهنده اثربخشی سلول های بنیادی مزانشیمی مغز استخوان در درمان پریتونیت عفونی گربه‌سانان می‌باشد.

کلید واژه ها: آلوژنیک، مغز استخوان، طوفان سایتوکین، مزانشیمی، سلول‌های بنیادی