Case Report

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

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ABSTRACT

Feline infectious peritonitis (FIP) caused by feline coronavirus (FCoV) is a common disease that leads to a cytokine storm and causes organ failure, with a high mortality rate in feline patients. This is the first case report on the detailed treatment of three cats with FIPs using allogeneic bone marrow mesenchymal stem cells. We aimed to evaluate the effectiveness of cell therapy in this disease in a shorter period with greater efficiency. Infected cats received five doses of bone marrow stem cells through intravenous infusion. During the treatment period, the subjects were kept in an isolated place and their clinical conditions were evaluated under the supervision of an internal specialist. This treatment resulted in the full recovery of all cats within 21 days. One cat was re-infected two months later after exposure to an infected cat, while two cats remained in remission at the time of writing this report. This case report suggests the effectiveness of using bone marrow mesenchymal stem cell therapy in the treatment of FIPs.

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

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Case History

History of feline infectious peritonitis (FIP)

In 1963, FIP was identified as a specific disease in cat, caused by a mutant feline coronavirus (FCoV) (Holzworth, 1963; Poland et al., 1996; Vennema et al., 1998; Pedersen et al., 2008). FIP can be categorized into two forms, including effusive (wet), characterized by 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, and lungs—along with virus-induced lymphopenia (Kennedy et al., 2020; Tasker et al., 2023). One of the outstanding features of FIP is lymphopenia, along with the apoptosis of T cells, which is associated with cytokine secretion by the virus-infected macrophages and other immune cells. The continuous replication of the virus causes an inflammatory response in FIP, leading to multiorgan failure. Currently, there is no effective licensed treatment for this disease (Takano et al., 2007; Tasker et al., 2023).

According to recent studies, there are similarities and differences between 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 conditions. In addition, the drug GS-441524, which is the active form of remdesivir and is recommended for the treatment of COVID-19, has also demonstrated good efficacy in treating FIP by controlling viral replication (Bearden et al., 2017; Decaro & 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 storms are found in FIP, it is essential to consider a combination of immunomodulatory agents for treating cytokine storms and antiviral agents for inhibiting viral replication. Mesenchymal stem cells (MSCs) offer more advantages than many other anti-inflammatory agents because, in addition to their regenerative properties, they have immunomodulatory effects on both the innate and adaptive immune systems. MSCs regulate cell function and downregulate inflammatory cytokines by secreting cytokines and modulating the immune response (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 authors' knowledge, there have been no reports of the successful use of BW-MSCs in the treatment of FIP.

Clinical Presentation

Three female domestic shorthaired cats with an average age of nine months were brought to Pouya Pet Clinic with a complaint of lethargy. Other reported symptoms were recurrent fever, weight loss, and anorexia. All cats were born on the street and had contact with feral cats. The condition of the animals at the time of presentation is shown in Table 1.

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

Diagnostic Testing

FIP was diagnosed by a combination of clinical signs (weight loss, recurrent fever, ascites, decreased appetite, and anemia) and laboratory tests, including blood analysis, the Rivalta test (for effusive cases), and reverse transcription-polymerase chain reaction (RT-PCR). Radiologic and ultrasonographic studies of the thoracic and abdominal cavities 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 observed in the abdomen in two cases (effusive), which was interpreted as ascites (Figure 1). No free fluid was observed in one case (none-effusive), and the finding indicated hepatomegaly (Figure 2).

In all cases, the A/G ratio was low (with a mean of 0.46), and total protein was high (with a 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 the surgeon on the first day.

Frozen bone marrow stem cells (BM-MSCs) were provided from Treatacaspian Co. stem cell bank with a total of 10^6 stem cells. For each dose administration, one vial was removed from the azoth tank. The outside of the vials was wiped with 70% ethanol, and then the cells were

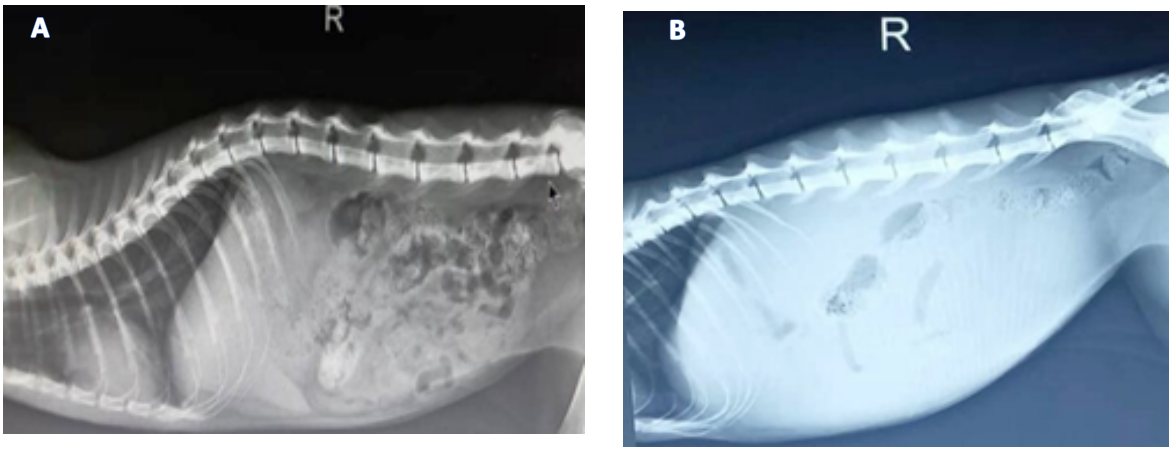

Figure 1. Radiography figures in the effusive cat

A) Clear peritoneal serosal detail after cell therapy, B) Free fluid in abdominal cavity before the cell therapy

quickly thawed in a 37 ˚C water bath by gently swirling the vials. A 20 µL aliquot of cells was mixed with 20 µL of the medium and then stained with Trypan Blue for cell counting. The remaining cell suspension was transferred to a conical tube using a pipette and rinsed with 1 mL of medium (DEMEM high glucose) by centrifuging the cell suspension at 300×g for 10 minutes at room temperature. The cell pellet was resuspended by gently flicking the tube, and then 5 mL of medium was added to the conical tube (Bearden et al., 2017; Zhang et al., 2018).

All patients received BM-MSCs on days 1, 4, 7, 14, and 21, with a dosage of 1×10^6 in 15 mL of normal saline, injected intravenously for one hour (Webb et al., 2013). Any reaction following the medical administration, such as skin rash or loss of appetite, was considered and noted in Table 3.

All treated cats underwent physical examinations and blood work, consisting of complete blood count (CBC) and serum biochemistry, each week (Table 2). Three days after the first treatment, the appetite was regained, and lethargy and fever regressed in all cats. Blood samples and radiological studies were performed to monitor the condition of the cats after recovery, and the blood factors in all cats showed normal levels.

After the 21-day treatment period, no detectable ascites or abnormalities in the organs were seen on ultrasonographic and radiographic images (Figures 1 and 2).

At the follow-up, two months after the treatment, two cats remained healthy, showing no signs of relapse, while one cat was reinfected after coming into contact with a severely diseased cat. At this stage, the continuation of the treatment with remdesivir at a dose of 30 mg/kg was continued but was not successful, and recovery was not achieved. The outcomes of this report are summarized in Tables 1, 2 and 3.

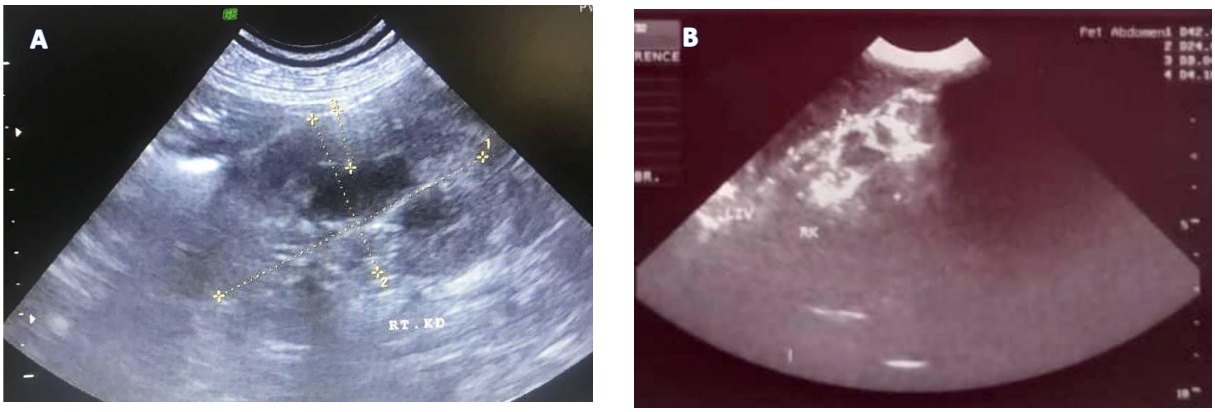

Figure 2. Ultrasonographic figures of cat with none effusive FIP

A) The size of liver and right kidney regressed to the normal range at the end of the treatment, B) Almost the entire enlarged kidney is embedded in the renal fossa due to liver enlargement before cell therapy

Table 1. The condition of cats at the time of presentation

Cat#	Breed	Age at disease onset (m)	Sex	Weight (kg)	Duration from Disease Onset to Treatment*	Effusion Type	Body Temperature (°C)	Heart rate (beats/min)	Breath rate (breaths/min)	Neurological/ Ocular Sign	Sample Isolation for PCR
1	DSH	7	Female	1.8	30	Non-effusive	40	160	20	+	Blood
2	DSH	8	Female	2.5	5	Effusive	39.6	170	30	-	Abdominal effusion
3	DSH	11	Female	2.2	65	Effusive	40.2	220	45	-	Abdominal effusion

DSH: Domestic short-haired.

*Based on the cat owner's report.

Table 2. Test values observed on the first visit and the final test results based on which the treatment discontinuation decision was made

Animal Examination	Cat 1	Cat 2	Cat 3
First visit	HCT: 23.8%	HCT: 40%	HCT: 20%
	HGB: 7.6 g/dL	HGB: 7.6 g/dL	HGB: 7.6 g/dL
	WBC: 51.8×10^3 µL	WBC: 21.1×10^3 µL	WBC: 43.5×10^3 µL
	Absolute lymphocytes: 518/µL	Absolute lymphocytes: 1488/µL	Absolute lymphocytes: 453/µL
	A/G ratio: 0.45	A/G ratio: 0.65	A/G ratio: 0.28
	Total protein: 10.7 g/dL	Total protein: 8.6 g/dL	Total protein: 9.5 g/dL
	AST: 504 U/L	AST: 35 U/L	AST: 119 U/L
	ALT: 530 U/L	ALT: 54 U/L	ALT: 112 U/L
	Total bilirubin: 2.35 g/dL	Total bilirubin: 0.26 g/dL	Total bilirubin: -
	PCR test: Positive	PCR test: Positive	PCR test: Positive
	Ultrasonographic/Radiology Report: Enlarged liver	Ultrasonographic//Radiology Report: Asci-tes observed	Ultrasonographic//Radiology Report: Asci-tes observed
Last visit before treatment discontinuation*	HCT: 30.2%	HCT: 42%	HCT: 42.5%
	HGB: 10.5 g/dL	HGB: 13.4g/dL	HGB: 14 g/dL
	WBC: 15.1×10^3 µL	WBC: 11.7×10^3 µL	WBC: 16.5×10^3 µL
	Absolute lymphocytes: 1812/µL	Absolute lymphocytes: 5967/µL	Absolute lymphocytes: 5940/µL
	A/G ratio: 0.91	A/G ratio: 0.85	A/G ratio: 0.6
	Total protein: 6.7 g/dL	Total protein: 7.4 g/dL	Total protein: 8.2 g/dL
	AST: 31 U/L	AST: 31 U/L	AST: 39 U/L
	ALT: 28 U/L	ALT: 26 U/L	ALT: 82 U/L
	Total bilirubin: 0.25 g/dL	Total bilirubin: 0.16 g/dL	Total bilirubin: 0.42 g/dL
	PCR test: Negative	PCR test: Negative	PCR Test: Negative
	Ultrasonographic/Radiology report: Normal	Ultrasonographic/Radiology report: Normal	Ultrasonographic/Radiology report: Normal

Abbreviations: HCT: Hematocrit, HGB: Hemoglobin, WBC: White blood cell count, A/G: Albumin/globulin ratio, AST: Aspartate aminotransferase, ALT: Alanine Aminotransferase.

*The tests were done two weeks after the last treatment.

Table 3. Summary of treatment in cats

Cat#	No. of Cells	Total Dose of Cell Administration	Outcome	Days Without Relapse	Adverse Events
1	1×10^6	5 on days 1,4,7,14,21	Died on day 102	-	Skin rash
2	1×10^6	5 on day 1,4,7,14,21	Remission	370	None
3	1×10^6	5 on days 1,4,7,14,21	Remission	180	None

BM-MSCs showed striking efficacy and treated cats responded to the treatment with rapid improvement of clinical signs. All cats achieved remission after 21 days of treatment. Follow-up on the cats in our report is currently ongoing, and more cats with FIP are being treated using this method.

Study Assessments

The main reason for the progression of FIP in patients is the immune abnormalities. When coronaviruses invade the body, they rapidly replicate and trigger the immune system to release inflammatory cells and antibodies (Akkoc, 2020; Addie et al., 2020). This leads to the immune regulatory network, to unbalanced, resulting in 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 systems (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 the immunomodulatory and anti-inflammatory effects of MSCs, these cells also can aid in the repair of the damaged tissues, significantly reducing the number of affected organs associated with FIP (Salari et al., 2020). The liver is one of the organs affected during FIP. Since remdesivir and GS-441524 are treatment options for FIP treat-ment, they will be metabolized in the liver and could worsen the liver function, potentially making it impossible to continue the treatment (Grein et al., 2020; Wang et al., 2020; Liu et al., 2023). Therefore, it is necessary to use a component to repair the damaged tissues during the treatment. The regenerative properties of stem cells, along with their immunomodulatory effects, make them the best option for FIP treatment with minimal side effects.

In our cell therapy results, the duration of treatment was shorter (21 days) compared to the duration of treatment with remdesivir (84 days) (Murphy et al., 2018; Pedersen et al., 2018). Since there is no access to oral remdesivir/GS-441524 for animals in the country under study, the need for daily visits for injectable medicine is eliminated. Additionally, since the majority of the infected cases are found among street cats, introducing a shorter treatment period will provide more cases with a chance to be cured. On the other hand, the costs associated with this treatment are much lower compared to the aforementioned treatment, which is considered a positive aspect of cell therapy.

The small number of studies using MSC products in cats reveals that this treatment is still largely in the discovery phase. Cell sources, administration routes, and dosages can have different results. In the current report, BM-MSCs were used for the first time in the treatment of FIP disease. Despite the failure of adipose stem cells in some reports regarding FIP treatment, this type of stem cells, when administered at the recommended dose and method, has enabled us to achieve positive results in the treatment of FIP in cats.

Conclusion

The results showed that the mesenchymal stem cells could 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. To check the long-term effectiveness of stem cells, more cases are needed.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles are considered in this article and all owners signed written informed consent before initiation of treatment.

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Authors' contributions

Conceptualization, methodology and validation: All authors, investigation, resources and data curation: All authors; Writing, visualization and supervision: Samira Mohamadian; Project adminestration and funding acquisition: Samira Mohamadian, Pejman Kazerooni, and Ali Taheri Mirghaed,

Conflict of interest

The authors declared no conflict of interest.

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گزارش موردی

درمان موفقیت آمیز بیماری پریتونیت عفونی گربه (FIP) با استفاده از سلول های بنیادی مزانشیمی

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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 ۵ 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 ۲۱ روز شد. در بین گربه‌های تحت درمان، یک گربه دو ماه بعد از اتمام درمان در مواجه با گربه آلوده، 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.

کلیدواژه‌ها: آلوژنیک، مغز استخوان، طوفان سایتوکین، مزانشیمی، سلول‌های بنیادی

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