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Case Report - Veterinary Medicine

Improvement of clinical and laboratory parameters in a canine with idiopathic chronic hepatitis after transplantation of allogeneic mesenchymal cells derived from bone marrow – case report

[*Melhora dos parâmetros clínicos e laboratoriais em um cão com hepatite idiopática crônica após transplante de células mesenquimais alogênicas derivadas da medula óssea – relato de caso*]

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ABSTRACT

Chronic hepatitis (CH) is characterized by the replacement of hepatocytes for fibrotic tissue after injury, and treatment is not curative. Stem cell therapy has shown potential to reduce liver fibrosis. This report describes the management of a dog with CH with a mesenchymal cell-based approach. A 13-year old dog was presented with weight loss, vomiting, diarrhea, and lethargy. She was previously diagnosed with CH. Blood analysis showed hypoalbuminemia, increased serum activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), and anemia. Three $2.5x10^6$ bone marrow-derived mesenchymal cells transplantations were performed. After the first transplantation, the owner reported an improvement in clinical signs. Biochemistry evidenced a reduction in ALP activity. After the second administration, serum biochemistry showed a decrease in serum ALT and ALP activities, which kept falling after the third transplantation. There was an increase in albumin and total plasmatic proteins concentration. After six months, the dog came back for control and further improvement in clinical signs, new decrease in ALT activity and a mild increase in ALP concentration was observed. Increase in total and plasmatic protein were also observed. Stem cell-based therapy may be considered a promising alternative for liver diseases. It was safe and showed efficacy in this report.

Keywords: liver disease, stem-cell, therapy, transplantation

RESUMO

A hepatite crônica (HC) é caracterizada pela substituição dos hepatócitos por tecido fibrótico após injúria, em vez de regeneração do parênquima funcional, e a terapia não é curativa. A terapia com células-tronco tem mostrado potencial para reduzir a fibrose hepática. Este relato descreve o manejo de um cão com HC com abordagem baseada em células mesenquimais. Uma cadela de 13 anos foi atendida com perda de peso, êmese, diarreia e letargia. Previamente, fora diagnosticada com HC. Exames de sangue mostraram hipoalbuminemia, aumento das atividades da alanina aminotransferase (ALT) e fosfatase alcalina (FA), e anemia. Três transfusões de $2,5x10^6$ células mesenquimais derivadas foram realizadas. Após a primeira transfusão, o tutor relatou melhora nos sinais clínicos. A bioquímica sérica evidenciou redução na atividade da FA. Após a segunda administração, houve redução nas atividades séricas da ALT e da FA, que continuaram caindo após a terceira transfusão. Houve aumento nas concentrações de albumina e proteína plasmática total. Após seis meses, o cão retornou para controle. Foram observados avanço na melhora dos sinais clínicos, nova redução na atividade da ALT e leve aumento da FA. A terapia baseada em células-tronco pode ser considerada alternativa promissora para doenças hepáticas crônicas. Neste relato, demonstrou segurança e eficácia.

Palavras-chave: células-tronco, doença hepática, terapia, transplante

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INTRODUCTION

Chronic hepatitis (CH) is characterized as a progressive and inflammatory injury with hepatocellular necrosis or apoptosis that culminates in regeneration and/or fibrosis (Webster *et al.*, 2019). In hepatic fibrosis, there is an accumulation of fibrillary extracellular matrix, due to an amount of collagen, which can have a content up to 10-fold higher than the normal (Eulenberg and Lidbury, 2018). Most causes of CH are classified as idiopathic, but exposure to toxins, infectious agents, metabolic or immunological abnormalities are possible etiologies (Webster *et al.*, 2019).

Dogs with CH may present a wide range of clinical signs, which can be nonspecific, e.g. decreased appetite, lethargy, anorexia, weight loss, vomiting, diarrhea, polyuria and polydipsia, or more specific to the loss of hepatic function, as hemostatic disorders, encephalopathy, icterus (Webster *et al.*, 2019), and gastroduodenal ulceration (O'Kell *et al.*, 2022). The therapy must be directed to the cause. Unfortunately, in most cases the etiology is not identified, and therefore limits the therapy to hepatoprotective agents, antifibrotics and antioxidants with little evidence of efficacy, and dietary management. Sometimes, attempting to presume an immune-mediated etiology, clinicians may try to evaluate the response to immunosuppressive therapy, aware about the adverse effects of these drugs (Webster *et al.*, 2019).

Stem-cell therapy is associated to antioxidative, anti-inflammatory and antiapoptotic effects and some studies have been shown their potential to reduce liver fibrosis (Shi *et al.*, 2021; Liu *et al.*, 2022; Wu *et al.*, 2022). This report describes the improvement of clinicopathological signs of a dog with CH after a mesenchymal stem cell-based approach.

CASUISTRY

On day 1, a 13-years old female Mixed Breed dog, 5.8kg of body mass was presented to a referral hospital in Southern Brazil with decreased appetite, weight loss, vomiting, yellowish diarrhea, and lethargy. The patient had been spayed six years before this presentation due to pyometra. The owner reported that the dog was previously submitted to a liver biopsy and received the diagnosis of chronic hepatitis. The prescribed treatment was silymarin (15mg/kg) and ursodeoxycholic acid (8.3mg/kg), both each (q) 24 hours (h) *per os* (PO) with no improvement of clinical signs. Due to heart murmur, the dog also receives pimobendane (0.25mg/kg) and benazepril hydrochloride (0.5mg/kg), both PO q 24 h.

At physical examination, the dog presented mitral valve murmur, but without tachycardia (108 beats per minute); the body condition score (BCS) was 3 of 9; it was difficult to evaluate the shape of the liver at abdominal palpation, probably due to the small size. No more alterations were observed. Serum biochemistry showed hypoalbuminemia, increased activities of alanine aminotransferase (ALT) and alkaline phosphatase (ALP); blood urea nitrogen (BUN) and creatinine were lower than their respective reference ranges, as well as the total protein (TP). The serum biochemistry results are shown in Table 1. A mild microcytic normochromic anemia was observed at complete blood count (CBC). Plasmatic protein (PP) was at its lower limit. Results of CBC are shown in Table 2.

Table 1. Serum biochemistry results of a female dog, 13-years old before and after allogeneic mesenchymal cells transplantation

Biochemistry/Days	01	03	17	30	36	50	240	Reference range
Albumin (g/dL)	2.2	2.2	2.2	1.5	2.5	2.4	2.25	2.5-3.6
ALT (UI/L)	161	121	137	75	113	73	44	17.2-63.0
ALP (UI/L)	219	233	184	157	129	112	167	15.2-190.4
BUN (mg/dL)	8	8	4	6	5	4	8	18.0-61.3
Creatinine (mg/dL)	0.4	0.4	0.5	0.5	0.5	0.5	0.6	0.7-1.5
TP (g/dl)	4.7	4.6	4.8	4.6	5.1	5.0	5.4	5.4-7.5

Reference: (Cornell University, 2017). ALT: alanine aminotransferase; ALP: alkaline phosphatase; BUN: blood urea nitrogen; GGT: gamma-glutamyl transferase; TP: total protein.

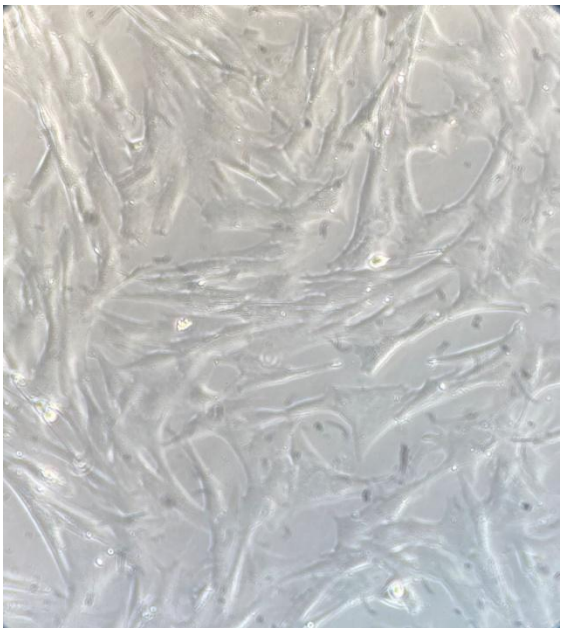
Table 2. Complete blood count results of a female dog, 13-years old before and after allogeneic mesenchymal cells transplantation

CBC/Days	01	03	17	30	36	50	240	Reference
Erythrocytes (x10^6 µL)	5.63	5.32	5.42	5.44	5.14	5.45	5.82	5.5-8.2
Hemoglobin (g/dL)	12.2	11.3	11.7	11.0	12.0	11.8	12.1	12.8-19.9
Hematocrit (%)	35.0	35.0	36.0	32.6	36.0	35.0	36.1	38.2-58.5
MCV (fL)	62.1	65.7	66.4	60.1	70.0	64.2	62.0	61.2-77.1
MCHC (%)	34.8	32.2	32.5	33.7	33.3	33.7	33.5	32.0-36.0
RDW (%)	12.9	13.3	12.6	13.9	15.0	15.0	14.9	11.1-16.3
PP (g/dL)	5.8	5.8	6.4	5.6	7.0	6.6	6.4	5.8-8.6
Platelets (x10^3/µL)	406	285	350	319	574	306	390	159-451
Leukocytes (/µL)	15,100	11,700	11,300	8,900	8,900	11,000	10,500	6,400-17,700
Neutrophils (/µL)	11,929	9,243	8,927	6,052	5,518	8,360	6,300	3,200-10,700
Lymphocytes (/µL)	1,963	1,755	1,582	1,602	2,670	1,980	3,465	1000-4,900
Monocytes (/µL)	-	234	226	267	178	110	315	0-1,100
Eosinophils (/µL)	1,208	468	565	979	534	550	420	100-2,500

Reference: (Bonamigo, 2022). CBC: complete blood count; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; RDW: red cell distribution width; PP: plasmatic protein; (-): not found.

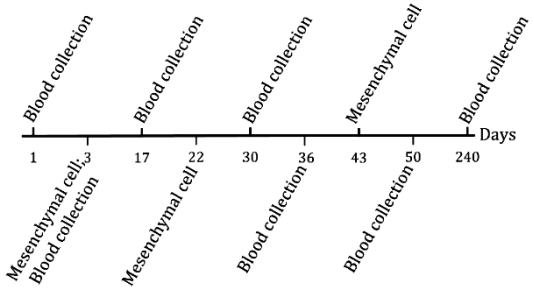
The cells were collected from bone marrow of a healthy dog – with approval from the Animal Ethics Committee on Animal Use (CEUA) of the Federal University of Santa Maria (protocol number 6702160621) – and cultivated *in vitro* in Dulbecco Modified Eagle Medium (DMEM) enriched with 10% Fetal Bovine Serum (FBS) in a chamber at 37°C with 5% CO_2 (Fig. 1). When the cells reached 80% confluence, were passed to other plates until the fifth passage, when they were cryopreserved at a -80° freezer until their use.

For application, cells were thawed at 37°C in water bath, washed twice in DMEM 10% FBS by gentle 10 min centrifugation (300 x g) and the pellet was resuspended with 1 mL of sterile 0.9% saline. The cell viability was checked by Trypan Blue dye method (1:1) in a Neubauer chamber and was higher than 90% in all days. The cell transplantations happened in days 3, 22 and 43. The dog received 2.5x10^6 cells in each application, diluted in 5mL of 0.9% saline and administered intravenously. A timeline with the events is available in Fig. 2. During the transplantations period, the patient didn't show any adverse effects.



Source: Santos *et al.* (2024).

Figure 1. canine bone marrow-derived mesenchymal cells.



Source: Santos *et al*. (2024).

Figure 2. Sequence of events during the therapy with mesenchymal cells in a dog with chronic hepatitis. Blood collection represents the days in which blood was collected for blood exams. Mesenchymal cells represent the days of cells transplantation.

Fourteen days after the first transplantation, a new blood collection was performed and a decrease in ALP serum activity could be observed (from 233UI/L to 184UI/L). The CBC showed a mild increase in plasmatic protein (PP), from 5.8g/dL to 6.4g/dL. The owner reported improvement in stool appearance (no more yellowish diarrhea) and the dog had a weight gain (from 5.8kg to 6.3kg).

Two blood collections were performed after the second transplantation and evidenced significant decrease in ALT (from 137UI/L to 75UI/L and 113UI/L respectively) and in ALP (157UI/L and 129UI/L, respectively) activities. A mild increase could be observed in total protein at the second analysis (from 4.6 to 5.1g/dL). On the other hand, a decrease in albumin serum concentration was present in the second sample after transplantation (from 2.2g/dL to 1.5g/dL). Nevertheless, six days after, in the following analysis, albumin concentration increased to 2.5g/dL. The same was observed for PP, which rose from 5.6g/dL to 7.0g/dL during the same period.

At day 50, seven days after the last cell transplantation, the dog kept gaining weight (6.6kg). The owner reported improvement in behavior, less vomiting, and no diarrhea. ALT activity decreased to 73UI/L. No significant alterations were evidenced in the CBC when compared to previous exams.

Six months later, the patient returned to a checkup. The owner referred to a good appetite, normal feces, and sporadic vomiting (the last episode happened three weeks before the day of appointment). The body weight remained the same (6.6kg). At physical examination, showed good BCS (5/9), euhydration, normal mucous membrane color, and a capillary refill time lower than two seconds. Blood chemistry evidenced a decrease in ALT activity (44 UI/L); and an increase in ALP activity (167UI/L), but still in the reference values. An improvement of total protein concentration was also observed (5.4g/dL). The CBC didn't show significant changes.

DISCUSSION

In chronic hepatitis there is an imbalance in the healing of chronic injury and inflammation. The extracellular matrix is deposited, but isn't adequately removed, which causes their accumulation. Some cells are reported as sources of extracellular matrix, like hepatic stellate cells, hepatic fibroblasts, epithelial cells that undergo epithelial-to-mesenchymal transition, bone marrow-derived fibrocytes and smooth muscle cells. There is an accumulation of fibrillary and nonfibrillary collagens, glycosaminoglycans and proteoglycans (Eulenberg and Lidbury, 2018). In cholestatic liver, bile acids also damage cells and produce oxidant injury, stimulating cytokine release. Portal hypertension may also develop and contributes to complications. To identify the etiology of hepatitis helps in the therapy institution (Webster *et al.,* 2019). However, in this report, as well as in the most reported cases, the patient was classified as idiopathic.

Even having slight and nonspecific signs, gastrointestinal system is one of the more affected in patients with CH, mainly in late stages (Webster *et al.*, 2019). Among those signs, decreased appetite, lethargy, vomiting, and diarrhea are common, and were manifested by the dog of this report. Gastrointestinal signs in hepatic disease have a mechanism poorly understood, with increased risk of developing a gastroduodenal ulceration (O'Kell *et al.*, 2022). However, in this report, the patient didn't presented signs of gastrointestinal bleeding.

The results suggest that cell therapy has a potential to ameliorate clinical signs in dogs with chronic hepatitis. The absence of adverse effects is indicative of safety in the procedure. Stem cell

approach in veterinary medicine has been studied as a potential and promising therapy for many diseases from different organ systems, like orthopedic, digestive, renal, cardiovascular, respiratory, neuromuscular and reproductive (Voga *et al.*, 2020).

There is a large number of studies appointing the role of stem-cells in regeneration of liver diseases in humans (Shi *et al.*, 2021; Liu *et al.*, 2022), but a small number in dogs (Matsuda *et al.*, 2017; Teshima *et al.*, 2017; Nishimura *et al.*, 2019). The cell-based therapy approach, however, showed success in reducing fibrosis area in a canine liver fibrosis model. Also resulted in an improvement in serum albumin (Matsuda *et al.*, 2017). The ALT activity was significantly lower in stem-cell group when compared to placebo (Matsuda *et al.*, 2017). Another study found similar results in the same injury model (Nishimura *et al.*, 2019). A decrease in ALT activity was found, but in addition, improvements in ALP, total and plasmatic proteins also were evidenced, with a mild increase in serum albumin.

Although the therapeutic role of cell therapy for liver diseases is described, several limitations are associated with this approach. For example, search for strategies to improve the capacity of the cells to reach the target organ (homing), and consequently potentialize their therapeutic effects. Gene-editing, alternative transplantation routes and cell culture improvements are potential strategies to increase the efficacy of cell therapy. Other aspects, such as the number of administrations, the optimal source of cells and the dosage need to be studied (Liu *et al.*, 2022). In a meta-analysis carried out from reports of human medicine, peripheral intravenous infusion seemed to be the ideal route due to the convenience and ease (Liu *et al.*, 2022), but a study in dogs, with a small number of participants, found longer effects in intra-arterial when compared to intravenous transplantation (Nishimura *et al.*, 2019). The dog of this report showed good results with three transplantations of $2.5x10^6$ cells intravenously, presenting weight gain, improvements in vomiting and diarrhea, and no more lethargy without adverse effects, what shows the safety of the cell-based therapy in this case. Under an inflammatory environment, mesenchymal stem-cells-derivate extracellular vesicles, which secret paracrine effects and secretory components, mediate immunosuppression, inhibiting proliferation of T and B cells and stimulating the activity of regulatory T cells. In liver tissue, these effects attenuate fibrosis by upregulation of matrix metalloproteinases (MMP) and anti-inflammatory factors, and downregulation of proinflammatory factors. Also inhibit epithelial-to-mesenchymal transition and hepatic stellate cells activation (Wu *et al.*, 2022). The exact role of mesenchymal cell therapy in tissue regeneration needs to be further investigated. Clinical and laboratory evidence of improvement and safety of a cell-therapy based approach for chronic hepatitis in a dog was shown in this report.

CONCLUSIONS

The transplantation of mesenchymal cells helped to improve clinicopathological signs of idiopathic chronic hepatitis in the dog reported here. The dosage of $2.5x10^6$ cells intravenously in three administrations showed safety and good therapeutic efficacy. This approach can be considered as a promising alternative for chronic liver diseases.

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