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Clinical safety for intravenous administration of allogeneic mesenchymal cells in healthy dogs

[*Segurança clínica à administração intravenosa de células mesenquimais alógenas em cães saudáveis*]

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ABSTRACT

Despite the significance of adipose-derived stem cells in complementary and regenerative medicine, studies regarding clinical safety for allogeneic intravenous administration in healthy dogs remain insufficiently conducted. This study aimed to assess cellular viability through laboratory and microbiological evidence, along with genetic markers, in addition to evaluating clinic-laboratory safety for allogeneic intravenous administration of adipose-derived stem cells in healthy dogs. To achieve this, two cell batches and eight dogs were included in the study. The results revealed positive genetic markers for CD29, CD44, CD105, SOX2, and OCT3.4. Both batches exhibited positive cell differentiation into adipocytes, chondrocytes, and osteoblasts. Microbiological evidence showed negative results, and cell viability after thawing indicated 92% and 88.5% viable cells after 30 minutes, and 86% and 83.5% after 24 hours, respectively, for both batches. Hematological cell counts and serum biochemical enzyme levels, before and after intravenous treatment, did not exhibit statistical differences between the time points ($p>0.05$). Median values remained within the reference range for the species during and after 30 days of treatment. Based on the cellular viability results, observed patterns, and the absence of hematological side effects, it can be concluded that intravenous therapy with allogeneic adipose-derived stem cells is clinically safe.

Keywords**:** hematology, stem cell research, patient safety, cellular therapy, cellular viability

RESUMO

Apesar da importância das células mesenquimais adiposas na medicina complementar e regenerativa, poucos estudos estão relacionados à segurança clínica de seu uso alógeno intravenoso em cães. Objetivou-se, por meio deste estudo, avaliar a viabilidade celular, mediante provas laboratoriais, microbiológicas e marcadores genéticos, e a segurança clínico-laboratorial da administração intravenosa de células-tronco derivadas do tecido adiposo em cães hígidos. Para tal, dois lotes celulares e oito cães saudáveis foram utilizados. Entre os resultados obtidos, os marcadores genéticos apresentaram-se positivos para CD29, CD44, CD105, SOX2 e OCT3.4. A diferenciação em adipócitos, condrócitos e osteoblastos foi positiva para ambos os lotes; as provas microbiológicas apresentaram-se negativas; e o teste de viabilidade após descongelamento resultou em 92% e 88,5% de células viáveis após 30min e 86% e 83,5% após 24h, respectivamente para os dois lotes. As contagens celulares hematológicas e as dosagens enzimáticas séricas, antes e após o tratamento intravenoso, não resultaram em diferença estatística entre os momentos (P>0,05), enquanto os valores medianos demonstraram-se dentro dos valores de referência para a espécie durante e após 30 dias de tratamento. Por meio dos resultados de padrão e viabilidade celulares, bem como da ausência de efeitos adversos hematológicos,

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concluiu-se que o uso intravenoso das células mesenquimais adiposas caninas alógenas é clinicamente seguro.

Palavras-chave: hematologia, pesquisa em célula-tronco, segurança do paciente, terapia celular, viabilidade celular

INTRODUCTION

The clinical use of mesenchymal cells (MC) in the clinical and surgical veterinary routine is relatively recent, showing a high growth in recent years, being previously used only for research and clinical tests. Through the studies and scientific evidence of their beneficial effects and the definition of some protocols for the cellular cultivation and administration as therapeutic approaches, commercial use became possible and popular worldwide, including the Brazilian veterinary commerce (Liu *et al.*, 2020). Their desirable effects are related to their power for promoting tissue regeneration and injury repair (Hu *et al.*, 2014), immunomodulation (Li, 2017), anti-inflammatory (Valdivel, 2020) and the stimulation of the target-tissue related inherent stem cells (Tieu *et al.*, 2020). Veterinary use is more widespread when compared to human medicine due to the commercial availability, which is restricted in human use, but the animal model can be a research method for human utilization (Pinheiro *et al.*, 2019b). Companion animals are a big target for the therapeutic usage of those cells and the clinical benefits can be related with a lot of tissues, organic systems, and pathological processes (Voga *et al.*, 2020). The cellular extraction can be done from a lot of tissues, but the adipose tissue is the main target because of the widespread availability, easy isolation, and absence of side effects for the donor, resulting in the adipose tissue derived stem cells (ADSCs) (Reshak *et al.*, 2013).

In view of its commercial dissemination, an important highlight has been done to the origin of the cells' extraction. Allogenic cells are those that are extracted from another animal of the same species and represent the main ones to the commercial and research use, but the laboratorial cultivation has to be done following the international recommendations and respecting all the quality control parameters for being securely used in the clinical routine (Przadka *et al.*, 2021), and the determination of those parameters are essential for the establishment of confidence in this therapeutic approach. These allogenic stem cells, mainly derived from the adipose tissue, in addition to the grater cellular availability, are also widespread because of their unethical responsibility related to the isolation and administration, compared to the embryonic stem cells, but even so, they are also subjected to the necessary proofs to emphasize the quality and the standard parameters (Voga *et al.*, 2020). On the other hand, there are some unanswered points in the field related to the clinical safety and possible side effects caused by the therapy, mainly referred to rejection signs or systemic side effects related to the inflammatory response through immunogenic reaction (Marx *et al.*, 2015)

Although the clinical efficacy and safety for the use of ADSCs for specific pathologic processes has been previously proven, either through intra-articular administration or local puncture (Park *et al.*, 2013; Cabon *et al.*, 2019; Liotta *et al.*, 2021), there is a lack of information about the clinical safety of the allogenic systemic administration, through intravenous therapy, in healthy dogs, in order to distinguish the possible laboratorial and clinical side effects from those derived from the active disease. Thus, there is a need to reinforce the research related to its commercial use from dog therapies, mainly for Brazilian quality control protocols and commercial standards, in relation to the cellular quality and safety for systemic use, due to its dissemination to all tissues and organs. Therefore, the aim of this research was to evaluate the quality control and standard characteristics in addition to the clinical safety for the intravenous therapy of commercial allogenic adipose derived stem cells in asymptomatic dogs and possible side effects through hematological parameters and dosage of kidney and hepatic biomarkers for function and injury.

MATERIALS AND METHOD

This prospective research was conducted under the permission of the Ethics Committee for Animal Use (CEUA) of the University of

Brasilia, under protocol number 66/2019. The study was developed in the clinical trial model, using eight healthy dogs (*Canis Lupus familiaris*), laboratorial and clinically asymptomatic, which were subjected to the intravenous administration of allogeneic ADSCs. Laboratorial parameters were evaluated, before and after the treatment, such as hematological cellular count and serum biochemistry, to evidence the possible changes in their values, compared to the pre-treatment parameters, resulting from ADSC use.

The selected animals were spontaneously inserted in the research through the legal consent of the owner. Eight dogs were inserted in the analysis, including four males and four females, to avoid sex-related discrepancies. The age was between one to seven years, without predilection to breed or weight. The inclusion criteria were absence of clinical, hematological and serum biochemistry alterations in relation to the parameters for the species, according to Thrall *et al.* (2012). The exclusion was performed when any clinical or laboratorial findings were related to pathological processes or infectious diseases.

This way, the eight dogs were included in the study, being inserted in one experimental group, which underwent serial evaluations of blood counts and serum biochemistry through the obtaining of a blood sample, collected through jugular or cephalic veins, and intravenous administration of ADSCs.

Five moments of blood sample analysis were defined: D0 was the screening moment, prior to the treatment and adopted as the control moment and values; D1 one day after the cellular therapy; D2 10 days after treatment; D3 21 days after; and D4 30 days after. The D0 was the Control Moment (CM), to be the pre-treatment period, and the other moments were defined as Treatment Moments (TM). The ADSC therapy was conducted in the D0 moment, after the screening by the inclusion criteria and through the physical exam during the patient's consultation.

The product utilized (ADSC, Mesenchymal cells, BIO CELL laboratories *Ltda.*, located at Brasilia, Federal District, Brazil) is commercial recognized by the Brazilian government and regulatory agencies as an official cellular therapy's seller. The cellular sample were obtained from the isolation of canine adipose tissue, extracted through elective sterilization surgeries, independent of being males or females. The adipose tissues were washed in DMPBS solution and underwent enzymatic digestion in collagenase and hyaluronidase solutions for 30 minutes at 38.5°C. In sequence, the mononucleated cells were isolated and added in culture bottles containing TCM 199 medium at 38.5°C and controlled atmosphere at 5% CO_2 for seven days, with exchange of the medium every 48 hours. Subsequently, the cells were divided into a greater number of bottles and cultivated until the confluence of 90% of the medium.

After the step mentioned, they were placed in specific culture medium, while two samples were destined to the quality assurance. The cellular culture was done through their allocation in a culture medium with 12 mL of Eagle's medium modified by Dulbecco (DMEM) without phenol red and 10% of fetal bovine serum was added in a 75 cm^3 culture's bottles, with a sample of approximately 300 hundred cells, making the medium exchange every 48 hours. For the commercial presentation, these cells were packet in 0.25 mL straws with the concentration of $1x10^6$ cells per packet, kept in a cryoprotector medium of dimethyl sulfoxide, fetal bovine serum and DMEM, frozen in liquid nitrogen until the use.

The samples sent to the quality assurance were evaluated according to the rules of the International Society for Cellular Therapy, being one for the molecular characterization and the other sent for cellular differentiation into osteoblasts, chondrocytes, and adipocytes for compliance with the standard rules. The osteoblasts differentiation was done through the allocation of a cellular sample in a specific culture medium, and, after, the Alizarin Red dye was added for the microscopic analysis, while the chondrocytes and adipocytes were studied through Oil red and Alcian blue dyes, respectively. The microscopic analysis was performed with a 100x magnification for the three studies. The genetics scanning was performed by the proportion determination for cellular biomarkers that are into the standard, such as: CD29, CD44 and CD105, besides SOX2 and OCT3.4, through flow cytometry and polymerase chain reaction (PCR), using specific

reagents for the canine species. Cell viability and sample sterility were also evaluated. The sterility was performed through the bacterial or/and mycological screening of the sample, making the specific cultivation of *Mycoplasma spp.* and, after, analyzed by PCR method, performed by an outsourced laboratory under specific and controlled conditions. Cell viability was verified through the Trypan blue dye, with 1:1 (cellular sample: Trypan blue) dilution ratio, which was added in Newbauer chamber and analyzed in microscope, making a characterization of 200 cells into viable or non-viable according to their color, in two moments, the first after 30 minutes and the second after 24 hours of thawing.

For the treatment administration, the cells underwent thawing at 37°C water bath for 20 seconds, with the exact quantity of straws per animal, determined by their body weight. After, the content of the straws was allocated in a test tube containing a specific thawing-medium provided by the laboratory, being centrifuged at 2000 rpm for three minutes. The supernatant on the surface was removed with a pipette and added to the washing-medium containing PBS, also provided by the laboratory, for the centrifugation under 2000 rpm for three minutes, repeating this act three times. At the end, the content was transferred and homogenized to the transportation-medium, which was added following the proportion of 1 mL for each million cells.

After manipulation and preparation of the ADSCs previously mentioned, the cells were packaged in sterile 10 mL syringes with a 13x0.45 mm hypodermic needle and stored with light protection and at ambient temperature up to 24 hours before the administration. The dogs were evaluated once more before the intravenous treatment referring to the physical examination, such as cardiac and respiratory frequency and rectal temperature.

The patients were subjected to peripheral venous access through the insertion of a catheter, with a caliber corresponding to and proportional to the dog's size and weight. So, the therapy was executed with a proportion of cells respecting the dog's weight, being $1x10^6$ cells per kilogram of body weight, and the total counting were diluted in sterile ringer lactate solution, with the final volume according to the body weight, varying from 50 to 150mL in the total volume, infusing the whole content in a maximum period of 40 minutes through a macrodrip infusion set. At the end of the infusion, the venous catheter was removed, and the dogs underwent a new physical examination.

The blood sample obtained from the patients was acquired through the jugular or cephalic veins puncture, with a 3mL syringe and 25x0.7mm needle. After the acquisition, the blood was divided into two tubes, one containing EDTA for the hematological count, and the other with clot activator for the serum biochemistry analysis. The cellular blood count (CBC) was used for the direct and proportional counting of the erythrocyte lineage, with red blood cells (RBC), hemoglobin (Hb) and hematocrit (Ht), and leukocyte lineages, being total leukocytes (TLeuk), segmented neutrophils (SN), total lymphocytes (TLym), besides platelets, through an automated analyzer and individual conference by blood smear. The biochemistry was performed in the blood serum through an automatic analyzer, to dose the kidney biomarkers for function, urea and creatinine, and the hepatic enzymes for lesion, as alkaline phosphatase (AP) and glutamic-pyruvic transaminase (GPT), and function, as total proteins (TP). The hematological analysis was performed by an outsourced veterinary laboratory (Santé Veterinary Laboratory, Brasília, Federal District, Brazil).

The samples were collected at the moments D0, D1, D2, D3 and D4 and analyzed to result in numeric dosages. The AP and GPT were given by the kinetic U.V. method with results in U/L, while the urea and creatinine were obtained by the kinetic method with results in mg/dL and TP by the colorimetric analysis and results in g/dL.

The results related to the blood analysis and cellular viability were inserted in data sheets for statistical analysis. First, the values were verified with median and sample standard deviation. After, the hematological and serum biochemistry values were analyzed in relation to ANOVA variance, correlating the TM with the CM, with a confidence interval of 95%, searching for any relations or significant differences between the moments and the variables. At the end, the average values of the TM related to CBC underwent linear regression to verify the

significance of the average values in relation to the CM, with the same 95% confidence interval. The analyses were done with the SAS Program (Cary, North Carolina, v.5.1) software and the results were shown in figures and tables.

RESULTS

This research evaluated the administration of ADSC intravenously in eight healthy dogs according to the hematological parameters, to prove the clinical safety of its utilization. For the cellular treatment, laboratorial proofs were also conducted in relation to cellular viability and sterility for commercial use. Both results were summarized below.

For the intravenous administration in the eight patients evaluated, two cell batches were used, which were acquired through other donators dogs that underwent elective surgeries for sterilization. The first proof was immunophenotyping of the cellular lineages isolated, according to the standard references internationally recommended for this kind of cell. The results of the genetic markers were presented in Table 1, showing the expected values and frequency for the standard biomarkers.

Table 1. Genetic and molecular immunophenotyping results for adipose tissue derived stem cells in two batches, used for the clinical safety tests in healthy dogs

Proof	Cell batch	
	1	2
Triple positive	(%)	(%)
CD29/CD44/CD105	80.27	94.67
Double positive		
CD29/CD44	8.4	0.04
CD29/CD105	0.3	0.63
CD44/CD105	9.59	3
Single positive		
CD29	0.83	0
CD44	0.3	0
CD105	0	0.1
SOX 2	60.2	94.36
OCT3.4	85.7	96.34

The sterility proofs were done in both batches, through PCR technique, and showed the same results. The three proofs (mycological and bacterial detection and *Mycoplasma spp.* identification), for both cell lineages, resulted in negative, showing the sterility of the samples that were used in this study. The least proof, of cellular viability, with the Trypan blue dye, showed the proportion of viable and non-viable cells after thawing, in two different periods. The results were demonstrated in Fig. 1, for both batches in the two moments of analysis, indicating that a high viability of the cells could be found even after 24 hours of their thawing and processing.

Both batches were subjected to cellular differentiation in osteoblasts, adipocytes, and chondrocytes, presenting a satisfied result for the three cellular types in both batches. Fig. 2 shows the differentiation results.

The inclusion criteria of the patients in this research considered the normal values in the laboratorial results before the treatment, at D0. The normal values, between the reference intervals for the sp,ecies, were considered as control-results in the first analysis moment. Table 2 shows the mean, median and standard deviation for the CBC and serum biochemistry at D0.

The sequential analysis was performed after the cellular treatment, to verify any deviation from the expected results of blood parameters. The mean values for TM and the final average of the analysis for each blood cellular group or enzymes were shown in Table 3. Table 4 represents the correlation between the before and after treatment values, considering the D0 moment as the reference one.

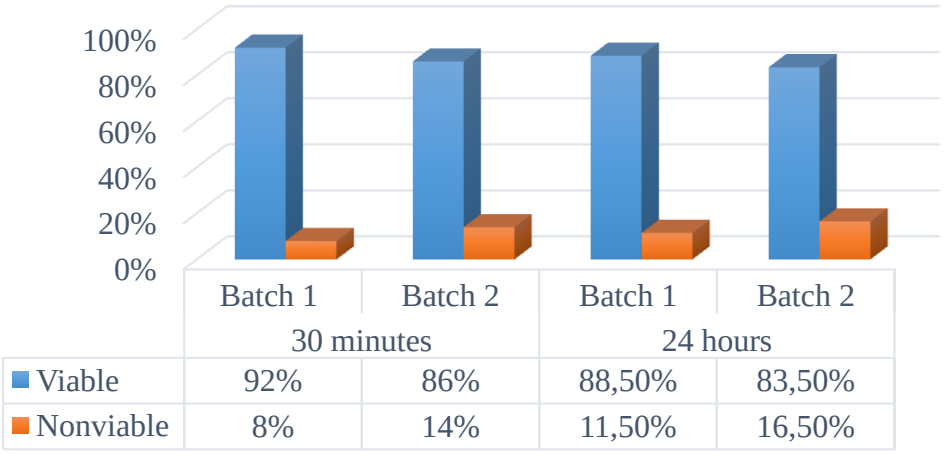

Figure 1. Cellular viability results, through Trypan blue dye, of allogenic adipose tissue derived stem cells utilized for clinical safety tests in healthy dogs.

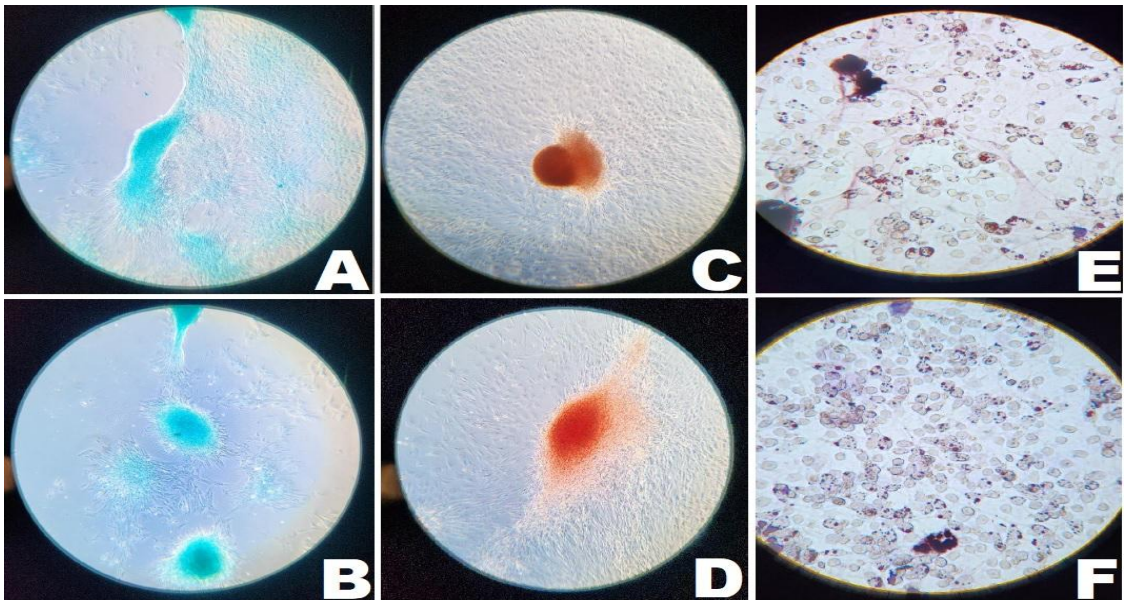

Figure 2. Cellular differentiation test result, applied in allogenic adipose tissue derived stem cells in two different batches. The first line represents batch 1 and the second, batch 2. 100X microscope magnification. A and B: chondrocytes differentiation, Alcian blue dye. C and D: osteoblasts differentiation, Alizarin red dye. E and F: adipocytes differentiation, Oil red dye.

Table 2**.** Control-results for Cellular blood count and serum biochemistry in healthy dogs that underwent cellular therapy with adipose tissue derived stem cells, at D0 moment

Variable	Mean	Median	Standard Deviation	Reference values (Thrall *et al*., 2012)
RBC	7.36	7.22	0.78	5.5 - 8.5
Hb	16.69	16.55	1.34	12 - 18
Ht	49.38	49.65	2.25	37 - 55
TLeuk	10.488	10.200	2.310	6000 - 17000
SN	7.623.13	7.794	2.318	3000 - 11500
TLym	2.528	2.416	858.99	1000 - 4800
Platelets	274.625	236.000	12.9811	180000 - 500000
TP	7.31	7.31	0.35	6 - 8
AP	37.14	25.65	25.23	20 - 156
GPT	47.29	44.1	19.29	21 - 73
Creatinine	0.86	0.86	0.20	0.5 - 1.5
Urea	36.77	37.6	3.89	21 - 60

Table 3. Serial results of healthy dogs underwent hematological and serum biochemistry analysis after the intravenous treatment with allogenic adipose tissue derived stem cells

	Mean±Standard Deviation				
	D1	D2	D3	D4	TOTAL
RBC	7.29±0.38	7.27±0.47	7.39±0.5	7.63±0.4	7.395±0.1652
Hb	16.78±0.63	16.84±1.21	16.55±1.1	16.8±1.13	16.7425±0.1307
Ht	48.5625±1.05	48.8875±2.84	50.3825±2.36	50.2625±3.27	49.52375±0.933
TLeuk	10900±2399	10750±709	9725±1614	10725±1646	10525±538.9032
SN	7724±1882	6656±793	6245±3225	7774±1568	7099.75±768.5078
TLym	3130±1176	3387±751	2371±991	2678±1180	2891.5±454.2044
Platelets	278250±133128	230625±101169	280488±112486	329250±114140	279653.25±40275.19
TP	7.191±0.22	7.329±0.43	7.276±0.26	6.998±0.46	7.1985±0.1452
AP	50.54±35.16	57.83±40.74	49.51±32.43	59.35±47.17	54.3075±5.0014
GPT	42.56±10.14	47.25±10.31	48.05±9.05	51.1±16.38	47.24±3.5335
Creatinine	0.9038±0.18	0.9625±0.22	0.95±0.17	0.9375±0.24	0.93845±0.0252
Urea	38.91±3.57	40.1±9.5	43.18±14.10	37.64±10.86	39.9575±2.3715

Table 4. Statistical correlation ANOVA between the D0 and after-treatment average values, for hematological and serum biochemistry analysis of dogs that underwent intravenous treatment with allogenic adipose tissue derived stem cells

	p Value (95% confidence interval)				
	D1	D2	D3	D4	**Conclusion**
RBC	0.8736	0.8736	0.8736	0.3845	No difference
Hb	0.999	0.999	0.999	0.999	No difference
Ht	0.9494	0.9494	0.9494	0.9494	No difference
TLeuk	0.9754	0.9955	0.823	0.9968	No difference
SN	0.9999	0.7545	0.4824	0.9997	No difference
TLym	0.577	0.2742	0.9936	0.9947	No difference
Platelets	> 0.9999	0.8712	0.9999	0.7662	No difference
TP	0.8962	0.9999	0.9985	0.2419	No difference
AP	0.8787	0.6295	0.9048	0.5723	No difference
GPT	0.8952	> 0.9999	0.9998	0.9472	No difference
Creatinine	0.9736	0.6872	0.7698	0.8445	No difference
Urea	0.9732	0.8853	0.4587	0.999	No difference

The average values of all after-treatment analysis and the total average were completely between the reference interval for the species. The variance analysis between the control and treatment moments did not identify any statistical difference in the values, showing that the patients have not suffered any deviation from normality values from cellular or enzymatic parameters, which is correlated with no relevant systemic side effects after the cellular therapy using allogenic and commercial ADSC intravenously, in healthy dogs.

DISCUSSION

The results evidenced in this paper are able to reinforce the hypothesis from the authors, that the intravenous administration of allogenic ADSC is clinically safe in dogs. This affirmation is proven by the absence of laboratorial abnormalities in the evaluated parameters, that are routinely used for determination of possible organic dysfunctions. All the hematological and serum biochemistry parameters analyzed, even before or after the treatment, were between the reference values for the species. Even so, it was possible to prove the commercial viability from these cells for intravenous therapy, because the positive results from the quality proofs, especially for the high cellular viability, absence of mycological or bacterial contamination and correct cellular characterization according to the standards. The sum of the results can lead to the affirmation of commercial viability of these batches analyzed and the clinical safety for the systemic administration in health dogs.

Although the clinical safety of ADSC's therapies has been proven through previous studies, which showed positive results in treatment of specific pathological processes from diverse origins and organs (Escalhão *et al.*, 2017; Villatoro *et al.*, 2018; Rhew *et al.*, 2021), there is a lack of studies that evaluate the safety for use in clinically and laboratory healthy dogs. These studies are important and necessary, to divide the possible laboratorial value deviations, which can be caused for the disease installed, associated pharmacological or surgeries therapies, or specific by the cellular therapy, which can lead to mistake in the interpretation. This way, the use in healthy patients is able to evaluate just the therapy effect, without interference of the disease or another treatment that the patient underwent.

The other aim was related to the commercial viability of those cells and their quality proofs, and the results of the tests were of great importance. Eventually, there were some doubts regarding the commercial availability and quality of those cells, mainly related to companion animals (Pinheiro *et al.*, 2019a). By the way, the results revealed in this paper are able to refute the hesitation in this point, proving that quality proofs and the standard parameters defined by the international society for Stem Cell Research (ISSCR) and International society for Cellular Therapy were completely within the expected results, making sure that the products tested in this study are high-quality and reliable for clinical routine use. Even more, the quality proofs inserted into the isolation and production process of the ADSCs, associated with the commercial regularization by the government in the Brazilian territory for the Ministry of Agriculture, Livestock and Food Supply, associated with the regulation and laws firmed by the Veterinary Medicine Federal Council (Resolution no. 1363, from October 22, 2020), it was possible to ensure the safety of the production processes.

Among the commercial criteria for cellular commerce, some abilities are desirable and mandatory, which are used for the specific characterization of the ADSCs. The biomarkers expression of CD44, CD105 and MHC I, and CD 29 through mRNA have to be positive, and negative for MHC II (Screven *et al.*, 2014). Those proofs are important for the cellular batches production and standardization and are an important portion of the quality proofs evidenced in the research, for the cellular characterization. Furthermore, in addition to the biomarkers, the ADSCs must be the ability to differentiate in chondrocytes, adipocytes and osteoblasts when allocated into a specific culture medium for each cultivation (Arnhold and Wenisch, 2015), methodology that were applied in this study during the cellular cultivation and proved the maintenance of those characteristics, reinforcing the power of regeneration in different tissues of those cells.

The ADSCs can modulate the inflammatory and immunologic response in animals and humans undergoing some diseases or systemic dysfunctions (Kornicka *et al.*, 2018). But, through the leukocytes count here identified,

there were no alterations in the absolute number between the treatment moments and the control one, when the first lineage of immune response was evaluated. In the other hand, the leukocytes functions were not evaluated, which can be a limitation of this kind of evaluation. The hypothesis that could be related to the ADSC's activity in the white blood cells is that the direct effect is conditioned for some initial disease in the cellular function or production (Tieu *et al.*, 2020), which is not evident in healthy patients, as verified in this research. Another highlighted result is the absence of pro-inflammatory or allergic response in the dogs treated. In some cases, it is expected that allogenic treatments may cause allergic or inflammatory response, because of the incompatible genetic recognition. But, when the treatment involves ADSCs, it is not observed due to the absence of MHC II, which is responsible for the allergenic reaction (Screven *et al.*, 2014). Consequently, it is expected that no allergic or inflammatory response may be found, and this result is confirmed for the absence of increasing leukocytes number in this evaluation, either in the initial days, then after four weeks of treatment.

In relation to the organs and systemic effects of the therapeutic approach, the ADSCs have already been evaluated in relation to their effectiveness in the treatment of canine hepatic diseases, demonstrating favorable results in relation to the structural lesions and functional recovery, being able to decrease the serum biomarkers of tissue injury (Gardin *et al.*, 2018). The kidney also showed good results. For chronic and acute kidney injury it had the power to increase the kidney function, being able to reduce the serum biomarkers for kidney lesion, such as urea and creatinine (Lee *et al.*, 2017; Allinson *et al.*, 2023). A limitation of those cited studies is that all of them were directed at previously affected organs, which were previously affected for some injury and had those biomarkers increased before the treatment with ADSC. In this study, we observed that either in healthy animals, that were not affected by any previous disease or impairment of organs' function, the therapy was not able to cause any dysfunction in hepatic or kidney functions, which were proved through the maintenance of the normal values in the four moments evaluated after the intravenous administration of the ADSCs. Knowing that, with the previous results and with the results observed here, it is possible that the cellular therapy did not cause any liver or kidney dysfunction and, at the same time, may be able to improve their function and structure (Lee *et al.*, 2017; Gardin *et al.*, 2018; Allinson *et al.*, 2023).

In the commercial field, the ADSC's therapy is continuously growing, principally in veterinary medicine. But, for that, many studies are needed involving the clinical safety and viability for the use of these cells, which is also important for human medicine, and most of them are developed in animal models (Pinheiro *et al.*, 2019b). Many new methods of cellular isolation, cultivation and administration are developed constantly (Yasumura *et al.*, 2023). All these facts are around the importance of studies involving this class of cells and the cellular treatment. This way, it's important to conduct other studies in healthy animals, then in sick ones. In both modalities, it's possible to evaluate the clinical safety of the therapy, through the analysis of any dysfunction caused after the cellular administration, and it is favorable too for the analysis of the cellular effectiveness for treatment of specific diseases or clinical signs, being able to confirm in the two fields, healthy and sick animals, the positive effects of the therapy.

Thus, analyzing the results verified in this study, it was possible to affirm the clinical safety for the ADSC's intravenous treatment in laboratory healthy dogs, without any identification of side effects in the post-treatment evaluations, through the maintenance of the reference values of the biomarkers selected for the analysis. In addition, there were no side effects in the kidney or hepatic function, even 30 days after the therapy. In the view for the cellular viability, the quality proofs executed here showed satisfactory results and evidenced the safety of the commercial availability of those cells. Although other research should be executed for evaluation of effectiveness and safety for allogenic administration of ADSCs in the various systemic disorders, the clinical safety has been proven for intravenous treatment of adipose derived stem cells in clinical and laboratory healthy dogs up to 30 days after administration.

CONCLUSION

Considering the scarcity of literature on the use of mesenchymal cells in healthy animals, we highlight the relevance of this study. Given that the evidence indicates that these cells exhibit the standard characteristics required for application, adequate viability and the absence of adverse hematological effects, we can conclude that intravenous administration of allogeneic canine adipose mesenchymal cells is clinically safe, even when administered to healthy dogs.

REFERENCES

ALLINSON, C.S.; POLLOCK, C.A.; CHEN, X. Mesenchymal stem cells in the treatment of Acute Kidney Injury (AKI), Chronic Kidney Disease (CKD) and the AKI-to-CKD Transition. *Integr. Med. Nephrol. Androl.*, v.10, p.1-11, 2023.

ARNHOLD, S.; WENISCH, S. Adipose tissue derived mesenchymal stem cells for musculoskeletal repair in veterinary medicine. *Am. J. Stem Cells*, v.4, p.1-12, 2015.

CABON, Q.; FEBRE, M.; GOMEZ, N. *et al*. Long-term safety and efficacy of single or repeated intra-articular injection of allogeneic neonatal mesenchymal stromal cells for managing pain and lameness in moderate to severe canine osteoarthritis without anti-inflammatory pharmacological support: pilot clinical study. *Front. Vet. Sci.*, v.6, p.1-14, 2019.

ESCALHÃO, C.C.M.; RAMOS, I.P.; HOCHMAN-MENDEZ, C. *et al*. Safety of allogeneic canine adipose tissue-derived mesenchymal stem cell intraspinal transplantation in dogs with chronic spinal cord injury. *Stem Cells Int.*, v.2017, p.1-11, 2017.

GARDIN, C.; FERRONI, L.; BELLIN, G. *et al*. Therapeutic potential of autologous adipose-derived stem cells for the treatment of liver disease. *Int. J. Mol. Sci.*, v.19, p.4064, 2018.

HU, M.S.; MAAN, Z.N.; WU, J. *et al*. Tissue engineering and regenerative repair in wound healing. *Ann. Biomed. Eng.*, v.42, p.1494-1507, 2014.

KORNICKA, K.; ŚMIESZEK, A.; WEGRZYN, A. *et al*. Immunomodulatory properties of adipose-derived stem cells treated with 5-azacytydine and resveratrol on peripheral blood mononuclear cells and macrophages in metabolic syndrome animals. *J. Clin. Med.*, v.7, p.383, 2018.

LEE, S.; RYU, M.; SEO, M. *et al*. Mesenchymal stem cells contribute to improvement of renal function in a canine kidney injury model. *In Vivo*, v.31, p.1115-1124, 2017.

LI, N. Interactions between mesenchymal stem cells and the immune system. *Cell. Mol. Life Sci.*, v.74, p.2345-2360, 2017.

LIOTTA, A.; BOLEN, G.; CEUSTERS, J. *et al*. Clinical safety of computed tomography-guided injection of autologous muscle-derived mesenchymal stem cells in the intervertebral disc in dogs. *J. Stem Cells Regen. Med.*, v.17, p.43-48, 2021.

LIU, G.; DAVID, B.T.; TRAWCZYNSKI, M.; FESSLER, R.G. Advances in Pluripotent Stem Cells: history, mechanisms, technologies, and applications. *Stem Cell Rev. Rep.*, v.16, p.3-32, 2020.

MARX, C.; SILVEIRA, M.D.; NARDI, N.B. Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cells Dev.*, v.24, p.803-813, 2015.

PARK, S.A.; REILLY, C.M.; WOOD, J.A. *et al*. Safety and immunomodulatory effects of allogeneic canine adipose-derived mesenchymal stromal cells transplanted into the region of the lacrimal gland, the gland of the third eyelid and the knee joint. *Cytotherapy*, v.15, p.1498-1510, 2013.

PINHEIRO, L.L.; LIMA, A.R.; BRANCO, E. Is stem Cell commerce in small animal therapies scientifically and morally justified? *Stem Cell Rev. Rep.*, v.15, p.506-518, 2019a.

PINHEIRO, L.L.; LIMA, A.R.; MARTINS, D.M. *et al*. Mesenchymal stem cells in dogs with demyelinating leukoencephalitis as an experimental model of multiple sclerosis. *Heliyon*, v.5, p.e01857, 2019b.

PRZADKA, P.; BUCZAK, K.; FREJLICH, E. *et al*. The role of Mesenchymal Stem Cells (MSCs) in veterinary medicine and their use in musculoskeletal disorders. *Biomolecules*, v.11, p.1141, 2021.

RESHAK, A.H.; SHAHIMIN, M.M.; BUANG, F. Comparative study on human and bovine AT-SC isolation methods. *Prog. Biophys. Mol. Biol.*, v.113, p.295-298, 2013.

RHEW, S.; PARK, S.; LI, Q. *et al*. Efficacy and safety of allogenic canine adipose tissue-derived mesenchymal stem cell therapy for insulin-dependent diabetes mellitus in four dogs: a pilot study. *J. Vet. Med. Sci.*, v.83, p.592-600, 2021.

SCREVEN, R.; KENYON, E.; MYERS, M.J. *et al*. Immunophenotype and gene expression profile of mesenchymal stem cells derived from canine adipose tissue and bone marrow. *Vet. Immunol. Immunopathol.*, v.161, p.21-31, 2014.

THRALL, M.A.; WEISER, G.; ALLISON, R.W.; CAMPBELL, T.W. *Veterinary hematology and clinical chemistry*. 2.ed. Oxford: Wiley Blackwell, 2012.

TIEU, A.; LALU, M.M.; SLOBODIAN, M. *et al*. An analysis of mesenchymal stem cell-derived extracellular vesicles for preclinical use. *ACS Nano*, v.14, p.9728-9743, 2020.

VALDIVEL, S. Inflammation in myocardial injury – Stem cells as potential immunomodulators for myocardial regeneration and restoration. *Life Sci.*, v.250, p.117582, 2020.

VILLATORO, A.J.; HERMIDA-PRIETO, M.; FERNÁNDEZ, V. *et al*. Allogeneic adipose-derived mesenchymal stem cell therapy in dogs with refractory atopic dermatitis: clinical efficacy and safety. *Vet. Rec.*, v.183, p.654-654, 2018.

VOGA, M.; ADAMIC, N.; VENGUST, M.; MAJDIC, G. Stem Cells in Veterinary Medicine - Current State and Treatment Options. *Front. Vet. Sci.*, v.7, n.278, p.1-20, 2020.

YASUMURA, Y.; TESHIMA, T.; NAGASHIMA, T. *et al*. Immortalized Canine Adipose-Derived Mesenchymal Stem Cells as a Novel Candidate Cell Source for Mesenchymal Stem Cell Therapy. *Int. J. Mol. Sci.*, v.24, p.2250, 2023.