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UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS AS AN ALTERNATIVE TO BONE MARROW IN IMMUNOSUPPRESSIVE THERAPY

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Background: Due to their immunomodulatory characteristics, mesenchymal stromal cells (MSC) have become an option in immunosuppressive therapy. HLA-G expression appears to play a key role in immunomodulation, inhibiting proliferation of immune cells and inducing activation of regulatory cells, modulating host’s immune response. Thus, identifying a source of MSC with high HLA-G expression can be a good strategy for future treatments.

Methods: MSC from umbilical cord tissue (UCT) (n=3) were compared to a sample of bone marrow (BM) (n=1) under the following growing conditions: (i) standard cultivation; (ii) stimulation of inter-feron-γ (IFN-γ); and (iii) hypoxia condition (2% O2). Expression of CD14, CD73, CD34, CD19, CD166, CD29, CD45, CD90, HLA-DR and CD105 markers and co-stimulatory CD40, CD80 and CD86 markers were analyzed. The analysis of CD152 molecule (intracellular isoform) and membrane HLA-G1 isoform was performed by flow cytometry to study the expression of immunosuppressive molecules. The analysis of soluble HLA-G5 isoform was performed by enzyme-linked immunosorbsent assay (ELISA). For analysis of the immunosuppressive potential of MSCs, a lymphocyte (PBMCs) inhibition assay was performed in proportions 1:2 and 1:1 (MSC:PBMC).

Results: The expression of cellular markers was following that required by the International Society for Cell and Gene Therapy. All samples showed negative expression for co-stimulatory markers CD40, CD80 and CD86 and positive expression for CD152. CD152 and HLA-G1 molecule inhibition assay was performed in proportions 1:2 and 1:1 (MSC:PBMC).

Conclusion: We observed that IFN-γ treatment increased inhibitory capacity of BM MSC, but not in UCT-MSC, being indicated in cultivation of BM MSC used for immunosuppressive therapy. In addition, UCT-MSC proved to be an efficient alternative to BM MSC. UCT-MSC showed an advantage in the expression of immunosuppressive

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USE OF MESENCHYMAL STEM CELLS IN THE TREATMENT OF BONE MARROW APLASIA IN DOGS

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Background: Bone marrow aplasia is a serious condition that affects dogs and cats. It is defined as a reduction or absence of all types of cells in the peripheral blood (pancytopenia) and hypoplasia of the three cell lines: erythroid, myeloid, and megakaryocyte in the bone marrow. The reduction or absence of one of these cell types is called hypoplasia, while the absence of the three cell types is called aplasia. Its causes can be infectious, hormonal, secondary to the use of drugs, toxins and radiation, neoplastic or idiopathic. The treatment of bone marrow aplasia in small animals is not well established, that way its objective is to stabilize the patient, who usually has profound anemia or significant thrombocytopenia, requiring transfusion of red blood cells or platelet concentrates, respectively. There is often no response to these therapies and alternatives are very few, making euthanasia an option. Thus, stem cell therapy has emerged as a new alternative for the treatment of bone marrow aplasia. Stem cells act by stimulating hematopoiesis, bringing important improvement to clinical signs and hematological exams, improving the patient’s prognosis. The present clinical study aimed to demonstrate the beneficial effects of the application of multiple doses of allogenous mesenchymal stem cells, derived from adipose tissue in dogs with bone marrow aplasia.

Methods: Eight dogs with bone marrow aplasia, confirmed by clinical and hematological exams, presenting persistent pancytopenia and refractory to treatment with antibiotics, corticosteroids and ethroproetin participated in the study. All animals had been receiving blood transfusions at variable intervals. The dogs received at least 3 intravenous applications of the solution containing mesenchymal stromal cells in intervals between 10 and 30 days. The doses administered contained at least 1 x 10^7 viable MSCs. The interval between the first and the second application of MSCs varied between 10 and 15 days.

Results: All treated animals showed improvement in the hematological condition, with consequent spacing or interruption of blood transfusions, which resulted in a substantial improvement in quality and expectancy of life.

Conclusion: Serial application of 1 x 10^7 MSCs proved to be a safe and good option of treatment, helping in the clinical improvement of dogs with bone marrow aplasia. These results present another therapeutic opportunity to be used in the treatment of this pathology.

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USE OF TOCILIZUMAB AND MESENCHYMAL STROMAL CELLS IN THE TREATMENT OF SEVERE COVID-19 – A CASE REPORT

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Background: Currently, there is no specific treatment for coronavirus disease, and some drugs and cell-based therapy have been tested as alternatives. This work aims to evaluate the effects of the combined use of humanized recombinant monoclonal antibody capable of binding the IL-6 receptor (Tocilizumab), and umbilical cord tissue-derived mesenchymal stromal cells (UCT-MSC) in the treatment of a patient with severe COVID-19 admitted to the intensive care unit (IUC) and submitted to mechanical ventilation.

Methods: This study is part of a project approved by the National Research Ethics Commission (CONEP); CAAE: 30833820.8.0000.0020. The patient had a diagnostic criterion for the severe acute respiratory syndrome resulting from infection with SARS-CoV-2 and received two 400 mg doses of tocilizumab, three infusions of 500,000 CTM / kg plus full anticoagulation. TCU-MSC were obtained from healthy donors. The following parameters were evaluated in the pre-infusion of cells (D1), on the day following each infusion (D2, D4, and D6), on the 14th and 60th day after the first infusion (D14 and D60): viral load, immune response (Regulatory T lymphocytes), C-reactive

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protein level in plasma, oxygen saturation, respiratory rate, total lymphocyte count and subpopulations (platelets, inflammatory cells, and reticulocytes), TGO / TGP, increased prothrombin time, D-dimer, creatinine, troponin.

**Results:** The relative viral quantification decreased gradually from 1 (D1) to 0.06 (D6) RdRP / RNApol, undetectable in D14. An increase in the relative viral quantitation, troponin.

**Conclusion:** The combined use of tocilizumab and MSC is safe, without adverse effects, and the results of this case report prove to be a promising alternative in the treatment of patients with severe acute respiratory syndrome due to SARS-CoV-2.

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**VALIDATION OF A CYTOTOXICITY ASSAY USING ADULT HUMAN MESENCHYMAL STEM CELLS AS AN ALTERNATIVE TO THE USE OF ANIMALS**

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**Background/Objective:** Russell and Burch, in 1959, made the first mention of the 3R's strategy involving animals in research: Reduce, Replace or Refine. In vitro methods with stem cells are being developed to predict drug potential as an alternative to animal use. Based on the assumption that a substance can disrupt the differentiation capacity, we investigated the ability of mesenchymal stem cells derived from adipose tissue (ADSCs) to predict drug toxicity, allowing the initial dose estimation the acute toxic class (ATC) method.

**Methods:** We evaluated ten chemicals representing the categories of toxicity 1-5 and unclassified toxicity of the Global Harmonized Classification and Labeling System (GHS) and determined the dose-response curves using the Hill function with a R2 > 0.85. The resulting IC50 (half-maximal inhibitory concentration of a chemical) values were used to predict LD50 (amount of a chemical which causes the death of 50% of a group of test animals) and estimate the ATC method's initial dose. Hybrid microplate reader (HMR) and High-content imaging microscope (HCl) were tested. HMR reading was optimized using the drug Sodium Dodecyl Sulfate (SDS) as control.

**Results:** Our results indicated that using the adipogenesis inhibition assay to estimate the initial dose for the ATC method would decrease the use of animals for 7 out of 10 tested substances, including all the most toxic substances (GHS 1-3 categories). We observed lower IC50 intensity and differentiation area parameters compared to nuclei quantification. Additionally, concentrations considered non-toxic inhibited adipogenesis, indicating that adipogenesis evaluation may be a sensitive method to evaluate in vitro toxicity. Regarding the standardization of reading in HMR, the ADSCs responded in a dose-dependent way to the SDS to evaluate adipogenesis. Both IC50 and LD50 were reproducible within the expected toxicity range, enabling reading in 3 x 3 fields (reading faster and less bulky).

**Conclusion:** These results show that the proposed method may be a complementary alternative assay to assess in vitro toxicity.

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**VIABILITY AND DISTRIBUTION EVALUATION OF MESENCHYMAL STEM CELLS DERIVED FROM ADIPOSE TISSUE MARKED BY NANOCRYSTALS IN HORSES WITH CHRONIC LAMINITIS 24 HOURS AFTER REGIONAL INFUSION - PRELIMINARY STUDY**

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**Background:** The objective of this study was to evaluate the viability, distribution and setting of previously characterized and differentiated equine Ad-MSC labeled with the Qtracker cell labeling kit after infusion into the forelimb of horses with chronic laminitis.

**Methods:** Subcutaneous fat from healthy horse was collected, and Ad-MSC were isolated and cultured. In the third pass, Ad-MSC were marked with fluorescent nanocrystals. Labeling was carried out according to the manufacturer's instructions. Briefly, two million Ad-MSC were marked by adding 2ul of nanocrystals, 2ul of PBS and 200ul of DMEM medium, followed by incubation at 37°C and 5% CO2 for 1 hour. Cell viability was determined by staining with trypan blue before staining and at 1 and 24 hours after staining. 20 million allogeneic Ad-MSC marked according to the manufacture's standards and suspended with 20 ml of PBS were infused in 2 horses with chronic laminitis. The infusion was performed only in one member (treated), previously drawn, by regional limb perfusion (RLP) and the tourniquet was maintained for 30 minutes. In the contralateral limb, only PBS was infused. Lamellar tissue biopsies were performed 24 hours later. The absorption of nanocrystals in the cell was observed through fluorescence microscopy in samples of labeled cells and lamellar tissue obtained by biopsy.

**Results:** Cytoplasmic absorption of nanocrystals was observed 1 and 24 hours after staining. The viability of Ad-MSC in culture before labeling was 91%. After 1 hour of incubation with nanocrystals, viability decreased slightly to 85% followed by 83% viable cells 24 hours after incubation. The results of this study demonstrate that Qdot nanocrystals can be successfully introduced into equine Ad-MSC with minimal cytotoxic effect on these cells. The cytoplasmic absorption of the nanocrystals was observed in the form of cell niches in the secondary laminae in both animals, but in greater quantity in the samples of the treated limb. Although lamellar tissue autofluorescence was observed in both limbs, there was a wide predominance of labeled Ad-MSC in the treated limbs.

**Conclusion:** The cell labeling protocol was successful, and the labeled cells remained in the lamellar tissue of horses with chronic laminitis 24 hours after the RLP. The preliminary findings of this study indicate that the cells remained in the limb that received the RLP, demonstrating therapeutic potential in the use of this technique.