Evaluation of the multidifferentiation of canine adipose-derived stem cells (C-ASC) in early passage ex vivo from different anatomic regions: preliminary results

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Abstract

Adipose-derived mesenchymal stem cells (ASC) exhibit three characteristics: multipotentiality, immunomodulation and angiogenesis, which makes them very attractive and suitable for tissue engineering. Those cells could be used as an alternative to traditional treatment in animal models and veterinary medicine. Domestic animal models offer a relevant contribution in advancing stem cell therapies for both human and clinical veterinary applications. Specifically in orthopedic medicine the usage of mesenchymal stem cell for cell therapy as an alternative treatment has been indicated because it could be in vitro differentiated into osteocyte and in vivo promoted osteogenesis. Furthermore, several studies adopting cell therapy with ASC in human and canine bone defects were able to accelerate and improve tissue regeneration. But there are not enough basic studies on in vitro multipotentiality and passages effect of canine ASC obtained from different anatomic regions upon cell therapy efficacy.

The objective of this study was to compare two different sources of ASC – subcutaneous fat (Sc) and visceral fat (Vs) and the passage effect in relation to its multipotentiality. During ovarectomy surgery, the ASC were obtained from the adipose tissue of 5 young female canines (4 – 11 months) from two anatomic regions: abdominal (from Sc) and periovaric (from Vs). The Sc and Vs ASC in passages 2 and 4 (P2 and P4) were induced to in vitro differentiation into adipocytes, chondrocytes and osteocytes. The osteogenic differentiation was certified by specific staining with Alizarin Red and microscopic images were randomic captured (9 fields/well) for analysis with ImageJ software. The isolated cells were confirmed as ASC when was possible to detected fibroblastic morphology, adhesiveness to plastic and ability to differentiate into adypocites, chondrocytes and osteocytes. The isolated cell population from ASC-Sc showed 7.84 ± 1.89 % area osteogenic differentiation and ASC-Vs 2.51 ± 0.15 %. These data demonstrated that ASC obtained from Sc had a higher capacity to in vitro differentiate into osteogenic lineage than Vs in both passages P2 and P4 ($p < 0.01$). Besides, when ASC from passages P2 and P4 were compared was possible to observe no interference in potential osteogenic differentiation. The adipogenic and condrogenic in vitro differentiation was confirmed in both anatomic ASC sources. The other tests to evaluate senescence,

chromosomic stability and immunomodulation capacity will be performed to complete ASC in vitro characterization. These finding suggests that the osteogenic differentiation potential can be influenced by the anatomic region of the tissue and ASC-Sc showed higher osteogenic differentiation capacity than ASC-Vs. These studies are still undergoing, with the purpose to better understanding the differences about mesenchymal cells obtained from different regions in order to optimize cellular therapy.

Ethics Committee: HCPA-POA. Protocolo 130510.