ABSTRACTS

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Comparison of canine and human adipose tissue derived mesenchymal stem cells (cAD-MSC) — Isolation, identification, differentiation and radiolabelling

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OBJECTIVES AND METHODS: The autologous mesenchmal stem cell (MSC) therapy is a promising novel treatment choice in the field of regenerative medicine and tissue engineering for both human and veterinary medicine (1). Canine MSCs can be derived from different tissue types including bone marrow, adipose tissue (2) and foetal adnexa (3). MSCs are self renewing and have the potential to differentiate into different mesodermal cell types. Human MSCs can be isolated by plastic adherence, they show fibroblast-like morphology, must differentiate to osteoblasts, adipocytes and chondroblasts in vitro and can be identified by the expression of specific cell surface antigens (4).

Our aim was to establish isolation-, identification-, differentiation- and radiolabelling method of canine adipose tissue derived MSCs (cAD-MSCs) and to prove the usefulness of the canine model for human biomedical sciences.

The adipose tissue samples were collected from visceral and different subcutaneous fat depots from Beagle dogs using standard sterile surgical procedures. The SVF (Stromal Vascular Fraction) was obtained by digestion with collagenase then after centrifugation were incubated in Dulbecco modified Eagle’s medium supplemented with 10% FBS. The differentiation capacity of isolated cells was investigated at passage 2 and 4. The cells were culturing in specific adipogenic, chondrogenic or osteogenic differentiation media.

The expression of cell surface markers of canine and human AD-MSCs was identified by flow cytometry with anti-human (CD44-PE, CD73-PE, CD90-APC) and anti-mouse (CD44-PE) antibodies. The SVF (Stromal Vascular Fraction) was obtained by digestion with collagenase then after centrifugation were incubated in Dulbecco modified Eagle’s medium supplemented with 10% FBS. The differentiation capacity of isolated cells was investigated at passage 2 and 4. The cells were culturing in specific adipogenic, chondrogenic or osteogenic differentiation media.

RESULTS: The adipose derived MSC cells — similarly to the human adipose derived cells — showed fibroblast-like morphology in light microscope. The phenotype of the isolated cAD-MSC was identified by detecting cell surface markers with flow cytometry (FACS). 100 % of the cells were positive with anti-human CD90 and anti-mouse CD44 antibody. Human CD44 and CD73 antibodies were negative in the canine MSC population which might be explained by the nonspecific staining of human antibodies in canine cells. We were able to differentiate the isolated cells into adipogenic, chondrogenic and osteogenic lineages in the presence of specific induction factors. The expression of cell surface markers of canine and human AD-MSCs was identified by flow cytometry with anti-human (CD44-PE, CD73-PE, CD90-APC) and anti-mouse (CD44-PE) antibodies. The expression of cell surface markers of canine and human AD-MSCs was identified by flow cytometry with anti-human (CD44-PE, CD73-PE, CD90-APC) and anti-mouse (CD44-PE) antibodies.

Intravenous application: 0-2 hours postapplication over 90% of the injected radioactivity detected in the lungs. Intraarticular application: 0-24 hours postapplication over 90% of injected radioactivity stayed within the knee joint.

CONCLUSION: Our preliminary results suggest that isolation-, identification-, differentiation- and radiolabelling of cAD-MSC are feasible. Canine adipose tissue represents an easily available source for veterinary stem cell therapies. Beside dog proved to be a promising biomedical model in the field of stem cell therapy too. On the bases of this study we have separated MSCs from canine foetal tissues. The detailed analyses are in progress.
