

Cell Therapy in Intervertebral Disc Degeneration

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademien, Göteborgs universitet kommer att offentlig försvaras i R-aulan, Sahlgrenska Universitetssjukhuset, Mölndal, den 7 maj 2021, klockan 09.00

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Avhandlingen baseras på följande delarbeten

- I. Papadimitriou N, Thorfve A, Brantsing C, Junevik K, Baranto A and Barreto Henriksson H (2014). "Cell viability and chondrogenic differentiation capability of human mesenchymal stem cells after iron labeling with iron sucrose." *Stem Cells Dev* 23(21): 2568-2580
- II. Papadimitriou N, Li S and Barreto Henriksson H (2015). "Iron sucrose-labeled human mesenchymal stem cells: in vitro multilineage capability and in vivo traceability in a lapine xenotransplantation model." *Stem Cells Dev* 24(20): 2403-2412
- III. Papadimitriou N, Hebelka H, Hingert D, Baranto A, Barreto Henriksson H, Lindahl A, Brisby H. "Intra discal injection of iron-labeled autologous mesenchymal stromal cells in patients with chronic low back pain. A feasibility study with 2 years follow up." *Manuscript, submitted*
- IV. Papadimitriou N, Hebelka H, Waldenberg C, Lagerstrand K, Brisby H. "Longitudinal MRI evaluation of disc and adjacent tissues up to 2 years after intervertebral disc injection of autologous mesenchymal stromal cells." *Manuscript*
- V. Henriksson H B, Papadimitriou N, Hingert D, Baranto A, Lindahl A and Brisby H (2019). "The traceability of mesenchymal stromal cells after injection into degenerated discs in patients with low back pain." *Stem Cells Dev* 28(17): 1203-12

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Background: Chronic low back pain (LBP) is the leading cause of disability worldwide. Intervertebral disc degeneration (IDD) is central in the pathogenesis. The injection of bone marrow-derived mesenchymal stromal cells (BM-MSCs) into degenerate intervertebral discs (IVDs) has been proposed as an alternative therapy. The aims of these studies were to investigate the iron labeling of human BM-MSCs *in vitro* and in an animal model, to assess the feasibility and efficacy of the intradiscal injection of autologous, iron-labeled BM-MSCs in patients with LBP and IDD, and to examine the survival of these cells post-injection.

The studies: In **studies I and II** BM-MSCs from human donors were labeled with iron sucrose (Venofer®). In study I, histology showed labeling of 98.1% of the cells. Flow cytometry showed good viability and somewhat lower expression of MSCs' surface markers (CD105) for the labeled cells. Cells cultured in the pellet mass system revealed: (i) traceability of labeled cells 28 days post-labeling and (ii) production of extracellular matrix (ECM). Immunohistochemistry (IHC) detected ECM components (coll2A1 and C6S). qRT-PCR (pellets) showed no differences between labeled and non-labeled cells for genes of chondrogenesis, ECM production and surface proteins.

In **study II**, the *in vitro* trilineage differentiation capability of the labeled cells was confirmed by detection of (i) GAGs (chondrogenesis) in pellets and (ii) calcium deposits (osteogenesis) and (iii) lipid droplets (adipogenesis) in cell cultures. Furthermore, a lapine animal model was used. Human BM-MSCs were injected in IVDs of 12 healthy animals (25x10⁴cells/IVD). One IVD received labeled and one non-labeled cells and the animals were sacrificed 1- and 3-months post injection. The presence of human cells and the traceability of iron-labeled cells were confirmed by means of IHC and histology respectively. Cell viability per IVD (all cells) decreased by 25% at 3 months for the injected IVDs (regardless of the labeling).

In **study III**, ten patients from the waiting list for lumbar surgery were recruited to receive an intradiscal injection of autologous, expanded, iron-labeled BM-MSCs in degenerate IVDs (1x10⁶ cells/IVD). The up to 2-year follow-up comprising of patient-reported outcome measures (PROMs) and magnetic resonance imaging (MRI) controls at regular intervals, revealed no adverse events and no evident amelioration of the PROMs on a group level over time. Five of the patients chose to proceed with the originally planned surgical procedure within 2 years from the injection.

Study IV was a longitudinal evaluation of the MRI investigations of the patients enrolled in study III. Injected and adjacent lumbar levels were assessed for multiple qualitative (Pfirrmann grade, IVD and endplate homogeneity, Modic changes) and quantitative (IVD height and angle, IVD signal intensity) parameters. A detailed baseline characterization was performed. No significant changes over time were seen.

In **study V** explanted tissues from injected IVDs were harvested from 4 patients from study III that proceeded to lumbar surgery, 8- (3 patients) or 28-months (1 patient) post-injection. Histological assessment showed the presence of iron-labeled cells in tissues explanted 8 months post-injection, with signs of metabolic activity in their vicinity. Expression of genes related to chondrogenesis (SOX9), ECM synthesis (COL2A1) and proliferation (PCNA) was confirmed by IHC investigations.

Conclusions: Iron sucrose labeling of BM-MSCs does not markedly affect cell viability and functionality. Intradiscal injection of autologous, expanded, iron-labeled BM-MSCs was a safe procedure. PROMs did not improve significantly in the present cohort; 5/10 patients could forgo surgery for a minimum of 2 years. Longitudinal MRI investigations revealed no adverse effects on the treated or the adjacent levels and no amelioration. Labeled BM-MSCs could be detected in IVD tissues explanted 8 months post-injection, indicating survival and engraftment of the injected cells in the IVDs.

Keywords: Low back pain, mesenchymal stromal cell, intervertebral disc, intervertebral disc degeneration, degenerative disc disease, cell therapy