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Publication details:
ORS Annual Meeting Transactions Vol.30
0149-6433 (ISSN)

Event details:
51st Annual Meeting of the Orthopaedic Research Society
Washington, DC

Publication Date:
2005

DOI:
https://doi.org/10.26190/unsworks/755

1837

SURVIVAL AND DIFFERENTIATION OF SYNGENEIC BONE MARROW-DERIVED MONONUCLEAR CELLS IN RAT INTERVERTEBRAL DISCS

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Introduction

Intervertebral disc degeneration may cause chronic low back pain. Disc degeneration is characterized by dysfunctional cells and a decrease in extra-cellular components. Bone marrow derived mononuclear cells (MNC´s) are a heterogeneous cell population which contains different stem/progenitor cells including mesenchymal stem cells. Transplantation of stem cells and other immature cell lines may provide a new approach to treat disc degeneration, however it is unclear whether transplanted cells can survive and differentiate in the non-vascularized disc tissue. The aim of the present study was to evaluate the feasibility of transplanting bone marrow derived MNC´s to the intervertebral disc in a syngeneic rat model.

Methods

Bone marrow was collected from syngeneic Sprague-Dawley rats and mononuclear cells were isolated by Ficoll density centrifugation. The cells were labeled with a fluorescence dye (CellTracker Orange, Molecular Probes, Eugene, OR) and suspended in phosphate saline buffer (PBS). 10-20µl of the cell suspension ($1\text{-}2\times10^5$ cells/disc) was injected into coccygeal discs in 12 syngeneic rats. For each rats two discs were injected and one disc served as control. The rats were sacrificed after 0, 7, 14 or 21 days. For each time point the discs from one animal was saved for routine histological staining. The injected discs of the other animals (i.e. 4 discs per time point) were formalin-fixed, frozen and sectioned together with the control discs. Frozen disc sections were visualized with fluorescence microscopy and the number of transplanted cells assessed. Expression of type II collagen, a marker of chondrocytes and chondrocyte-like cells in the disc, was assessed in the transplanted cells using immunofluorescence technique.
The study was approved by the University of New South Wales animal care and ethics committee. Numbers of cells are expressed as mean ± standard error of the mean (SEM). Mann-Whitney test was used to compare number of cells at different time points with baseline.

Results

No major histological changes (including changes in disc height and infiltration of inflammatory cells) could be detected after injection of MNC´s into intervertebral discs. All cell-suspension injected discs contained transplanted bone-marrow cells (Fig 1). The discs within each time-group demonstrated a large variation in number of detected cells. There was a significant decrease in detected cells at day 14 compared to baseline (day 0) (Fig 2). No difference was detected between detected cell number at 7, 14 and 21 days. Transplanted MNC´s expressed collagen II at 21 days but not at 7 and 14 days.

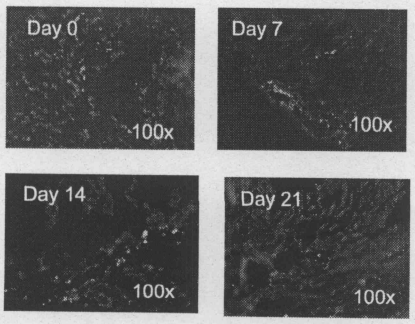

Figure1: *Injected bone marrow derived mononuclear cells (red dots) were detected within the intervertebral discs at thel investigated time points.*

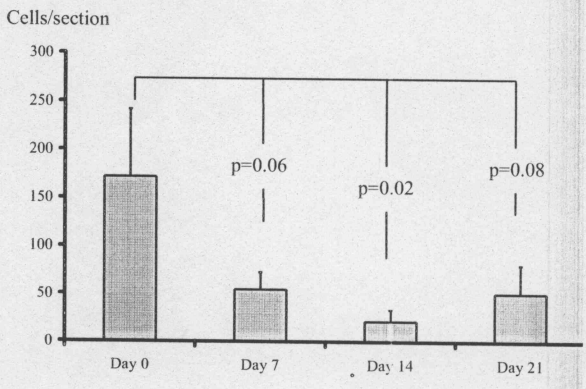

Figure 2. *Number of transplanted cells/section at different time points. Four discs were investigated at each time point and the cells counted in 10 sections per discs.*

Discussion:

The results suggest that transplanted bone marrow-derived mononuclear cells can survive and differentiate within the intervertebral disc in rats. The expression of a chondrogenic marker, type II collagen, in injected cells 21 days after transplantation, indicate that the micro-environment influences the transplanted cells to differentiate into cell types normally present within the intervertebral disc. One of the advantages of using unselected bone marrow derived mononuclear cells, instead of selected populations of stem cells, is that the preparation of cells after extraction from the bone marrow is minimal and therefore quick. This may be desired in a clinical setting. Further studies in models of disc degeneration are warranted to investigate the regenerative potential of the disc following bone marrow derived MNC´s transplantation.

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51st Annual Meeting of the Orthopaedic Research Society
Poster No: 1075