Protocol

Osteogenic Potential of Mesenchymal Stem Cells (MSCs) based on *in vitro* studies in Man and Horse: A systematic Review

Line Nymann Thomsen[1], Lise C. Berg[1] and Else Marie Bartels[2]

[1]Department of Large Animal Sciences, University of Copenhagen, Taastrup, Denmark

[2]The Parker Institute, Department of Rheumatology, Copenhagen University Hospital Bispebjerg and Frederiksberg, Denmark

Corresponding Author:

Else Marie Bartels

The Parker Institute

Frederiksberg Hospital

Ndr. Fasanvej 57

2000 Frederiksberg

else.marie.bartels@regionh.dk

Tel. +45 38164168

Short Title: Osteogenic potential of MSCs in OA

Abstract

Objective: Mesenchymal stem cells have been suggested as a method of treatment in bone-related diseases, including serious bone fractures. To facilitate the development of these treatment methods, our aim is to define osteogenic differentiation of mesenchymal stem cells from horse and man *in vitro* with regards to origin, culture environment and quality of outcome, and to compare cells originating the two types of donors.

Methods: A systematic search in the bibliographic databases: Medline via Pubmed, EMBASE, CAB Abstracts, Agricola, and Agris, all via OVID, and BiosisPreviews and Web of Science via ISI Web of Knowledge, followed by selection selection of studies according to set criteria concerning cell type and function. Data will be analysed according to origin of cells, culture methods used, and quality of osteogenic differentiation.

Results: Out of X retrieved references Y studies were selected for analysis.

Conclusion:

Prospero No:

Introduction

Mesenchymal stem cells facilitate bone healing and fracture repair (Bruder *et al.*, 1998) and show promising results when used as therapy in bone-related disease (Horwitz *et al.*, 1999). Humans and horses share development of bone- and joint-related disorders (Devas, 1967;Kawcak *et al.*, 2001;McIlwraith *et al.*, 2012).

According to the US Department of Health, a large numbers of horses are subject to treatment and therapeutic intervention for bone-and joint related disorders every year (http://www.aphis.usda.gov/animal_health/nahms/equine/). Due to this, horses are subject to intense research, and much knowledge exists concerning equine bone and joint diseases (Chu *et al.*, 2010). The number and nature of bone and joint defects in horses are similar to those in humans, making the horse a very relevant model for bone and joint related disorders in humans (Chu *et al.*, 2010).

In both horse and man, mesenchymal stromal cells or mesenchymal stem cells (MSCs) have been investigated for possible cell-based treatment of bone- and joint-related disorders. MSCs are multipotent cells which can differentiate into mesenchymal lineages (Pittenger *et al.*, 1999). It has been shown that recruitment of MSCs plays a crucial role in bone repair (Milner *et al.*, 2011), where local mobilization of MSCs occurs from both bone marrow and periosteum. This local recruitment could be boosted by injection of culture-expanded MSCs (Milner *et al.*, 2011). Yet culture expansion of MSCs and correct choice of MSC cell type require extensive understanding of the nature of MSCs.

MSCs have been found in a range of tissues, including adipose tissue, bone marrow, periosteum, peripheral blood, placenta, skin, synovium, and umbilical cord blood (De Bari *et al.*, 2001;De Bari *et al.*, 2006;Kassis *et al.*, 2006;Pittenger *et al.*, 1999;Shih *et al.*, 2005;Yen *et al.*, 2005;Zuk *et al.*, 2001). The minimal criteria to characterize human MSCs are tri-lineage potential (adipogenic, chondrogenic and osteogenic), adherence to plastic in culture, and surface expression of a set of defined markers (Dominici *et al.*, 2006). Although these minimal criteria apply to all MSCs regardless of their different tissue origin, MSCs from various tissues appear to possess different potential for osteogenic and chondrogenic differentiation (Im *et al.*, 2005). As reviewed by Boeuf and Richter (Boeuf & Richter, 2010), differences in chondrogenic potential could be caused by the origin of the cells from different tissues, the isolation methods used to segregate cells, or medium composition used to culture the cells. Likewise, osteogenic potential must be influenced by these factors.

Based on the hypotheses that MSCs from different tissues are comparable but not identical and that MSCs have an unequal osteogenic potential, the tissue origin and culture micro-environment will play a role in the osteogenic differentiation potential of a particular MSC. The aim of this

systematic review is to define the possible cell types, the culture conditions used *in vitro* to induce a particular cell type (osteocyte) in equine and human MSC cultures, and the results obtained. In addition, the cell performance from horse and man in the included studies will be compared to investigate, if the findings support the hypothesis that horse is an applicable model for man in terms of bone regeneration in bone-related diseases.

Methods

A systematic literature search followed by study selection and data extraction according to pre-specified eligibility criteria. Data analyses will be performed according to cell type, environmental factors for growth and other influencing factors. The protocol, will be made publicly available via PROSPERO (XXXX): www.crd.york.ac.uk/PROSPERO/

LITERATURE RETRIEVAL

The following bibliographic databases will be searched up to XXXX 2015: MEDLINE via PubMed from 1950, EMBASE via OVID from 1980, CAB abstracts via OVID from 1910, Agricola via OVID from 1970, Agris via OVID from 1975, and BiosisPreviews via ISI Web of Knowledge from 1969, and Web of Science from 1900.

The search strategy will be: (mesenchymal stem cell* OR mesenchymal stromal cell* OR multipotent stromal cell* OR multi-potent stromal cell* OR cord blood stromal cell*) AND (osteogenic OR osteogenesis OR bone formation) AND (culture* OR in vitro) AND (human OR horse OR equine). All words will be searched as free text and, where applicable, also as keywords. Reference lists from reviews will be screened for further studies.

ELIGIBILITY CRITERIA

Inclusion criteria: Studies of defined MSCs from horse or man *in vitro* which demonstrate osteogenic activity.

Two authors (LNT and EMB) will screen the retrieved literature for possible inclusion in the study, and disagreement will be solved by LCB.

DATA EXTRACTION

Data from the selected studies will be extracted by two reviewers (LNT and LCB) and questions will be solved together with EMB. A standard data-extraction form is developed for data collection:

Study	Subject	Origin of cells	Methods	Results	Potential bias

ANALYSES

The included studied will be compared according to origin of cells (human, horse, tissue type), methods applied for growing and measuring cell responses, as well as stimulation of cells in the culture.

Following this, studies of horse cells and human cells in the method groups will be compared.

RESULTS

From XXXX retrieved references, Y were reviews, editorials and similar, Z did not fulfill the inclusion criteria, and W studies were included in our analyses. See Figure 1 for the selection process.

Figure 1 – Flow Diagram

The selected studies are shown in Table 1.

Out of the selected studies, U were studying cells originating from horse, and V were originating from humans.

References

Boeuf S & Richter W (2010). Chondrogenesis of mesenchymal stem cells: role of tissue source and inducing factors. *Stem Cell Res Ther* **1**, 31.

Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, & Kadiyala S (1998). Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. *J Orthop Res* **16**, 155-162.

Chu CR, Szczodry M, & Bruno S (2010). Animal models for cartilage regeneration and repair. *Tissue Eng Part B Rev* **16**, 105-115.

De Bari C, Dell'Accio F, Tylzanowski P, & Luyten FP (2001). Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* **44**, 1928-1942.

De Bari C, Dell'Accio F, Vanlauwe J, Eyckmans J, Khan IM, Archer CW, Jones EA, McGonagle D, Mitsiadis TA, Pitzalis C, & Luyten FP (2006). Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. *Arthritis Rheum* **54**, 1209-1221.

Devas MB (1967). Shin splints, or stress fractures of the metacarpal bone in horses, and shin soreness, or stress fractures of the tibia, in man. *J Bone Joint Surg Br* **49**, 310-313.

Dominici M, Le BK, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, & Horwitz E (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **8**, 315-317.

Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WW, Gordon PL, Neel M, Sussman M, Orchard P, Marx JC, Pyeritz RE, & Brenner MK (1999). Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nat Med* **5**, 309-313.

Im GI, Shin YW, & Lee KB (2005). Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? *Osteoarthritis Cartilage* **13**, 845-853.

Kassis I, Zangi L, Rivkin R, Levdansky L, Samuel S, Marx G, & Gorodetsky R (2006). Isolation of mesenchymal stem cells from G-CSF-mobilized human peripheral blood using fibrin microbeads. *Bone Marrow Transplant* **37**, 967-976.

Kawcak CE, McIlwraith CW, Norrdin RW, Park RD, & James SP (2001). The role of subchondral bone in joint disease: a review. *Equine Vet J* **33**, 120-126.

McIlwraith CW, Frisbie DD, & Kawcak CE (2012). The horse as a model of naturally occurring osteoarthritis. *Bone Joint Res* **1**, 297-309.

Milner PI, Clegg PD, & Stewart MC (2011). Stem cell-based therapies for bone repair. *Vet Clin North Am Equine Pract* **27**, 299-314.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, & Marshak DR (1999). Multilineage potential of adult human mesenchymal stem cells. *Science* **284**, 143-147.

Shih DT, Lee DC, Chen SC, Tsai RY, Huang CT, Tsai CC, Shen EY, & Chiu WT (2005). Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. *Stem Cells* **23**, 1012-1020.

Yen BL, Huang HI, Chien CC, Jui HY, Ko BS, Yao M, Shun CT, Yen ML, Lee MC, & Chen YC (2005). Isolation of multipotent cells from human term placenta. *Stem Cells* **23**, 3-9.

Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, & Hedrick MH (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* **7**, 211-228.