Available online at www.sciencedirect.com

SciVerse ScienceDirect

British Journal of Oral and Maxillofacial Surgery 51 (2013) 937–941

BRITISH Journal of Oral and Maxillofacial Surgery

www.bjoms.com

Recruitment of exogenous mesenchymal stem cells in mandibular distraction osteogenesis by the stromal cell-derived factor-1/chemokine receptor-4 pathway in rats

Jian Cao [a,b,1], Lei Wang [a,*,1], Zhao-jie Du [a], Peng Liu [a], Ya-bo Zhang [a], Jian-fu Sui [a], Yan-pu Liu [a], De-lin Lei [a,*]

[a] *Department of Oral and Maxillofacial Surgery, School of Stomatology, Fourth Military Medical University, Xi'an, China*
[b] *Department of Oral and Maxillofacial Surgery, The Military General Hospital of Lanzhou Command, Lanzhou, China*

Accepted 2 May 2013
Available online 6 June 2013

Abstract

Distraction osteogenesis is widely used in orthopaedic and craniofacial surgery. However, its exact mechanism is still poorly understood. The purpose of this study was to find out whether there is systemic recruitment of mesenchymal stem cells (MSC) to the neocallus in the distraction gap by the stromal cell-derived factor-1 (SDF-1)/CXC chemokine receptor 4 (CXCR4) axis during osteogenesis. We examined the migration of MSC towards a gradient of SDF-1 *in vitro*. We also transplanted MSC labelled with green fluorescent protein (GFP) intravenously, with or without treatment with CXCR4-blocking antibody, into rats that had had unilateral mandibular distraction osteogenesis, and investigated the distribution of cells labelled with GFP in the soft callus after 24 h. We found that SDF-1 facilitated the migration potency of MSC both *in vitro* and *in vivo*, and this migration could be inhibited by AMD3100, an antagonist of CXCR4, and promoted by local infusion of exogenous SDF-1 into the distraction gap. This study provides a new insight into the molecular basis of how new bone is regenerated during distraction osteogenesis.

Keywords: Distraction osteogenesis; Mesenchymal stem cells; Homing; Stromal cell-derived factor-1; CXC chemokine receptor 4

Introduction

Distraction osteogenesis is a unique technique for regenerating endogenous bone, which has been widely used in orthopaedic and maxillofacial surgery.[1,2] However, the exact mechanism by which the mechanical stimulus is translated into biological signals remains unclear. If we understood the molecular mechanism we might be able to shorten the rather long treatment period and minimise inconvenience and morbidity.[3]

During the process of distraction osteogenesis the formation of intramembranous bone is the predominant mechanism of ossification in which neocallus is formed through the direct differentiation of mesenchymal stem cells (MSC) into osteoblast lineages.[4] MSC are multipotent, non-haematopoietic stromal cells that not only reside in haematopoietic bone marrow, but also circulate in peripheral blood.[5] Evidence suggests that MSC can home in on injured or ischaemic tissues, and this involves migration across layers of endothelial cells.[6] It is likely that injured tissue expresses

* Corresponding authors at: Department of Oral and Maxillofacial Surgery, School of Stomatology, Fourth Military Medical University, Xi'an 710032, China. Tel.: +86 29 84772501; fax: +86 29 83224470.
E-mail addresses: j.cao@live.com (J. Cao), wanglzyh@fmmu.edu.cn (L. Wang), duzhaojie222@yahoo.cn (Z.-j. Du), liupeng@fmmu.edu.cn (P. Liu), zhangyabo@fmmu.edu.cn (Y.-b. Zhang), suijianfu@fmmu.edu.cn (J.-f. Sui), liuyanpu@fmmu.edu.cn (Y.-p. Liu), leidelin@fmmu.edu.cn, hmwkcj@gmail.com (D.-l. Lei).
[1] These authors contributed equally to this work, and should be regarded as joint first authors.

0266-4356/$ – see front matter
http://dx.doi.org/10.1016/j.bjoms.2013.05.003

specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSC at the site of injury, as in the case of recruitment of leucocytes to sites of inflammation.[7] These ligands include a key stem cell homing factor called stromal cell-derived factor-1 (SDF-1 or CXCL12), which belongs to the CXC subfamily of chemokines and is widely expressed in many tissues, particularly bone marrow. It has many important roles through activation of its unique receptor CXCR4, which is a G protein-coupled receptor that has been reported to be expressed on the surface of MSC.[8]

Accumulating evidence has supported the hypothesis that SDF-1 is upregulated at sites of injury and attracts circulating MSC that express CXCR4 to help to repair the liver, heart, and brain.[9,10] However, it has not been confirmed whether the circulating MSC can be recruited to the site of distraction osteogenesis and participate in the formation of new bone.

We hypothesised that, in response to the gradual stress of distraction, there is systemic mobilisation of MSC to the neocallus in the distraction gap through the SDF-1/CXCR4 axis. To test this possibility we used MSC labelled with exogenous green fluorescent protein (GFP) systemically, and tracked them during distraction osteogenesis. We found that SDF-1/CXCR4 axis may play an important part in the recruitment of MSC from the circulation to the neocallus in the distraction gap during distraction osteogenesis.

Materials and methods

Model of mandibular distraction osteogenesis in rats

All the animal protocols were approved by the Animal Care and Use Committee at the Fourth Military Medical University. The model of mandibular distraction osteogenesis in rats was established as previously described.[11] Briefly, male Sprague-Dawley rats aged 10–12 weeks old and weighing 280–320 g were anaesthetised with 1.0% pentobarbital sodium 30 mg/kg injected intraperitoneally. After exposure of the right mandibular body and ramus through a submandibular incision, a titanium distractor (Zhongbang Titanium Biomaterials Corporation, Xi'an, China) was fixed along the buccal surface of the mandible. A vertical corticotomy was then made at the midmandibular level (Fig. 1). The incision was sutured carefully, with the distraction rod exposed on the outside. After a latency period of 5 days, the rods were distracted gradually at a rate of 0.2 mm twice a day for 5 days, and the regenerated bone was then allowed to consolidate for an additional 14 days. Animals were killed with an overdose of pentobarbital sodium, and the callus from the distraction gap was harvested, demineralised, and prepared for staining with haematoxylin and eosin (H and E).

Culture of MSC and flow cytometric analysis

Rat GFP–MSC (Oricell^{TM}) were purchased from Cyagen Bioscieces, Guangzhou, China. Cells were cultured for 5 passages. Cell surface antigens including CD34, CD45, CD90, CD44, and CD29 were analysed on a FACS^{Calibur} flow cytometer (Becton Dickinson) with CellQuest software. For detection of CXCR4 expression in MSC, the cells were stained with rabbit polyclonal antirat CXCR4 (Abcam), followed by goat antirabbit IgG-FITC (Sigma). For intracellular staining, cells were first blocked with non-conjugated antirat CXCR4 polyclonal anti-body (10 μg/ml for 1 h at 4 °C), then fixed with 4% paraformaldehyde (for 15 min at 4 °C) and made permeable with 0.5% Triton X-100 (Sigma) for 1 h at room temperature.

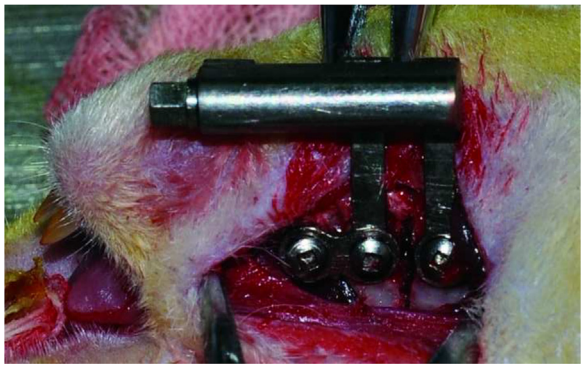

Fig. 1. The vertical corticotomy at the level of the midmandible in the model of mandibular distraction osteogenesis in rats.

In vivo chemotaxis assay

Twenty-four male Sprague Dawley rats (weight 280–320 g) were distributed randomly into 3 groups and had distraction osteogenesis on the right mandible as described above: the first group were given GFP–MSC systemically through the tail vein (GFP–MSC alone group); The second group were given GFP–MSC pretreated with AMD3100 5 μg/ml (Sigma) (pretreated group); and the third group had recombinant SDF-1 100 ng locally injected into the distraction gap 1 h before GFP–MSC were injected (local injection group) (Fig. 2). The GFP–MSC were collected in phosphate buffered saline at a cell concentration of 2.0×10^6/ml, and a 500 μl aliquot of this cell suspension was infused into each rat through the tail vein for 5 days after the start of distraction.

Animals were killed with an overdose of pentobarbital sodium 24 h after transplantation. The soft callus from the

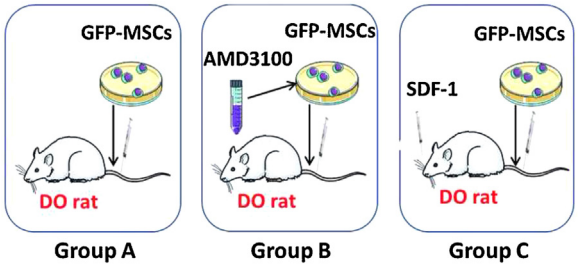

Fig. 2. Diagram showing the chemotaxis assay groups.