Injury, Int. J. Care Injured 42 (2011) 814–820

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Human adipose derived stem cells reduce callus volume upon BMP-2 administration in bone regeneration

Claudia Keibl [a,c,*], Alexander Fügl [b,c], Gerald Zanoni [a,c,d], Stefan Tangl [b,c], Susanne Wolbank [a,c], Heinz Redl [a,c], Martijn van Griensven [a,c]

[a] Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Austrian Cluster for Tissue Regeneration, Donaueschingenstrasse 13, A-1200 Vienna, Austria
[b] Department of Oral Surgery, Medical University of Vienna, Währingerstrasse 25A, A-1090 Vienna, Austria
[c] Austrian Cluster for Tissue Regeneration, Donaueschingenstrasse 13, A-1200 Vienna, Austria
[d] Baxter Innovations GmbH, Industriestrasse 20, A-1220 Vienna, Austria

ARTICLE INFO

Article history:
Accepted 7 March 2011

Keywords:
Bone regeneration
Drill hole model
Tissue Engineering
Human adipose derived stem cells
BMP-2
Fibrin
Micro Computer Tomography

ABSTRACT

Introduction: The demand for new therapeutic approaches to treat bone defects and fractures is increasing in trauma surgery and orthopaedics because the number of patients with degenerative diseases is continuously growing. "Tissue Engineering" offers promising new technologies that combine the three components – cells, growth factors and matrix. Efforts are targeted at improving and accelerating recovery, especially for long bone fractures, and reducing the risk of delayed bone healing or pseudoarthrosis. Adult human adipose-derived stem cells (ASC) can differentiate into osteoblasts in an osteogenic surrounding. Bone morphogenetic protein-2 (BMP-2) accelerates and initiates this differentiation. Fibrin, a matrix that promotes wound healing, is a promising carrier for ASCs and BMP-2.

Materials and methods: In this study, a 2 mm transcortical drill hole in the femur of male rats served as a small non-critical size defect model for fracture simulation. In vivo bone healing was investigated upon administration of the growth factor BMP-2 embedded with ASCs in a locally applied fibrin matrix. Groups with the components alone were also investigated. After 2 and 4 weeks, μCT and histology were performed to determine the bone and callus volume.

Results and discussion: After only a short period of time (2 and 4 weeks), this animal model discloses comparative information about the osteogenetic potential and bone regeneration with little effort (no osteosynthesis necessary). The most significant result found in this model is that the combination of ASCs and BMP-2 in a fibrin matrix significantly reduces callus formation after 2 weeks compared to BMP-2 alone. BMP-2 alone significantly increased callus formation. ASCs embedded alone in the fibrin matrix did not lead to increased bone regeneration.

Conclusion: Transplantation of ASC modulated the callus induction by BMP-2 to a normal volume.

Introduction

Bone defects caused by fractures or osteoporosis represent an enormous therapeutic challenge in trauma surgery and orthopaedics. The problems and difficulties resulting from trauma and operation such as blood loss, injury of blood vessels and nerves etc. is associated with a number of complications and with an increased length of treatment. So far, autologous spongiosa has been used as the gold standard for bone regeneration as well as bone substitutes such as collagen or composites.[38,48] Besides the morbidities associated with the harvesting of spongiosa, these materials can cause adverse reactions, the formation of fistula or an interference of the healing process.

Giannoudis et al. described in the "Diamond Concept of Bone Fracture Healing Interactions" the mechanical environment as an essential factor for bone restoration along with cells, growth factors and scaffolds.[15]

Mesenchymal stem cells are able to differentiate in different lineages such as bone, cartilage or nerve. Adult human adipose-derived stem cells can be isolated from liposuction material.[1,16] Since availability of autologous bone marrow is limited, ASC can easily be obtained as a suitable and cost-saving source without any ethical problems.[33] Zuk et al. noted that these cells show a stable

* Corresponding author at: Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Austrian Cluster for Tissue Regeneration, Donaueschingenstrasse 13, A-1200 Vienna, Austria.
Tel.: +43 133 110 464; fax: +43 133 110 460.
E-mail address: claudia.keibl@trauma.lbg.ac.at (C. Keibl).

0020–1383/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.injury.2011.03.007

growth and proliferation in vitro and that they have the potential to differentiate under certain conditions into lineages including bone, cartilage, fat and muscle.[51] In 2004 it was already demonstrated in a clinical study that the implantation of these multipotent mesenchymal stem cells in a mixture of fibrin and autologous spongiosa could heal a defected skull bone of a 7 year old girl.[23] A further advantage of the application of these autologous ASC is that they lack immunogenicity to some degree.[25,49]

Fibrin, which was used in our study as a matrix, plays an important role in wound healing.[2] Furthermore, fibrin shows excellent cell adhesive characteristics and it also binds and sets free growth factors.[36,42] It is an excellent scaffold material for injectable formulations.

BMPs are also well known to be osteoinductive. BMP-2 is commercially available for clinical use.[40] Rosen et al. described that BMP-2 is responsible for the direct differentiation of mesenchymal stem cells in osteoblasts which accelerates and improves the bone regeneration.[37] BMP-2 is a potential bone growth factor which is applied on a fleece in clinical routine.[5,8,9] Govender et al. described 2002 a safe, effective and quick healing of open tibia fractures by applying BMP-2.[17] Termaat et al. underlined the importance of local application and dosis of BMP-2 due to the fact that the dissimilation of BMP-2 takes place in a relatively short period of time.[46] Besides its positive effects this signal protein and member of the transforming growth factor (TGF)-β superfamily has a significant disadvantage. On the one hand it may induce a surplus of callus and on the other hand bone may develop in muscles (heterotopic ossification).[12,24]

Thus, the matrix, growth factors and cells all play an important role. However, their interaction has not been studied in detail. Therefore, we tried to investigate the role of the ASC embedded in fibrin in the interaction with BMP-2 in a load-bearing drill hole model. The purpose of our studies was to investigate a new and efficient method of bone treatment which improves and accelerates the healing process in order to reduce clinical costs and complications. The interaction of cells and mediators are known as an important factor of fracture healing.[13,14] For this reason we applied all three components of Tissue Engineering in our study.[26,34]

Materials and methods

The animal protocol review board of the City Government of Vienna, Austria approved all experimental procedures in accordance with the Guide for the Care and Use of Laboratory Animals as defined by the National Institute of Health (MA58/05766/2007/8).

A transcortical 2 mm non-critical size bone defect was created under sterile conditions bilaterally in the middle of the femur diaphysis of 50 male Sprague–Dawley rats (Animal Research Laboratories, Himberg, Austria) (Fig. 1A). The rats weighing between 350 and 450 g were randomised and divided into five groups ($n = 10$ each): Control; Fibrin (F); Fibrin + ASC; Fibrin + ASC + BMP-2 (bone morphogenetic protein-2); Fibrin + BMP-2.

Anaesthesia was induced by intramuscular injection of a mixture of 110 mg/kg ketaminhydrochlorid (ketamidor, Richter Pharma AG, Wels, Austria) and 12 mg/kg xylazin (rompun 2%, Bayer AG, Vienna, Austria). The rats received a preoperative 2 mL liquid depot (ringer solution (Mayerhofer Pharmazeutika GmbH, Linz, Austria) mixed with 0.3 mL butafosan (catosal, Bayer Health Care Austria GmbH, Vienna, Austria)) subcutaneously (s.c.) and carprofen (rimadyl, Pfizer Corporation GmbH, Vienna, Austria) (4 mg/kg) s.c. once a day for four days as an analgetic. A 2 mm drill hole was created in the middle of the femur shaft and irrigated with 0.9% sodiumchlorid solution (Mayerhofer Pharmazeutika GmbH, Leonding, Austria) (Fig. 1B and C). According to the groups, the defect was filled with 0.2 mL filling compound and in the control group no filling was used (Fig. 1D).

Subcutaneous adipose tissue was obtained during outpatient tumescence liposuction under local anaesthesia (IRB consent obtained). ASCs were isolated and cultured in DMEM-low glucose/HAM's F-12 supplemented with 2 mM L-glutamine, 10% foetal calf serum (FCS, PAA, Pasching, Austria), 100 U/mL penicillin, 0.1 mg/mL streptomycin and 1 ng/mL recombinant human basic fibroblast growth factor (rhFGF, R&D Systems, Minneapolis, USA) at 37 °C, 5% CO_2 and 95% air humidity to a subconfluent state.[49]

We applied cells in passages 3 and 4 embedded in fibrin at 2×10^5 cells/200 μl clot. Fibrin (Tisseel, Baxter AG, Vienna, Austria) was used in 1 mL duploject syringes (100 mg/mL fibrinogen solved in 3000 KIU/mL aprotinin and 500 U/mL thrombin solved in 40 mmol calcium chloride). 10 μg BMP-2 (Induct OS, Wyeth Europe Ltd, Berkshire, UK) was mixed with 1 mL of the thrombin component for the BMP-2 groups. For the ASC groups, a cell pellet of 2×10^6 cells was dissolved in 1 mL fibrinogen. Thus, no dilution effect concerning other components in the gel by adding the cells was present.

In order to exclude fractures, dorso-ventral and latero-lateral X-rays were performed on days 7, 14 and 21 under short inhalation anaesthesia with 2 vol.% isoflurane (forane, Abbott GmbH, Vienna, Austria) and 3 L/min air.

Two and 4 weeks after the surgery the rats were sacrificed in general anaesthesia (see above) by an overdose of thiopental-sodium (thiopental sandoz, Sandoz GmbH, Vienna, Austria) (120 mg/kg) by intracardiac injection. The femora were harvested and stored in 4% formaldehyde solution (VWR International, Vienna, Austria) for 1 week until further processing.

Two rats showed a fracture; in the fibrin group on day 9 and in the control group on day 2. These rats were euthanised and excluded from further analysis.

Micro Computer Tomography (μCT) analysis

All explanted femora were subjected to Micro Computer Tomography analysis (μCT) (μCT 20, Scanco Medical AG,

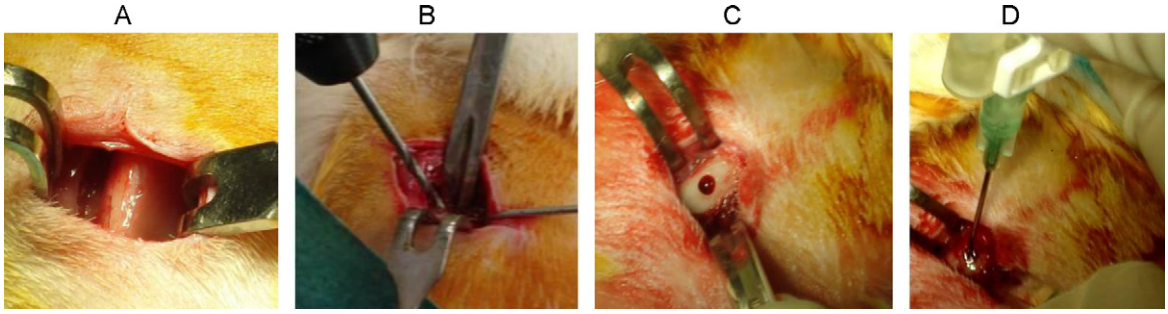

Fig. 1. Drill hole model: (A) surgical approach; (B) drilling; (C) 2 mm drill hole; (D) filling.