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Review

The rational use of animal models in the evaluation of novel bone regenerative therapies

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ABSTRACT

Bone has a high potential for endogenous self-repair. However, due to population aging, human diseases with impaired bone regeneration are on the rise. Current strategies to facilitate bone healing include various biomolecules, cellular therapies, biomaterials and different combinations of these. Animal models for testing novel regenerative therapies remain the gold standard in pre-clinical phases of drug discovery and development. Despite improvements in animal experimentation, excessive poorly designed animal studies with inappropriate endpoints and inaccurate conclusions are being conducted. In this review, we discuss animal models, procedures, methods and technologies used in bone repair studies with the aim to assist investigators in planning and performing scientifically sound experiments that respect the wellbeing of animals. In the process of designing an animal study for bone repair investigators should consider: skeletal characteristics of the selected animal species; a suitable animal model that mimics the intended clinical indication; an appropriate assessment plan with validated methods, markers, timing, endpoints and scoring systems; relevant dosing and statistically pre-justified sample sizes and evaluation methods; synchronization of the study with regulatory requirements and additional evaluations specific to cell-based approaches. **This article is part of a Special Issue entitled "Stem Cells and Bone".**

Contents

Abbreviations: EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; OECD, Organisation for Economic Co-operation and Development; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.

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Introduction

The already high incidence of bone trauma in the human population will inevitably increase as the human population ages. Osteoporosis as the major underlying condition makes nearly 27.6 million men and women in the EU (6% of men and 21% of women aged 50–84 years) susceptible to a bone fracture [1]. In 2010, approximately 3.5 million bone fractures were reported in the EU with direct healthcare costs of 37 billion € (approx. 50 billion $) and 1,180,000 quality adjusted life years lost [2]; these costs are expected to undergo a 25% increase by 2025. Large bone defects, as well as non-unions and extensive bone loss after fractures still remain significant challenges for efficient clinical interventions and require additional support of the damaged site. Since current therapeutic approaches are often accompanied with prolonged treatments, pain and risk of infection, hemorrhage, nerve damage and loss of function, there is a significant unmet medical need for the development of new therapeutic options for bone repair and prevention of bone non-unions. Various animal models are available to study the efficacy, safety and tolerability of new therapies.

The objective of this review is to provide an overview of bone defect animal models and available tools for the assessment of bone healing. Additionally, we are suggesting guidelines for rational animal use in an attempt to advance bone research, as well as to support the development of investigational products in bone regeneration.

Bone regenerative strategies: biomolecules, cells and biomaterials

Bone healing is a precisely orchestrated regenerative process, which restores the bone quality in essence by mimicking embryological cascade of events. Bone healing process is traditionally divided into three stages: an early inflammatory stage, a repair stage and late remodeling [3]. A schematic presentation of a long bone healing stages and grades are presented in Fig. 1A.

Although bone possesses endogenous self-repair mechanisms [4–8], in conditions such as impaired blood supply, excessive damage to the periosteum, inadequate immobilization, infection at the affected area, mineral and vitamin deficiencies, underlying diseases and side effects of certain medications and radiation, the enhancement of the regenerative processes is necessary to ensure the rapid and adequate restoration of skeletal functions [9–11]. The standard therapy to treat bone fractures/defects includes mechanical support such as a cast and/or mechanical devices (*e.g.* nails, plates and screws). Additional strategies being used and currently developed to further support bone healing are primarily based on the use of: (1) active ingredients (biomolecules), (2) cellular therapies and (3) biomaterials.

Biomolecules

Biomolecules used in the regenerative therapies for bone are mainly various growth factors [15]. Osteogenic factors primarily belong to the TGF-β superfamily, and the most studied factors are bone morphogenetic protein BMP2, BMP4, BMP6 and BMP7 [16,17]. Due to the fact that vascularization is essential for bone regeneration, angiogenic factors VEGF, PDGF, FGF and IGF are also being extensively tested for their usefulness in bone repair [18–24]. Immunomodulatory and anti-inflammatory agents, such as selective anti-cytokine therapies, corticosteroids and non-steroidal anti-inflammatory drugs, are used to direct specific effects on the regeneration and resorption pathways during bone healing [25,26]. Additionally, the use of parathyroidal hormone (PTH), growth hormone, steroids, calcitonin and vitamin D in systemic applications has also been shown to advance bone healing through stimulating osteogenesis, angiogenesis and osteoblast differentiation [27–30]. Various combinations of biomolecules have also been extensively evaluated in pre-clinical models with mostly positive results [27,31–37].

As one of the first regenerative strategies translated to clinical practice, biomolecules, mainly BMPs, showed substantial benefits in conditions where physiological mechanisms of bone healing fail. Thus, standard treatment of bone non-unions, open tibial fractures, spinal fusions and maxillofacial injuries and conditions has been expanded with additional therapeutic option. With the wide clinical use of BMP devices there were also reports on the increased risk for heterotopic bone formation, osteolysis, radiculitis, and retrograde ejaculation [38–41].

Cell-based therapy

Cell-based therapy utilizes multipotent mesenchymal stromal cells (MSC) originally identified among the bone marrow stromal cell population [42] and defined as plastic-adherent, fibroblast like cells expressing mesenchymal non-hematopoietic phenotype, with the potential to differentiate into osteogenic, chondrogenic and adipogenic lineages [4,43–46]. The putative MSC population used for therapeutic purposes is heterogeneous and, depending on the isolation procedure, contains variable percentages of multipotent stem cells, committed progenitors and differentiated cells. Although most studies have been conducted with bone marrow derived MSCs, other tissues have been described to comprise a corresponding osteoprogenitor population including adipose tissue, muscle, umbilical cord blood, periosteum, dental pulp and periodontal ligament [4,47–53]. Various tissue-specific MSC-like populations differ in morphology, phenotype, proliferation and differentiation potential, with the bone marrow-derived MSCs suggested to be superior to adipose or muscle tissue-derived MSCs in osteoregenerative capacity *in vivo* [7,43,45,49,52,54–56]; study details presented in Table 3. Besides direct contribution to tissue repair by differentiation into osteogenic lineages, transplanted MSCs exert beneficial therapeutic effects by secreting cytokines, growth and differentiation factors with the ability to modulate immune response, angiogenesis and endogenous reparative processes [7,43]. The multilineage differentiation ability, paracrine osteogenic and angiogenic effects and immunomodulatory properties of MSCs make them an ideal for tissue engineering and regenerative purposes [5,7,45,46,57]. Under appropriate *in vitro* conditions MSCs could be differentiated into a variety of mesenchymal tissues such as bone, cartilage, tendon, ligament, marrow stroma, muscle, fat and dermis [4,56,58–61]. To induce fracture healing, MSCs are expanded *ex vivo* prior