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Bone morphogenetic proteins and tissue engineering: future directions

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ABSTRACT

As long as bone repair and regeneration is considered as a complex clinical condition, the administration of more than one factor involved in fracture healing might be necessary. The effectiveness or not of bone morphogenetic proteins (BMPs) in association with other growth factors and with mesenchymal stem cells in bone regeneration for fracture healing and bone allograft integration is of great interest to the scientific community. In this study we point out possible future developments in BMPs, concerning research and clinical applications.

Introduction

In a bone regeneration setting such as delayed fracture, aseptic bone necrosis or other critical defect, bone morphogenetic proteins (BMPs) have proved key in enhancing the natural ability of the surrounding tissues to produce bone healing. If the mechanical conditions are fulfilled, these molecules are able to address progenitor cells in the bone-forming cascade to allow the repair of the damaged tissue. This action seems efficient when a considerable number of mesenchymal stem cells are available in the local environment.

In the complex clinical conditions associated with bone repair and regeneration, the involvement of more than one healing factor is needed. The more difficulty in healing expected, the availability of more factors, such as an adequate osteosynthesis device and application of growth factors and progenitor cells, are required. On the other hand when local damage is limited, mechanical stabilisation is unnecessary and the site is rich in progenitor cells, correct healing will require at most only the application of growth factors.

In this chapter we point out possible future developments in the application of BMPs. The rationale of the use of protein, starting from the relationships among the different type of factors applied in the system, is considered. The first issue is to improve the protein carrier, and the use of bovine collagen is discussed as well as the possible application of different carriers in different preparations. The usual preparation time, which includes mixing the protein with the carrier through an aqueous system, may be inappropriate in some circumstances. An initial rapid efflux ('dumping') of the protein was suggested upon the observation of heterotypic ossification. Thus we discuss various preparations and methods of application. An injectable material is now foreseen as the best product to obtain early application of the protein in difficult clinical conditions.

Finally, we explore the possibility of coupling the protein with other growth factors and/or with mesenchymal stem cells to obtain a more reliable biological therapeutic product. We conclude by looking at gene delivery of the BMP in allograft healing and delayed union.

BMP carriers and local delivery systems

Despite the significant evidence for stimulation by BMPs of bone healing that has been demonstrated in animal models, future clinical investigations will need to better elucidate some open questions, i.e. the ideal delivery system for human BMPs, the determination of suitable dosage and the real concentration of BMPs at the graft site, and future developments and applications.

To exert their biological effect, BMPs need to be combined with carriers for controlled release.[88] Carriers act as delivery systems for BMPs by retaining these growth factors at the site of injury for a prolonged period and by providing initial support for the attachment of cells and formation of regenerated tissue.[138] Controlled delivery systems are necessary in order to avoid uncontrolled ectopic bone formation in non-bony tissues.[110,154,161]

Essential requirements of a suitable carrier are the ability to provoke the best possible inflammatory responses, the formation of an interface with the surrounding biological tissue, and ideal porosity in order to allow first the infiltration of cells and then vascular ingrowth. In addition, carriers should be biodegradable but allow protection to BMPs from degradation for a period sufficient to induce a specific amount of bone mass at the treatment site. Finally, carriers should be sterile, immunologically inert, non-toxic and user-friendly.[7,134] The incorporated factors should be continuously released and controlled because of the very short half-life of most growth factors *in vivo*.[93]

Various formulations of delivery system may be designed to meet different mechanical requirements according to the type of tissue to be regenerated. Vascular ingrowth is essential in bone formation whereas, in cartilage, carriers should deal with compressive and

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shear stresses. For these reasons scaffolds have increased in complexity, mimicking the properties of the extracellular matrix in some cases, or responding to physiological modification of pH in others. The manufacture of the carrier also defines its ability to successfully deliver BMPs at the injured site.[66]

At present, the clinically available delivery devices for rhBMPs are far from ideal, because large doses of BMP are required for the desired osteogenic result.[45] In fact there is more BMP in a single dose than in 1000 humans, and objections can be made as to cost and safety.[59,88] Finding suitable carriers for BMPs is a great challenge for researchers. To date, despite the ready availability of rhBMPs for clinical use, the dilemma facing clinicians and the biotechnology industry is how to achieve optimal delivery systems that can decrease the dose of BMP, maintain a more sustained release pattern and be effective for osteoconduction.[102] Taking all these factors into consideration, workers have put their efforts into searching for efficient, simple and cheap delivery systems for drug targeting. Delivery systems can be divided into four major categories.[134]

Of natural carriers such as collagen, hyaluronans, fibrin, alginate, silk and agarose, the most commonly used is bovine collagen for delivery of rhBMPs.[59] The advantage of these materials is good biocompatibility; drawbacks are the natural source, processing, possible disease transmission and immunogenicity.[138]

Inorganic materials, such as calcium phosphates, calcium sulphates and bioglass, can mimic the natural bony structure and are, for example, produced as injectable paste, granules and blocks.[138]

Synthetic materials such as polylactic acid (PLA) and polyglycolic acid (PGA) or their copolymers such as polylactic-co-glycolic acid (PLGA) are widely used as biodegradable implants for orthopaedic application.[84]

Composites consist of a combination of the materials mentioned above, the advantages of individual materials optimising those of another material class. The ideal composite material should combine osteogenic (cells), osteoinductive (growth factors) and osteoconductive (structural) properties to promote tissue regeneration.[52]

Recently great attention has been paid to the subject of nanoparticles and microparticles for drug delivery. Most common materials in the design of nanodevices as delivery carriers are synthetic materials, natural polymers and hydroxyapatite-based particles.[73] PLA, PGA and their composite PLGA have been used in animal models as carriers for nanoparticle-based delivery systems for BMPs.[71,86,128,129,136,156] In a study by Ruhe et al., rhBMP-2 release was observed to depend on composite composition and nanostructure, as well as on the pH of the release medium.[128]

Microspheres based on collagen–hydroxyapatite have also been evaluated as rhBMP-4 carriers in rabbits.[155] Bone regeneration was observed in the animal group treated with BMP-4 particles whereas, in the group receiving the carrier alone, the defects were filled with fibrous tissue and inflammatory cells. Chitosan-sodium alginate microspheres have been studied *in vitro* in bone-marrow-derived cells.[121] Chen et al. evaluated dextran-based microspheres and nanospheres for BMP delivery[20–24] and demonstrated how much rhBMP release was influenced by changing the ratios of the components.[23]

Nanoparticle technology applied to BMP delivery appears the most promising approach in the future of bone tissue engineering, and further investigations must focus on this field in order to find ideal carriers for growth factors.

Dosage and concentration of BMPs

In clinical practice, BMPs are used for acute fracture treatment and healing of bony defects, delayed unions and non-unions. Two growth factors of the TGF-β superfamily, BMP-2 and BMP-7, have received approval for restricted clinical administration;[14,44,57] rhBMP-7 (Osigraft®, Stryker-Biotec, Hopkinton, MA) is available as 1 g lyophilised powder containing 3.5 mg eptotermin-α with bovine collagen 1 and can be applied as a suspension. According to the manufacturer, not more than 2 g (7.0 mg eptotermin-α) should be administered to any one individual.[38] BMP-2 (InductOs®, Wyeth, Gosport, UK) is available as a kit containing 12 mg dibotermin-α (1.5 mg/ml), to be applied in a bovine collagen 1 matrix. According to the manufacturer, not more than 24 mg dibotermin-α should be administered to any one individual.[38]

Both growth factors have also been applied 'off label' in delayed healing with promising results,[35] although only BMP-7 is approved for the treatment of non-unions. Pharmacokinetic studies showed that BMP release is characterised by an initial burst effect, followed by a more gradual release; in the initial phase the carrier can lose up to 30% of its BMP.[45,50] In addition, the high dose resulting from this initial rapid release determines a supraphysiological concentration of BMPs, which can be related to severe complications such as ectopic bone formation within the spinal canal, generalised haematomas in soft tissue and bone resorption around implants.[18,49,53,59,125] Therefore the effective dosage of BMP required in humans are fairly high. One pack of Osigraft® (rhBMP-7) contains 3.5 mg of eptotermin-α and, since 1 kg of human bone yields &1 mg of BMP, the application of one vial is equivalent to the total amount of BMP-7 in the skeleton of two people.[9] As a result, the high local and consequently low systemic concentrations of incorporated growth factors may reduce the overall dosage per application. Furthermore, because of the very short half-life of growth factors (60–240 min), direct and continuous application of the factors at the required site is necessary.[163,167]

Preclinical and clinical studies have revealed little evidence of toxic effects and few adverse events have been reported. A low rate of antibody formation following administration of BMPs has been observed in some cases, without clinical consequences.[60] In another study, antibody responses to rhBMP-2 were detected in less than 1% of people treated for spinal problems. For rhBMP-7, low immune responses have been observed in 38% of cases without adverse clinical effects.[119] Long-term effects are yet to be demonstrated.

To date the effective dosage of BMPs related to the size of the gap to be filled has not been established, i.e. the use of one 3.5 mg vial of Osigraft® (OP-1) in recurrent non-union without osseous gap and two vials for non-union with bone loss has not yet been validated. In addition, we do not know the retention rate of the OP-1 in the application site. Retention of the growth factor depends on BMP immobilisation in the delivery system,[7] and much effort is currently being put into finding and producing delivery carriers for BMPs that do not cause loss of their activity. Immobilisation of the BMPs in a delivery system may be achieved by adsorption, entrapment, immobilisation or covalent binding.[99]

In the case of adsorption, conformational changes may occur and the release of the protein may be less sustained. With entrapment, hydrophobic polymeric matrices are known to release bioactive agents over extended periods of time;[83] however, during carrier material processing, pH and temperature conditions can lead to denaturation of the protein. Covalent binding to the carrier may be performed by production of a fusion BMP protein with a domain of specific binding to a biomaterial.[145] Anyway, covalent immobilization may negatively affect the binding of the growth factors to their receptors as it could lead to subsequent dimerization of the receptors in the plane of the membrane.[99]

Animal models have been studied to evaluate systemic distribution and pharmacokinetics and the retention of BMP at the site of orthopaedic injury, through specific BMP targeting using radiolabelled [125]I OP-1 associated with different carriers.[6,97] Human studies are difficult because of legal problems in combining