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Bone morphogenetic proteins in soft-tissue reconstruction

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ABSTRACT

Different options are reviewed in the field of musculoskeletal tissue reconstruction, from the addition of biological actors (cells, growth factors, biological or artificial scaffolds) to the application of gene therapy or tissue engineering. Growth factors can enable innovative solutions to treat such disease if we can extrapolate to soft tissue the promising results obtained in bone reconstruction with bone morphogenetic proteins. However, as in bone reconstruction, soft-tissue regeneration will depend on the drug delivery carrier, the scaffold for the newly formed tissue, the dose of growth factor and the animal model, which must all be explored before extrapolation to clinical problems.

Introduction

Degenerative cartilage, tendon or disc disease remains a major cause of disability among elderly people and is one of the greatest challenges in orthopaedic surgery. A range of treatments have been explored in the field of musculoskeletal tissue reconstruction, from the addition of biological actors (cells, growth factors, biological or artificial scaffolds) to the application of gene therapy or tissue engineering. Growth factors may enable innovative solutions to such problems if we can extrapolate to soft tissue the promising results obtained in bone reconstruction with bone morphogenetic proteins (BMPs). Induction of the differentiation of cells of osteoblastic cell lineages BMP-2 and BMP-7 has been investigated in animal models to meet the clinical challenges of long-bone problems, and has reached a good level of proof in resolving resistant septic non-union or fresh open fracture. However, for bone reconstruction using BMPs, all the results highlighted the necessity for two pre-existing conditions: a matrix for the BMP itself and a scaffold for the new bone. Both bone and soft-tissue reconstruction will depend on the drug delivery system, the scaffold for the newly formed tissue, the dose of BMP and the animal model.

Cartilage and BMPs

Cartilage is avascular and is submitted to hydrostatic, compressive and shear forces. Chondrocytes embedded in a matrix where oxygen tension is low receive nutrients only by diffusion and have limited potential to synthesise new proteoglycan. Complex anatomy to reproduce (four zones with different properties can be described in mature articular cartilage), lack of vascularity and low metabolic activity are the three main difficulties in cartilage reconstruction.

Osteochondral defects can heal following the migration of mesenchymal stem cells (MSCs) from bone marrow proliferation and their differentiation into chondrocytes. Such cell migration is obtained in clinical practice by drilling or, in the case of cartilage defect, by abrasion of subchondral bone. However, the ensuing capacity for repair is very limited and the resulting cartilage contains a high proportion of fibrous elements. Modern trends in cartilage reconstruction aim to obtain a cartilage with properties as close as possible to native tissue.

Whatever the chosen method to regenerate cartilage (application of stem cells with or without growth factor, gene therapy or tissue engineering), the creation of a defect in animal cartilage is a reliable model, a three-dimensional scaffold remaining a pivotal to success. A large number of growth factors have already been tested,[10] i.e. BMPs 2–7 and cartilage-derived morphogenetic proteins (CDMPs) -1, -2 and -3 expressed in articular cartilage.[4] Maintenance of adult chondrocytic phenotypes, extracellular matrix production and chondrocyte differentiation are all stimulated by BMPs.[36]

Majundar et al. demonstrated that BMPs -2 and -9 can induce the chondrogenic differentiation of human MSCs.[27] Tamai et al., using rhBMP-2 combined with porous hydroxyapatite (HA) and synthetic polymer, reported complete repair at 6 weeks of a cartilage defect in an animal model.[44] Sellers et al., studying rhBMP-2 in an animal model, showed acceleration of subchondral bone and cartilage formation.[43] A type 1 collagen sponge with rhBMP-2 was used to fill a cartilage defect of the trochlear groove (3 mm in diameter and 3 mm deep) in 89 New Zealand White rabbits; evaluation of the repair of the defects at 4, 8, 24 and 52 weeks proved that rhBMP-2 stimulated faster repair and better cellular morphology with more abundant hyaline-like cartilage than that seen in the control defects. Filling osteochondral defects in dogs with rhBMP-7, Cook et al. reported significant improvement of bone and cartilage healing.[5] A putty formulation (type I collagen particles and carboxymethylcellulose) with 200 μg of rhBMP-7 filled voids 5 mm in diameter and 6 mm deep in the femoral condyles, with evaluation at 16 weeks in the great majority of cases. In the cases treated with rhBMP-7, the defects contained cartilage of thickness

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similar to that of the surrounding intact cartilage. In the control group, defects were filled by fibrous tissue or fibrocartilage. Jelic and colleagues reported the capacity of rhBMP-7 in a liquid formulation to repair a chondral defect (without damage of subchondral bone) in sheep knee;[21] two defects 10 mm deep were created, one on the medial condyle and one on the trochlea of the femur. The joints were infused with a pump over a 2-week period with either 55 μg or 170 μg of rhBMP-7 or an acetate buffer control. Evaluation at 3 months showed that the control knees had no signs of cellular growth into the defect area but, in the rhBMP-7 group, the condylar defects were 40% to 62% filled and the trochlear defects were 56% to 81% filled. The authors concluded that the delivery of rh BMP-7 in the first 2 weeks after surgery was sufficient to stimulate MSCs and obtain cartilage in the chondral defect by 3–6 months.

Regeneration of cartilage by gene therapy has followed two routes, enhancing the formation or suppressing the loss of cartilage. Gene therapy has been used to deliver various regenerative molecules in two different modes (*in vivo* or *ex vivo*) into cartilage defects of animal distal femurs. Di Cesare et al. reported similar but incomplete healing of cartilage defects when comparing the use of plasmid DNA encoding BMP-2 with the use of collagen matrix with BMP.[7] Kuroda et al. showed incomplete reparative capacity of *ex-vivo* gene therapy with muscle-derived stem cells and BMP-4.[25] Combinations of BMPs -2, -4 and -7 with gene therapy have been tested in bone reconstruction with some exciting results, but not in cartilage regeneration.[10] Reconstruction of cartilage by tissue engineering has already tested the effect of different growth factors in maintaining chondrogenic differentiation, either alone (if BMP-2 has been most commonly used, BMP-7 has shown chondrogenic differentiation properties) or in combination (although BMP-2 + transforming growth factor β1 [TGF-β1] work together for chondrogenesis, TGF-β3 + BMP-6 or TGF-β3 + IGF-1 are the most effective combinations).[10]

Tendon and ligament

We can take the structures of tendon and ligament as similar (rows of collagen type 1 and fibroblasts), although ligament is composed of more chondroitin and keratin sulfate than tendon. Vascular ingrowth confers on tendons and ligaments a general capacity for regenerative repair at a level between the capacities of bone and of cartilage, although tendons differ in this, covered tendons receiving more vascularity than sheathed tendons.[14]

In tendon and ligament reconstruction, as in bone and cartilage reconstruction, the types of lesion (trauma, degeneration, laceration, complete defect, avulsion), of carrier used and of animal model remain important factors. Cellular infiltration of the BMP carrier will depend on the orientation of matrices, and controlled stress during healing can help to regain the original tensile stress.[6,41] During tendon healing, BMP signalling remain key but is influenced by mechanical loading. Eliasson et al. reported an analysis of the evolution of the expression of the BMP signalling system during healing of a transected rat Achilles tendon. They proved that the mechanical loading had influenced the gene expression of four BMP genes (OP-1/BMP-7, GDF-5/CDMP-1/BMP-14, GDF-6/CDMP-2/BMP-13, and GDF-7/CDMP-3/BMP-12).[11]

As to the involvement of growth factors in tendon and ligament repair,[20,45] only a few studies have tested the capacity of BMPs -12, -13, and -14 in animal models to enhance the repair process in cases of laceration or defect.[15,30,37]

The enthesis (bone–tendon and bone–ligament interfaces) remains difficult to repair surgically because of the complex arrangement of tissue layers between soft structures and bone (i.e. between tendon or ligament and fibrocartilage, calcified fibrocartilage, and bone). BMPs have been shown to promote ectopic endochondral ossification, and induction of bone into tendon by BMP has been reported in a clinical situation as a secondary effect. According to Nawata, development of the bone–tendon junction is similar to endochondral ossification.[35] Hashimoto reported the regeneration of a direct enthesis in a rabbit model.[19] RhBMP-2 was injected into the flexor digitorum communis tendon of the hind limb in order to induce ectopic bone formation; after 1 month, the resultant tendon–bone complex was transplanted onto the surface of the rabbit tibia. One month later, radiological and histomorphological examination confirmed direct insertion of tendon–bone structures in the new metaphyseal bone site. In this animal model, a population of cells can be stimulated by BMP along the chondro–osseous pathway. In the same way, BMPs have been found to enhance the repair of anterior cruciate ligaments when bone tunnelling was performed.[31,40]

In a rat model of an Achilles tendon defect, Forslund et al. reported the use of CDMPs to evaluate the capacity of four different doses of three different CDMPs on tendon healing and on osteogenesis.[16] CDMPs belong to the family of BMPs and can induce growth of cartilage, bone tendon and ligament, and CDMP-1 and CDMP-2 are the human analogues of GDF-5 and GDF-6. CDMPs were injected 6 h postoperatively to fill the 3 mm defect in the tendon, and the animals were sacrificed after 8 days to test tendon regeneration. The authors concluded that the three CDMPs showed equal capacities to improve tendon repair and osteogenicity, with a significant dose-related increase in strength and stiffness.

A very critical problem remains today in the repair of the rotatory cuff, with an prevalence of tears between 7% and 37% among the general population in the USA. The high recurrent tear rate (20% to 70% of tears) has highlighted the fact that surgical repair is not sufficient. In 50 cases operated by open or athroscopic technique, ultrasound evaluation at 6 months showed a defect, but without correlation with functional assessment.[13]

Nicotine and non-steroidal anti-inflammatory drugs (NSAIDs) are known to diminish healing at the tendon–bone interface, and together with further factors influencing repair can be controlled (number of involved tendons, size of the tear, degenerative fatty atrophy, tendon quality, postoperative rehabilitation protocol). Because growth factors can improve proliferation, differentiation of cells, neovascularisation and extracellular matrix synthesis, treatment with biological factors and carriers can play a role in the improvement of clinical results.[2,24] Gerber et al.[17] and Rodeo et al.[39] demonstrated in animal models that normal tendon–bone insertion at the rotatory cuff site was very difficult to regenerate owing to lack of regeneration of calcified cartilage.

The choice of animal model also remains a difficult question: the extrinsic impingement and the fatty infiltration seen in clinical practice are both infrequently reproduced in the animal. If rat rotator cuff tendons are under an arch, as in humans, no fatty infiltration can be observed and this model is not large enough to allow reproducible surgical technique. This was pointed out recently by Gupta et al., who described a rabbit model (not so well-known in terms of knowledge of biomarkers and genome as the rat).[18] Kobayashi et al. identified four growth factors critical to tendon healing: basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF-1), platelet-derived growth factor (PDGF) and TGF-β.[23]

Würgler-Hauri et al. evaluated the temporal expression of eight growth factors – bFGF, bBMP-12, BMP-13, BMP-14, cartilage oligomeric matrix protein (COMP), connective tissue growth factor (CTGF), PDGF-B and TGF-β1 – in tendon–bone healing in an animal model of supraspinatus detachment and repair.[47] An increase in the expression of all growth factors was observed at 1 week, decreasing to normal or undetectable levels at 16 weeks. Nakase et al. performed the first study of the local regulating factors of tendon injury in the human rotatory cuff.[34]

BMP-12 and BMP-13 are known to induce the formation of tendon and fibrocartilage. Rodeo documented an animal study in 72 sheep