REVIEW ARTICLE

The role of growth factors and related agents in accelerating fracture healing

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In vivo studies have shown that bone morphogenetic proteins (BMPs), transforming growth factor (TGF) beta, insulin-like growth factor (IGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are all present during normal healing of fractures. They are all naturally-occurring agents, each of which has a range of molecular variants which have been identified in vivo.7,9,10

The BMPs are members of the larger TGF-beta super family. To date, over 20 different BMPs have been isolated, but only some have the potential to induce formation of new bone.11,12 TGF-beta, another of the TGF-beta super family, has five isoforms and is found in many types of tissue.9 Animal studies have demonstrated varying results in its influence on the healing of fractures.3,13-15 The IGF family consists of two polypeptide members; IGF-II is the most abundant in bone, but IGF-I has the greater osteogenic potency.9 In vitro, IGF-I has been shown to stimulate chemotaxis and activity of osteoblasts, whilst in vivo, increased bone formation has been observed in bone-repair models in animals. IGF-I is of greatest effect when used in combination with TGF-beta.16,17 To date, nine members of the FGF family have been identified, the most abundant in human tissue being FGF-1 (alpha) and FGF-2 (beta).9 The former has been shown to promote an osteogenic response in progenitor cells by providing cytotoxic resistance to inflammatory oxidants,18-20 and the latter has been found to accelerate fracture healing directly.21-23

VEGF is stored and secreted by osteoblasts and endothelium and has an important role in angiogenesis during repair of fractures.24-27 The role of PDGF in the healing of fractures has not been clarified. It is released from platelets during the formation of haematoma3 and has been shown to stimulate the migration of osteoblasts28 and mesenchymal progenitor cells.29

In addition to these agents, various hormones such as growth hormone and parathyroid hormone (PTH) have an effect on the repair of fractures.30-33 Growth hormone (GH) has been shown to increase the ultimate load, stiffness and callus in a model of fracture repair,30,34 while PTH was found to increase the bone mineral content, density and strength in a model of repair in long bones and to accelerate healing.32,33

Agents such as the statins, PTH-related peptide (PTH-rP), TP508 (a thrombin-derived peptide) and pleiotrophin may also have the potential to affect the healing of fractures. Statins have been shown to induce BMP-2 and VEGF, inducing earlier bone formation in repair models.35-37 PTH-rP is a hormone which acts on the same receptors as PTH and was found to improve fracture repair greatly in a model of delayed healing.5,38 TP508 is a synthetic peptide with a similar amino-acid sequence to thrombin, and accelerates fracture repair and bone formation.39,41 Pleiotrophin is an extracellular matrix-associated protein which has recently been found to have a role in osteoblastic recruitment, motility and proliferation.42

Clinical situations
There are a number of clinical situations for which the application of therapeutic agents should be considered. These include:

1) simple closed fractures;
2) delayed healing (open-fracture/high-velocity injuries and older osteoporotic fractures);
3) nonunion; and
4) segmental defects.

These different situations almost certainly lead to the recruitment of different cells, growth factors and growth-factor inhibitors within the site of the fracture. Receptor expression may also be influenced. Models of nonunions have demonstrated the influence of growth factors on their receptors and blood vessels which are present in varying degrees in the fracture gap, but not necessarily in the correct temporal and spatial arrangement.43,44 The endogenous levels of the various growth factors and the receptiveness of the cells are
likely to vary under these different circumstances. This needs to be kept in mind when considering the experimental evidence and the studies carried out with the various growth factors on patients, since it may not be possible to extrapolate from one situation to another.

**Time of delivery**
The type of cells present within the site of the fracture varies at the different stages of healing. The growth receptors on the cells also vary in number at different stages. It is therefore possible that if a growth factor is given at an early stage of attempted repair, it may have less influence or be completely ineffective than when given at a later stage when receptive cells are present. The surrounding environment of the cells, particularly the degree of vascularity and their mechanical stability, will also have an effect on whether the growth factor can initiate an improvement in healing.

The options to be considered are:

1) open insertion versus injection;
2) suspension in a carrier or buffer vehicle;
3) local application versus systemic treatment; and
4) peptide versus gene treatment.

The major advantage of a carrier over a buffer is that it can release the active agent in the exact area required, delivering it over a sustained period of time. The carrier also prevents the growth factor leaching away from the site into the surrounding tissues. Collagen is a popular choice of carrier since it is biocompatible, degradable and osteo-conductive. It has been used in sponge and particle forms in human trials with BMP. The disadvantage of collagen sponge is that it requires an open procedure for insertion and is a xenogeneic material. Various synthetic biodegradable polymers have been created and tested and recently, injectable forms have been investigated. Hyaluronic-based gels, gelatin gels and calcium-phosphate pastes have also been tested.

In a small animal study, retention of recombinant human BMP-2 (rhBMP-2) at the site of an osteotomy was determined after injection of nine different carriers and buffer. There was a vast difference in local retention of BMP-2 between products, but despite the diverse range of concentrations of BMP-2, seven of the nine carriers produced statistically significant acceleration of healing. Studies using injection of growth factor alone or in a buffer have shown significant enhancement of healing in both large and small animal models even when given as a single injection. This may indicate that the growth factors have the potential to direct uncommitted cells down the osteogenic lineage to produce a beneficial effect over an extended period of time.

Most therapeutic agents, in particular BMPs, are delivered locally to the site of the fracture. The effect of systemic subcutaneous injection has also been tested using growth hormone, PTH and PTH-rP, with acceleration of bone healing. Although injections are simple to administer, they are required daily or three times per week for several weeks.

An alternative method of delivery which is currently under investigation is gene therapy. Here the complementary DNA sequence of a particular growth factor is delivered to cells at the site of the fracture. Once the cells have incorporated this DNA into their own they can produce the desired growth factor. There are two types of vector for delivery of the DNA into the cell; viral, using an adenovirus or retrovirus; and non-viral, liposomes or polypeptides. The vector can be administered directly to cells at the defect or fracture site, or may be transferred to cells ex vivo, and these are subsequently delivered to the site. Each method has its own advantages and disadvantages.

Gene therapy has the following potential advantages:

1) a prolonged expression of the protein;
2) high local concentrations;
3) greater effectiveness;
4) low cost of manufacture;
5) reduced systemic effects; and
6) longer shelf life and easier storage.

There are, however, some potential disadvantages:

1) a host immune response, which may require suppression;
2) immunosensitisation;
3) non-specific inflammatory response;
4) variation of gene expression;
5) the potential to revert to the wildtype virus;
6) ectopic bone formation; and
7) excess growth of bone.

*In vivo* studies of gene therapy are still in their infancy. Delivery of TGF-beta, BMP-2, BMP-6 and BMP-7 into osteotomy models by adenovirus have been shown to produce accelerated healing. Cells genetically engineered to express BMP *ex vivo* have been administered to segmental and other defects resulting in osseous repair. The ability to manipulate expression of cellular genes has opened up the possibility of engineering hybrid cells *ex vivo* to express multiple types of BMP. IGF-1-modified cells administered systemically were found to localise at the site of the fracture and enhance repair. Percutaneous delivery of a marker gene into a model of avascular atrophic non-union showed sustained local expression of the adenoviral vector for over four weeks. This could potentially overcome the problems of leaching of growth factor, molecular degradation and protein delivery in an avascular defect. However, these methods of delivery by gene therapy have yet to be fully explored. There is a limited amount of research available comparing the different forms of gene delivery.

**Current levels of research**
Over the last 20 years there has been an immense rise in the number of publications on the osteogenic potential of...
growth factors. There are now over 3000 articles in the literature on BMPs. Evaluation of the agents tends to go through several stages. These are:

1) the effect on cell culture;
2) the effect in small animal experiments;
3) the effect in large animal experiments;
4) observations on uncontrolled series of patients; and
5) randomised, controlled trials in patients.

All the growth factors discussed have been tested in small animal trials,\(^{5,25,32,39,41,42,83}\) GH, IGF, TGF-beta, and FGF are further on, and have been studied in large animal experiments.\(^ {14,16,22,31,34}\) The gold standard is patient randomised control trials; however, only BMP-2 and BMP-7 have reached this level of scrutiny.\(^ {59,60,83}\) Even at this stage, there is considerable variation in their assessment. BMP-2 was evaluated in open fractures,\(^ {59}\) whereas the effect of BMP-7 was examined in nonunion\(^ {60,84}\) and open fractures of the tibia.\(^ {83}\) Variation is also a factor in the animal models that have been used. IGF, TGF, FGF and TP508 have all been assessed in fracture and defect models whereas PTH and PTH-rP have only been tested in simple fracture models. Models of nonunion have only been utilised for studies with VEGF and BMP.

**Clinical studies**

The best clinical evidence for BMPs we have to date is derived from randomised, controlled trials. Two large trials have been undertaken which tested rhBMP-7, otherwise known as osteogenic protein 1 (OP1). One trial, by Friedlaender et al,\(^ {60}\) studied 122 patients with 124 established nonunions of the tibia. All the patients had an intramedullary nail and were then randomised to receive either fresh autograft or rhBMP-7 in a type-1 collagen carrier. After nine months, 81% of the rhBMP-7 group and 85% of the autograft group had achieved clinical union and 75% of the rhBMP-7 group and 84% of the autograft group were considered to have united radiologically. At two years there was no statistical difference between the control and treatment groups. The authors concluded that rhBMP-7 was equivalent to autograft in the management of nonunion, but noted that 20% of the autograft group had chronic pain at the donor site.

The other trial using rhBMP-7 was carried out by McKee et al.\(^ {83}\) Only the preliminary results are available. They studied the effect of rhBMP-7 applied without a collagen carrier in fresh open fractures of the tibia. All 124 patients had debridement, irrigation and intramedullary nailing. The treatment group (n = 62) also received rhBMP-7. They found that at six months the treatment group had a significantly lower rate of secondary intervention and had an improved functional outcome as assessed by pain and the ability to fully weight-bear.

A prospective pilot study by McKee et al\(^ {84}\) evaluated the administration of rhBMP-7 in 15 patients with established complex nonunion of a long bone with a mean of 2.8 previous unsuccessful operations. Within three months 87% had healed.

Treatment with BMP-2 was studied in 450 patients with open fractures of the tibia.\(^ {59}\) This was a randomised, prospective trial stratified for the grade of open fracture. The patients were managed by irrigation and debridement of the wound, initial reduction of the fracture within 24 hours and final closure of the wound and reduction of the fracture within 14 days. The patients were stabilised within 24 hours. In addition to a control group of 250 patients, there were two further groups who had a collagen sponge impregnated with BMP-2 placed at the fracture site. One received a low dose of BMP (0.75 mg/ml) and the other a high dose (1.5 mg/ml). The baseline profile for the type of fracture and the characteristics of stabilisation were similar. At 12 months 94% of the patients were followed up. Those treated with high-dose BMP-2 had statistically significant (p = 0.0022) accelerated healing, fewer invasive interventions and a lower rate of nonunion than the control group.

There has been a rhBMP-7 clinical trial in the United States with 653 cases.\(^ {85}\) There was an 82% success rate with rhBMP. However, it was observational, non-randomised and incorporated a wide variety of clinical scenarios and anatomical differences.

**Cost-effectiveness**

Before these agents come into active clinical practice, we need to be clear as to whether we are using them to try to accelerate healing or to reduce the rate of nonunion. In the former case we need to consider by how many weeks we have to reduce the healing time to justify the cost of treatment. In the case of BMP, when delivered as a protein the cost may be £1000 or more, but the use of thrombin peptide may be considerably less. However, it may be simpler to justify the use of the more expensive therapies in the treatment of nonunion, as the cost of the current management of such patients can be in excess of £10 000. With the development of gene therapy, which is potentially much cheaper to produce than BMP, the application of growth factors in the management of high-risk or even common fractures may become economically advantageous.

**Conclusion**

We now have agents including BMP-2 and BMP-7 which have been demonstrated to have a beneficial effect on the healing of fractures and nonunions in randomised, controlled trials. However, caution must be used in extrapolating these results. Other agents, such as GH, FGF and PTH-rP have shown promise in experiments on large animals, and others, including PTH and VEGF, in small animals. Further studies are needed to evaluate the range of agents available and to determine the optimum time and means of delivery to achieve success.

**References**


