Growth factors: Possible new clinical tools

A review

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Bone tissue contains numerous cell-to-cell signaling peptides called growth factors with potent effects on bone cell metabolism. In vivo studies over the last 5 years have demonstrated that growth factors can stimulate bone formation and bone healing and these results have made them candidates for use in orthopedic surgery. In numerous clinical conditions enhanced bone formation and bone healing could improve the results of surgery; clinical trials using growth factors to stimulate bone formation in spinal surgery, and to stimulate healing of bone defects, have been initiated. Growth factors for clinical use will become commercially available in the near future.

This review describes the main growth factors and their actions in vitro and in vivo in relation to bone tissue and bone healing. Possible areas for clinical use are also discussed.

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Inadequate bone stock and impaired bone healing are problems in orthopedics. Complicated fractures may result in pseudarthrosis. Surgery for bone tumors often leaves large defects which need extensive bone grafting. Fusion of the spine requires large amounts of trabecular bone grafts. Loosening of endoprostheses is ascribed to inadequate initial bone ingrowth, which may be improved by enhanced bone healing.

At present, problems with impaired bone healing are primarily solved by extensive bone grafting. Autogenic bone graft has a well documented osteoinductive capacity, but graft harvest prolongs operations and subjects the patient to postoperative discomfort and morbidity. Allogenic bank bone is also extensively used, but the bone graft often disintegrates and provides only osteoconductive properties. A number of newly developed artificial bone materials are presently being evaluated as graft material. These include bioactive glass ceramics, porous synthetic and coral hydroxyapatite ceramics, biodegradable calcium carbonate and combinations of collagen and ceramics. These products may have osteoconductive properties, but their clinical use is not clear.

Chemical stimulation of bone healing is a new approach to the above-mentioned clinical problems and this concept has become increasingly relevant with the discovery of peptide regulator molecules, called growth factors. Growth factors have been found in all tissues and are today known to regulate local cell-to-cell metabolism and to mediate cellular effects of different hormones. Bone matrix is a large reservoir for numerous growth factors that have been suggested as regulators of bone remodeling and initiators of the bone healing process (Baylink et al. 1993). In vitro studies have documented that bone growth factors have numerous regulating effects on bone cells, while in vivo studies have shown that some growth factors can stimulate the bone healing process in animals. These findings are promising for the clinical use of growth factors as stimulators of bone formation and healing. This review concerns the growth factors which are of major importance for bone tissue physiology and it describes some of the future possibilities for the clinical use of growth factor-stimulated bone formation and bone healing.

Bone chemistry

Bone consists of cells and extracellular matrix; the latter is composed of one third organic and two thirds inorganic components (Martin et al. 1988). The organic components of bone matrix are traditionally divided into collagen and noncollagenous proteins. Type I collagen constitutes more than 90% of the organic material in bone matrix and is the major structural protein in bone. The remaining 10%, the noncollagenous proteins, have different regulatory functions for mineralization, mediation of cell-to-matrix binding and various interactions with structural proteins, such as collagen (Rodan 1992). Bone growth factors consist of less than 1% of the noncollagenous proteins, but they are the main regulators of bone-cell metabolism (Mohan and Baylink 1991).

Table 1. In vitro effects of growth factors on osteoblasts

	Prolife-ration	Alk. phos.	Coll. synth.	Noncoll. synth.	Chemo-taxis
TGF-ß1	+++	—/+	++	+/–	+++
BMP-2,3	0	++?	0	ND	+
BMP-7	+	+++	+	0	ND
PDGF-BB	++	0/–	0	0	+++
PDGF-AA	+	0	0	0	+
IGF-1	++	0	+	0	+
IGF-2	+	0	0	0	+
FGF-basic	+	0	0	+	+
FGF-acidic	0	0	0	0	0
EGF	+	0	0	0	0

Table 2. Growth factors in bone matrix

Growth factor in bone	Size kD	Bone content mg/kg	Source in bone osteobl. / serum
TGF-ß 1–5 [a]	25	200	+ / +
BMP 1–12 [a]	16–30	2–5	+ / –
PDGF AA.AB,BB	36	50	+ / +
IGF I, II	7.6	400 [b]	+ / +
FGF [c]	16–17	20	+ / –

[a] Members of the TGF-ß super family.
[b] IGF–II
[c] Acidic, basic 1–7

Growth factors

The bone-derived growth factors are mainly produced by osteoblasts and incorporated into the extracellular matrix during bone formation, but small amounts can also be trapped systemically from serum and be incorporated into the matrix. At present, the main hypothesis is that the growth factors are located within the matrix until remodeling or trauma causes solubilization and release of the proteins (Canalis et al. 1988, Joyce et al. 1991). After release, the growth factors can regulate osteoblast and osteoclast metabolism during bone remodeling and initiate and control a healing response after bone trauma. Bone growth factors affect only the local cellular environment, thereby stimulating neighboring bone cells to proliferate and increase matrix protein synthesis (paracrine effects). Likewise, the osteoblasts which produce the growth factors can stimulate themselves and cause additional metabolic activity (autocrine effect). The total number of growth factors that can affect proliferation, differentiation and secretory functions of bone-related cells is unknown, but the number increases continually as a result of new advanced techniques in protein biochemistry and molecular biology. Normal skeletal growth and bone remodeling result from a balance between bone-matrix formation and resorption which are regulated by both systemic and local factors. Several systemic hormones are known to have important effects on bone metabolism and have been studied extensively (Raisz and Kream 1981). The pathways triggered by these hormones in the target cells, however, are much less well-known. A number of polypeptide growth factors have substantial effects on bone and cartilage metabolism; this suggests an important role for these growth factors in mediating hormonal responses locally. But they also suggest a local metabolic regulation of bone metabolism, without the influence of systemic hormones. Growth factors augment cell replication and help to stimulate the differentiation and metabolic functions of bone cells (Table 1). They produce their effects through binding to membrane-bound receptors. This leads to a cascade of intracellular events which affect the expression of genes that encode for metabolic functions, such as cell division and protein synthesis.

There are several bone-related growth factors (Table 2). Bone morphogenetic protein (BMP) exists in 12 subtypes and is the only growth factor known to stimulate the mesenchymal stem-cells to differentiate into osteoblastic and chondroblastic lineage. The transforming growth factor-beta (TGF-ß) is found presently in 5 subtypes and bone and platelets contain high amounts of this growth factor. TGF-ß is probably the most potent multifunctional regulator of bone cell metabolism. Platelet-derived growth factors (PDGF) exist in 3 isotypes and are potent stimulators of both proliferation and matrix protein synthesis. The insulin-like growth factors (IGF-I and -II) are produced by osteoblasts, and IGF-II is the growth factor found in its highest concentration in bone matrix. The synthesis of IGF-I is mediated through growth hormone.

IGFs primarily stimulate the proliferation of osteoprogenitor cells. Fibroblast growth factors (FGFs) are present in bone matrix and are secreted by isolated osteoblasts. Basic-FGF is found in bone matrix in a tenfold higher concentration than acid-FGF. FGF is primarily a mitogen in normal bone cells and a powerful angiogenic factor.

Bone morphogenetic proteins (BMPs)

In 1965, Marshall Urist discovered that demineralized bone matrix (DBM) could induce bone formation when placed subcutaneously (Urist 1965). He observed that DBM formed an ossicle with mineralized woven bone and bone marrow. The ability of demineralized bone matrix to induce bone formation was ascribed to a protein, which Urist named "Bone

Morphogenetic Protein (BMP)" (Urist et al. 1983, 1984). In 1988, Elizabeth Wang and John Wozney from the Genetics Institute purified 3 different BMPs and characterized their amino acid sequences (Wang et al. 1988, Wozney et al. 1988). Later, 9 additional BMP genes were identified, so that today BMP 1–12 have been identified (Celeste et al. 1990, D'alessandro 1991, Wozney 1992).

BMPs are the only growth factors that can stimulate differentiation of the mesenchymal stem-cell into a chondro- and osteoblastic direction (Urist et al. 1983, Chen et al. 1991, Yamaguchi et al. 1991). The proteins should therefore be involved in the maintenance of a differentiated bone-cell population. During bone healing, the release of BMPs from traumatized bone stimulates differentiation of the mesenchymal stem-cells which will participate in the healing process. Recent studies have demonstrated that BMPs are expressed during the early phases of fracture healing (Jin et al. 1994, Nakase et al. 1994). The novel recombinant BMPs have intact bone-inducing capacity, but need special carriers in order to maintain their activity at low doses (Wang et al. 1990, Cox 1991, Luyten et al. 1992, Ripamonti et al. 1992). Functional carriers for BMP include collagen matrix, demineralized bone matrix and various synthetic polysaccharide matrices (Yasko et al. 1993). The function of the carrier matrix is to immobilize the bone-inducing protein at a particular site for a sufficient amount of time to allow bone induction to occur.

In vivo studies have primarily focused on the usage of BMPs for stimulating the healing of bone defects. In long bone defect-models in rats, rabbits, sheep and monkeys, BMP-2, -3 and -7 have been powerful stimulators of bony healing (Cook et al. 1992, 1993, Stevenson et al. 1992, Yasko et al. 1993, Kirker-head et al. 1994). In muscle diffusion chambers, BMP-2 stimulates bone formation in monkeys (Aspenberg et al. 1993, Miyamoto et al. 1993). In monkeys, BMP-3 also stimulates bony healing of large skull defects (Ripamonti et al. 1992). Cook et al. (1994) used BMP-7 and collagen as a substitute for autologous bone in spinal fusions in dogs. Although the cellular mechanisms for BMP-stimulated bone induction are vaguely understood, the in vivo bone induction activity of this group of growth factors is unique and the bone morphogenetic proteins are promising for clinical use in any situation where bone defects require stimulation for proper healing.

TGF-ß (Transforming growth factor-beta)

TGF-ßs are multifunctional cytokines with a broad range of activities in bone tissue, connective tissue and the immunological system. These include regulation of growth and differentiation of many cell types. In general, TGF-ß stimulates cells of mesenchymal origin and inhibits cells of ectodermal origin. TGF-ß belongs to a family of related proteins, which demonstrates various degrees of amino acid sequence homology. This protein family is called the TGF-ß superfamily (Burt 1992). Other members of this family include the bone morphogenetic proteins (BMPs) and the embryonical growth factors inhibin, activin and müllerian substance (Mohan and Baylink 1991). The secreted precursor protein is biologically inactive and is called latent TGF-ß (Lawrence et al. 1985). 5 subtypes, TGF-ß 1–5, have been found (Derynck et al. 1988, Jakowlew et al. 1988, Rosa et al. 1988, Kirker-head et al. 1994).

Bone and platelets contain almost 100 times more TGF-ß than any other tissue, and osteoblasts bear the highest amount of TGF-ß receptors (Robey et al. 1987, Sporn and Roberts 1990b). These findings suggest that TGF-ß is of major importance for bone metabolism. TGF-ß has profound in vitro effects on osteoblasts (Table 1). In the mouse calvarial osteoblasts and murine osteoblastic cell-lines, TGF-ß inhibits proliferation and alkaline phosphatase activity (Noda and Rodan 1986, Pfeilschifter et al. 1987). In the rat calvarial and human osteoblasts and corresponding cell-lines, TGF-ß increases cell proliferation (Canalis 1985, Rickard et al. 1993). The effects of TGF-ß on bone-cell differentiation is controversial. Both collagen production and collagen gene expression are stimulated by TGF-ß (Wrana et al. 1988, Centrella et al. 1992). Alkaline phosphatase activity and expression are generally decreased by TGF-ß stimulation (Noda and Rodan 1986, Wrana et al. 1988), and in vitro mineralization is also inhibited by TGF-ß (Antosz et al. 1989).

The first in vivo studies demonstrating stimulatory effects of TGF-ß on bone formation used injections of TGF-ß into fetal rat and mice calvaria and found a marked increase in bone formation (Noda and Camilliere 1989, Joyce et al. 1990). A more recent study has demonstrated that this effect was not present in older animals (Critchlow et al. 1994). In a study using a calvarial defect model in rabbits, TGF-ß in a methyl cellulose gel carrier could stimulate bone healing (Beck et al. 1991). Systemic administration in rats and rabbits causes endosteal bone formation and generalized osteoblast hypertrophy, with high matrix protein synthesis activity (Miyaushi et al. 1990). In monkeys, TGF-ß had no effect on bone ingrowth into a titanium bone ingrowth chamber, but the newly formed bone showed increased osteoblastic activity (Aufdemorte

et al. 1992).

TGF-ß, applied continuously to an osteotomy in rabbits, stimulated increased callus formation and increased maximal bending strength of the osteotomy (Lind et al. 1993). Most recently, TGF-ß has been demonstrated to enhance bone ingrowth and mechanical fixation of implants inserted in trabecular bone in mature dogs (Lind et al. 1995b, Sumner et al. 1995). TGF-ß along with the BMPs and FGFs are probable candidates for clinical use.

Fibroblast growth factors (FGFs)

Fibroblast growth factors are polypeptide growth factors that show potent mitogenic activities for cells of mesodermal and neuroectodermal origin (Sporn and Roberts 1990a). The FGF family currently consists of 7 members. FGF-1 and FGF-2 are also called acidic and basic FGF (aFGF and bFGF), respectively. FGFs mainly have proliferative effect on osteoblasts, but less effect on protein synthesis. Consequently, they probably enhance bone formation by increasing the number of cells capable of synthesizing bone collagen (McCarthy et al. 1989c, Rodan et al. 1989). bFGF is generally more potent than aFGF (Canalis et al. 1991). TGF-ß synthesis by osteoblasts can also be stimulated by bFGF, and FGF may therefore exert some stimulatory effects through other growth factors (Noda and Vogel 1989). FGFs are also angiogenic factors which are important for neovascularization during bone healing.

Several studies have used bFGF for in vivo stimulation of bone formation and bone healing. Basic-FGF has been incorporated into demineralized bone matrix and implanted intramuscularly in rats leading to increased new bone formation (Aspenberg and Lohmander 1989). Bone graft incorporation can also be enhanced by bFGF in bone chambers both by treatment of the graft with bFGF and by continuous use of a miniosmotic pump (Wang and Aspenberg 1994, Wang et al. 1996). Fracture healing in rats has been stimulated by bolus doses of bFGF that caused increased callus formation and bone mineral content (Kawagushi et al. 1993). Acidic FGF has also been shown to stimulate callus formation in rats (Jingushi et al. 1990). Systemic administration of bFGF to rats increased osteoblast proliferation and endosteal bone formation (Mayahara et al. 1993). FGFs along with BMPs and TGF-as are interesting for possible future clinical use, especially since FGFs have both osteogenic and angiogenic properties.

Platelet-derived growth factors (PDGFs)

Platelet-derived growth factor (PDGF) was discovered in serum as the major mitogenic activity responsible for growth of cultured mesenchymal cells (Sporn and Roberts 1990a).

The main effect of PDGF on bone cells is mitogenic and has been found in both human and rat osteoblasts and various osteoblastic cell-lines (Canalis et al. 1989b, Abdennagy et al. 1992, Canalis et al. 1992). PDGF is also a powerful chemotactic factor for mesenchymal cells, including osteoblasts from both rat and human tissue (Tsukamoto et al. 1991, Hughes et al. 1992, Lind et al. 1995a). PDGF have only slight effects on the metabolic functions of bone cells. Demineralized bone matrix treated with PDGF and implanted in muscle in rats, showed an increase in calcium content and alkaline phosphatase activity (Howes et al. 1988). In rat calvarial defects, PDGF inhibited BMP-3-stimulated bony healing (Marden et al. 1993). One study has used a combination of PDGF and IGF in a gel formulation to stimulate bony ingrowth into dental titanium implants (Lynch et al. 1991).

Insulin growth factors (IGFs)

2 IGFs have been characterized: IGF-I and IGF-II. Their original designations were somatomedin-C and skeletal growth factor (Mohan and Baylink 1991). These peptides are synthesized by multiple tissues, including bone (McCarthy et al. 1989a, McCarthy et al. 1992). IGF-II is the growth factor found in highest concentration in bone matrix (Finkelman et al. 1990). IGF-I and -II have similar biological properties, but IGF-I is 4–7 times more potent than IGF-II. Bone cells also secrete IGF-binding proteins, which bind IGF and modulate biological activities (Ernst and Rodan 1990, Mohan et al. 1992, Conover and Kiefer 1993, Mohan 1993). IGF production in bone tissue is stimulated by PTH and growth hormone (GH) (Canalis et al. 1989a, Spencer et al. 1989, Chenu et al. 1990). The major effect of IGF in bone tissue is probably on the cartilage in the growth plate. Here it is assumed that GH controls longitudinal growth via local stimulation of chondroblastic IGF production and IGF subsequently regulates chondroblastic growth and metabolism (Isgaard et al. 1986, Nilsson et al. 1990, Scheven and Hamilton 1991).

IGF-I and -II stimulate preosteoblastic cell replication, which increases the number of cells capable of synthesizing bone matrix (VandePol et al. 1989, Scheven et al. 1991). But their mitogenic effect is less pronounced than those of other growth factors, such

as TGF-ß and PDGF-BB (Pfeilschifter et al. 1990). IGFs also have independent effects on the differentiated functions of the osteoblast, increasing bone collagen production and inhibiting collagen degradation (McCarthy et al. 1989b, Strong et al. 1991). As a result of these effects, IGFs increase the bone mass.

Several studies have investigated the use of IGFs for stimulation of in vivo bone healing and systemic bone formation, but with limited success. One study used a bone ingrowth chamber model with IGF-1 applied by minipumps to callus tissue, but found no increase in bone formation (Aspenberg et al. 1989). Another study used a continuous local application of IGF-1 to heal osteotomy, but no effects were found (Kirkeby and Ekeland 1992). 2 studies performed on rats found increased bone formation after the systemic administration of IGF-1 (Skottner et al. 1990, Spencer et al. 1991).

Epidermal growth factors (EGFs)

The epidermal growth factor (EGF) was originally discovered in crude preparations of nerve-growth factor prepared from mouse submaxillary glands. EGF is related to TGF-α and these 2 growth factors share the same receptor. In vitro EGF is a mitogen for fibroblasts and endothelial cells and in vivo EGF induces epithelial development and promotes angiogenesis. EGFs have very modest effects on osteoblast in culture. Systemic administration of EGF to mice resulted in increased periosteal and endosteal bone formation and osteoblastic activity (Marie et al. 1990).

Other cytokines and factors

Hematological cells secrete cytokines which are mainly regulators of immunological responses, but they may also function as regulators of bone cell function. Because of the close proximity of the marrow cells and bone, some cytokines could act as paracrine regulators of bone cell metabolism. The cytokines that affect bone cells can be divided into interleukins (IL-1, IL-3, IL-6), colony-stimulating factors (M-CSF, GM-CSF) and the tumor necrosis factor (TNF-α). These factors can be produced by osteoblasts (Canalis et al. 1991) and probably exert their main actions in osteoblast-osteoclast interactions, where the colony-stimulating factors stimulate the monocyte/osteoclast lineage of cells, while the interleukins and tumor necrosis factors inhibit osteoblastic activity and stimulate osteoclastic activity. Their general effect on bone tissue is therefore stimulation of bone resorption, although some studies have indicated stimulatory effects in low doses of IL-1 on isolated osteoblasts (Goldring and Goldring 1990, Mohan and Baylink 1991).

Actions of growth factors during bone-healing and bone-remodeling

Research over the last decade has revealed that growth factors regulate and control many of the complex cellular events during bone healing and remodeling. Besides having individual effects, the growth factors also mediate the effects of hormones (Canalis et al. 1988).

In bone remodeling, 2 major events, bone resorption and bone formation, are closely regulated in which growth factors are thought to play a critical role. The initiation of osteoclastic bone resorption is mainly under the hormonal control of PTH. During osteoclastic bone resorption, increasing amounts of growth factors are released from the resorbed bone matrix (Canalis 1983). TGF-ß probably participates in the inhibition of continued osteoclastic activity (Chenu et al. 1988). In concert with other released growth factors, BMPs initiate the differentiation (Reddi et al. 1987), whereas other factors like TGF-ß and IGFs stimulate proliferation of osteoprogenitor cells on adjacent periosteal surfaces (Mohan and Baylink 1991, Sandberg 1991) (Figure 1). Then PDGF and TGF-ß stimulate chemotactic migration of osteoblasts to the resorption pit (Lind et al. 1995a). The differentiated osteoblasts in the resorption pit maintain bone matrix synthesis through auto- and paracrine secretion of regulating growth factors (Mo-

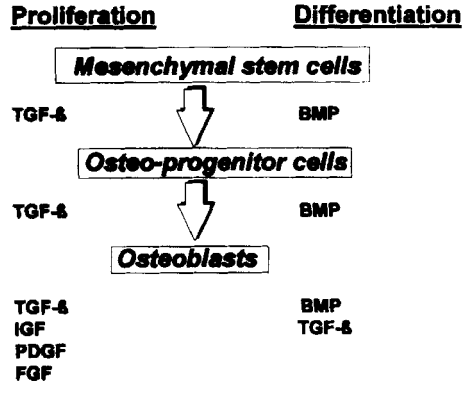

Figure 1. Growth factors that stimulate the different phases of osteoblast differentiation and proliferation.

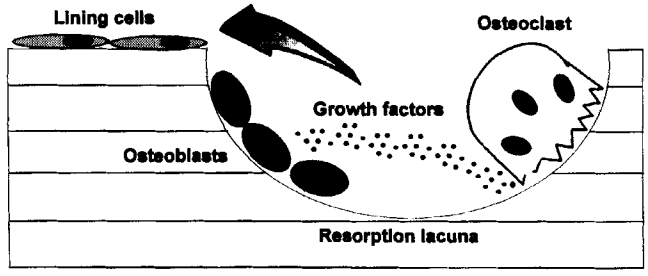

Figure 2. Growth factors released during osteoclastic bone resorption stimulate osteoblasts and osteoprogenitor cells to migrate to the resorption pit and here form new bone matrix. This could be the so-called coupling effects between bone resorption and bone formation during bone remodeling.

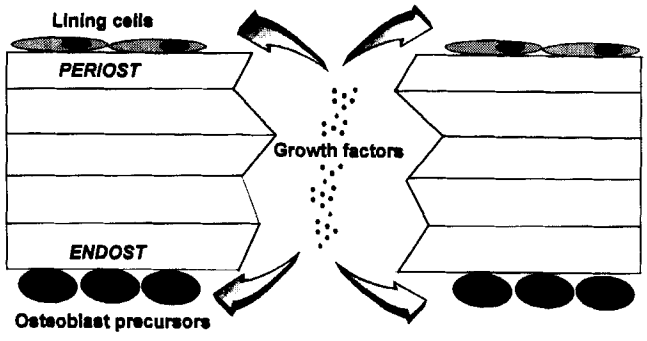

Figure 3. During bone-healing, growth factors released from both the blood clot and the traumatized bone ends can stimulate preosteoblasts from periosteal and endosteal surfaces to differentiate and proliferate to form a healing response.

han and Baylink 1991, Lian and Stein 1992) (Figure 2). Because of the stimulatory effects of growth factors during bone remodeling, there is intensive research to use systemic administration of growth factors to reverse the negative bone formation balance in patients with osteoporosis.

During the bone-healing process, growth factors are important for initiation and maintenance of differentiation and proliferation of the osteoprogenitor cells and osteoblasts which contribute to the new bone formation. In the early phases of healing, TGF-ß and PDGF released from platelets from the blood clot initiate differentiation of osteoprogenitor cells towards an osteoblastic lineage (Centrella et al. 1989). Growth factors released from the traumatized bone ends can contribute to continued stimulation of osteoblastic activity as well (Figure 3). The growth factor released from platelets and bone tissue stimulates specific expression and synthesis of additional growth factors by the osteoblasts, thereby maintaining the healing process (Joyce et al. 1991, Bolander 1992). Very early, after 2 days, BMP is expressed by periosteal osteoblasts during fracture-healing (Nakase et al. 1994). This synthesis may contribute to the continuous osteoblastic differentiation of mesenchymal stem cells. After 7–12 days, other growth factors, like TGF-ß , FGF and PDGF, are synthesized by osteoblasts in order to maintain a high proliferative and metabolic level of the osteoblasts that are involved in the healing process (Sandberg 1991, Joyce et al. 1992). FGFs are important for the angiogenesis in the newly-formed bone (Bolander 1992).

In vivo effects of growth factors in clinically related studies

Extensive efforts have been made to find methods by which growth factors can be used to stimulate local bone healing and bone formation in various clinical situations. The growth factors from the TGF-ß superfamily (TGF-ßs and BMPs) and basic-FGF are the only growth factors which have been demonstrated to possess substantial in vivo bone stimulatory capacity (Table 3). Stimulation of bone formation in bone defects at various anatomical sites has been investigated for both BMPs and TGF-ß. Beck et al. (1991) found that a single dose of TGF-ß in methyl cellulose gel could stimulate bony healing of large calvarial defects in rabbits. Ripamonti et al. (1992) demonstrated similar effects of BMP-3 in large calvarial defects in monkeys.

Defects in long bones can be used as another type of model. In this case different BMPs, in particular, have shown a potent capacity for inducing bony healing in otherwise non-healing defects. BMP-2 has

Table 3. In vivo studies using growth factors in clincally related models

Author	Year	Animal	Model	Dose	Effects
Beck S L	1991	Rabbit	Calvaria defects	3 µg TGF-ß, one dose	Induction of bony healing
Lynch S E	1991	Dog	Dental implants	PDGF & IGF in gel	Improved bone ingrowth
Ripaomonti U	1992	Monkey	Calvaria defect	100 µg BMP-3	Healing compared to nonunion in controls
Yasko A	1992	Rat	Femoral defects	15 µg BMP-2	100% healing and restoration of strength
Cook S D	1992	Rabbit	Ulna defect	3-400 µg BMP-7	Healing compared to nonunion in controls
Lind M	1993	Rabbit	Tibial osteotomy	1 and 10 µg/day 6 weeks (TGF-ß)	Increased callus formation Higher bending strength
Cook S D	1993	Monkey	Ulna defect	1–5 mg BMP-7	Healing compared to nonunion in controls
Wang J S	1994	Rat	Bone chamber with	bFGF-treated graft	Enhanced graft incorporation
Kirker-head H	1994	Sheep	Femur defect	1–5 mg BMP-2	Healing compared to nonunion in controls
Cook S D	1994	Dog	Spine fusion	1 mg BMP-7	Healing comparable to autologous bone
Lind M	1995b	Dog	Ceramic-coated	TGF-ß-treated implants	Increased bone ongrowth, gap healing, and mechanical fixation
Sumner R	1995	Dog	Ceramic coated	TGF-ß-treated implants	Increased bone ongrowth, gap healing
Beck S	1995	Rabbit	Tibial defects	Intramedullary nail TGF-ß in TCP ceramic	Enhanced healing

been found to stimulate bony healing and restore mechanical strength when applied in a collagenous matrix carrier to femoral defects in rats and sheep (Yasko et al. 1992, Kirker-head et al. 1994). BMP-7 has exhibited similar potent stimulatory effects in ulna defects in rabbits and monkeys (Cook et al. 1992, 1993). A recent study by Beck et al. (1995) has revealed that a tibial defect fixated by an intramedullary nail demonstrates enhanced bone healing when the defect is stimulated with TGF-ß adsorbed to a tricalcium-phosphate (TCP) ceramic. In a spine fusion model in dogs, Cook et al. (1994) have shown that BMP-7 together with a collagenous matrix carrier can stimulate bone formation comparable to autologous bone. Enhanced incorporation of bone graft has been demonstrated by basic FGF stimulation (Wang and Aspenberg 1994).

Chemically enhanced fracture healing was expected soon after the potency of growth factors was first recognized, but only a few experimental studies have successfully demonstrated enhanced fracture-healing. In a plated rabbit tibia osteotomy model stimulated by a continuous administration of TGF- ß, the growth factor stimulation increased strength and callus formation (Lind et al. 1993). In a similar model, IGF-1 had no effects on bone healing (Kirkeby and Ekeland 1992). It seems likely that many other researchers have unpublished data showing that growth factors fail to stimulate the healing of fractures and other conditions. Growth factors are probably best able to exert a stimulatory effect when they are used in conditions associated with impaired healing.

Growth factor-enhanced healing of implants is another area of interest. An early study by Lynch et al. (1991) showed increased bone ingrowth to dental implants stimulated by a combination of PDGF and IGF-1 in dogs, where the implants were implanted in press fit in root canals. Recent studies have demonstrated that TGF-ß, adsorbed to the ceramic coating of implants inserted into the trabecular bone of mature dogs, can stimulate anchorage, bone ingrowth and gap healing of the implants (Lind et al. 1995b, Sumner et al. 1995). In conclusion, research during the last 5 years has shown increasing evidence that growth factors can be used as in vivo stimulators of bone healing and bone formation.

Possible clinical applications

The growth factors from the TGF-ß superfamily, BMPs and TGF-ß, have shown potent stimulatory effects on bone healing and bone formation in vivo.

The major problem for clinical use of these growth factors is appropriate delivery systems. BMPs have very few biological effects in solution and a carrier is needed for adequate in vivo activity. Demineralized bone matrix, collagen matrix, hydroxyapatite ceramic and various polysaccharide matrices are some of the known effective carriers for BMPs (Lindholm and Gao 1993). The autoinductive capacity of BMPs for bone formation makes this group of growth factors ideal for bone-filling of large bony defects. BMPs

could therefore successfully be used to fill large bone cavities after tumor resections and to facilitate bony healing of pseudarthroses or complicated long-bone fractures, where bone loss can compromise normal healing. For these purposes, BMP can be used alone in an appropriate carrier or as an adjuvant to a biological or nonbiological graft material if initial mechanical stability is desired.

In future, the use of bone grafts could probably be reduced considerably by the use of growth factors in collagenous or other matrices. Animal experiments have demonstrated that BMPs can form bone to the same extent as autologous bone for spine fusions in dogs (Cook et al. 1994).

TGF-ß appears to have activity without the same carrier requirements as the BMPs. Moreover, TGF-ß has activity at much lower doses than BMPs. TGF-ß has its optimal effects on bone healing at µg levels whereas BMPs are required in mg levels. These low doses have made it possible to adsorb TGF-ß to ceramic coatings of implants and to ceramic beads that can stimulate bone healing of defects (Beck et al. 1995, Lind et al. 1995b, Sumner et al. 1995). This method of delivery makes it possible to produce ceramic-coated cementless prosthetic components, which have TGF-ß adsorbed to the ceramic surface for enhanced bone ingrowth. For revision arthroplasties, both TGF-ß and BMPs could be used to enhance bone formation, where the revision implant is placed cemented or uncemented in a sheath of packed bone powder. At present, only animal data exist about clinically relevant growth factor effects. The number of positive results from such studies has steadily increased, which indicates a future clinical use of growth factors. However, it is not known if the results of the animal studies can be applied to humans. Many studies have probably not shown positive effects of growth factors on bone healing but they have not been published (Kirkeby and Ekeland 1992, Estevez et al. 1993). The lack of effect may be due to the high sensitivity of growth factor stimulation to proper delivery systems and correct doses.

A few studies have indicated possible side-effects of short-term local growth factor stimulation. Healing of defects in dogs was associated with heterotopic bone formation and bone-cyst formation, when high doses of rhBMP-2 were used (Sciadini et al. 1995).

In rabbits, subjected to subperiosteal injections of rhTGF-ß2, bone stimulatory effects were present only in neonatal animals and edema occurred in surrounding connective tissues (Critchlow et al. 1994). Finally, growth factors may have unknown long-term side-effects. BMPs are expressed by some tumor cell lines and this has caused concern that treatment with BMPs could be carcinogenic. No data dealing with this problem are available today.

Acknowledgement

Thanks are due to Professor Cody Bünger for his careful review of this manuscript.

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