



**2009 OTA Annual Meeting
Manchester Grand Hyatt
San Diego, CA**

Basic Science Focus Forum

Symposium II: Growth Factors: Beyond BMPs

Douglas D, Level I

**9:45am – 10:55am
Wednesday, October 7, 2009**

**Moderators: Peter V. Giannoudis, MD
Emil H. Schemitsch, MD**

**Faculty: Thomas A. Einhorn, MD
Ralph Marcucio, PhD
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Disclosure: Faculty disclosure can be found starting on page 67 of the 2009 OTA Annual Meeting program.

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The current state of growth factors in orthopaedic trauma

Peter V Giannoudis MD, FRCS

Ongoing research at the molecular level has expanded our understanding of the physiological processes that regulate the complex phenomena of fracture healing and bone regeneration. A number of key molecules have been identified and shown to facilitate the progression of healing from one stage to another, leading to an uneventful outcome. Among these candidate molecules, bone morphogenetic proteins (BMPs) possess potent osteoinductive properties. They interact with osteoprogenitor cells, regulating both mitogenesis and differentiation potential.

Factors such as the optimal therapeutic dosages, delivery systems and local conditions for bone repair are still under investigation. Basic surgical management to provide adequate environmental conditions of the implantation site, such as soft-tissue coverage, host-bed vitality and biomechanical stability, remains essential. A better understanding of the mechanisms that regulate BMP expression and signalling is needed to develop the best carrier systems and possibly combination therapies with other BMPs or growth factors.

The osteoinductivity of a single BMP vial has a dose–response ratio unaffected by the individual characteristics of the recipient. However, BMPs must be administered to humans in much higher doses than to other species to achieve osteoinductive activity, and the subsequent bone

formation is not consistent. It is believed that the dose must overcome a certain threshold before effective induction of bone formation can take place. The dose–response curve becomes steeper with the progression from rodent to nonhuman-primate models.

Since the discovery of BMPs, a number of experimental and clinical trials have supported their safety and efficacy of their use in therapy. Nonetheless, at times their efficacy falls short of expectations. Several factors have been identified as contributing to this result. It is anticipated

that, as our knowledge expands and we understand better the complex pathways and cascades of molecular events attributable to BMPs, the application of these molecules in the clinical setting will continue to increase and to show more favourable outcomes.

Latest studies-Outcomes

Author, Year, Journal, Type	N of cases with OP-1	Efficacy	Safety	Comments
<i>Friedlaender et al, 2001, JBJS Am</i> RCT	63 TIBIAL nonunions	<u>In 9 months</u> 81% clinical union 75% radiological union	No Adverse Events	Statistically comparable results with AUTOGRAFT
<i>Maniscalco et al, 2002, Acta BioMed</i> RCT	7 TIBIAL shaft closed fractures	90% union	No Adverse Events	Small sample, no benefit for fresh fractures.

<p><i>Deloye et al, 2004 Acta Orthop Bel</i></p> <p>CASE SERIES</p>	<p>3 FEMORAL ALLOGRAFT</p> <p>NONUNIONS</p>	<p>0% healing</p>	<p>No Adverse Events</p>	<p>Tumour resection-allograft transplantation-nonunions</p>
<p><i>Bong et al, 2005, Bull Hosp Jt Dis</i></p> <p>CASE SERIES</p>	<p>23 HUMERAL NONUNIONS</p>	<p>100% union in 4.8 months</p>	<p>No Adverse Events</p>	<p>Combination with various grafting techniques</p>
<p><i>Giannoudis et al, 2005, Injury</i></p> <p>CASE SERIES</p>	<p>26 TIBIAL, FEMORAL, HUMERAL, ULNAR, PATELLAR, CLAVICLE</p> <p>nonunions</p>	<p>92.3% Union In <u>4.2 months</u> clinical In <u>5.6 months</u> radiological</p>	<p>No Adverse Events</p>	<p>Uncontrolled data.</p>
<p><i>Bilic et al, 2006, Int Orthop RCT</i></p>	<p>11 SCAPHOID</p>	<p>90% union clinical-radiological-scintigraphic</p>	<p>No Adverse Events</p>	<p>In combination to Allograft minimises the healing time.</p>

<i>Zimmerman et al, 2006, Unfallchirurg</i> CASE SERIES	23 TIBIAL, FEMORAL, HUMERAL, FOREARM	95.6% union rates	No Adverse Events	Uncontrolled data.
<i>Ristiniemi et al, 2007, JBJS Br</i> RCT	20 TIBIAL Pilon fractures	100% union in 15.7 wks	No Adverse Events	Faster healing
<i>Giannoudis et al, 2007, Injury</i> CASE SERIES	9 PELVIC nonunions	89% union rates	No Adverse Events	Uncontrolled data.
<i>Zimmerman et al, 2007, Unfallchirurg</i> CONTROLLED SERIES	26 TIBIAL persistent nonunions	92% union rates	No Adverse Events	Non Randomised – retrospective controls
<i>Desmyter et al, 2007 Acta Orthop Bel</i> Retrospective CS	62 TIBIAL nonunions	In <u>7,5 months</u> 79.6% clinical union 84.9% radiological union	No Adverse Events	Uncontrolled retrospective data

<i>Ekrol et al, 2008, Injury</i> RCT	15 RADIAL distal metaphyseal OSTEOTOMIES	In <u>12 months</u> 84% union rates	2 osteolysis	<u>Not as good</u> as Autograft when combined with Ex Fix
<i>Kanakaris et al, 2008, Injury</i> CS	68 TIBIAL	In <u>6.5 months</u> 89.7% union rates	No Adverse Events	Uncontrolled data
<i>Dohin et al, 2009, CORR</i> CS	19 <u>PEDIATRIC</u>	73.9% union rates	No Adverse Events	Uncontrolled data
<i>Giannoudis et al, 2009,</i> CORR CS	45 TIBIAL-FEMORAL-HUMERAL	In <u>5 months</u> 100% union rates	No Adverse Events	OP-1 +AutograftGRAF T EXPANSION

<i>Kanakaris et al, 2009, Injury CS</i>	30 FEMORAL	In <u>6 months</u> 86.7% union rates	No Adverse Events	Uncontrolled data
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Orthopaedic Trauma Association

Basic Science Focus Forum

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“Systemic Agents for Fracture Healing: The PTH Story”

Thomas A. Einhorn, MD

Over the past several years, there has been increasing interest in developing technologies for the enhancement or acceleration of fracture healing. Although the use of locally implanted or injected growth factors has received most of the attention, the ability to enhance bone repair by systemic means is an attractive alternative. Parathyroid hormone (PTH) treatment is an anabolic therapy for osteoporosis and has been shown to increase bone density and prevent fractures. A growing body of evidence supports the notion that PTH may also be an effective anabolic therapy for the enhancement of bone repair.

Parathyroid hormone is an 84 amino acid peptide that has been associated with both anabolic (with pulsatile administration) and catabolic (with continuous exposure) effects on the skeleton. The first thirty-four amino acids of the molecule represent its active site and recombinant gene technology has produced a recombinant human PTH (1-34) also known as teriparatide. Experiments in models of fracture healing have demonstrated that PTH (1-34) consistently enhances the stiffness and strength of experimental fractures when used at doses slightly higher than those currently approved for the management of osteoporosis. Thus, one question regarding the clinical utility of PTH is whether doses capable of enhancing skeletal repair in animals would be well-tolerated by patients.

Investigations in models of endochondral bone formation, including those on growth plate and fracture healing biology, have shown that PTH has direct effects on both chondrogenesis as well as osteogenesis. Indeed, analyses of experimental fractures treated with PTH (1-34) show enlarged calluses based primarily on increased formation of cartilage. Moreover, micro CT analysis demonstrates that PTH increases callus size but not density suggesting that the mechanism by which PTH enhances skeletal repair may be based on its chondrogenic effects. Expression lineage determination for transcription factors associated with chondrogenesis and osteogenesis support this hypothesis and show that PTH treatment leads to an earlier and more robust expression of SOX9 when compared with controls. Moreover, expression of matrix metalloproteinases occurs earlier in fractures treated with PTH suggesting an enhancement of cartilage turnover and an acceleration of the transition from calcified cartilage to bone.

One of the major intracellular pathways associated with bone formation and skeletal repair is the Wnt signaling pathway. Wnt's comprise a family of secreted signaling proteins that regulate diverse developmental processes. Recent data have shown that Wnt's 4 and 5B are thought to be associated with chondrogenesis. Using experimental models of fracture healing, our laboratory has demonstrated an increase in the expression of these two Wnt molecules in animals treated with PTH (1-34).

Finally, Wnt signaling is modified by the co-receptor LRP-5. Studies have shown that gain-of-function mutations in LRP-5 increase Wnt signaling and result in higher bone mass. Data from our laboratory have shown that PTH (1-34) enhances and accelerates the expression of LRP-5. Conversely, Wnt receptor antagonists such as DKK-1 and sclerostin down-regulate Wnt signaling. Its action to increase the expression of DKK-1 and sclerostin appears to occur later in fracture healing suggesting a regulatory role in skeletal repair.

To date, only one clinical trial has been conducted investigating the use of PTH (1-34) in patients with fractures. Post-menopausal women who had sustained a dorsally angulated distal radial fracture in need of closed reduction but not surgery were randomly assigned to eight weeks of once-daily injections of

placebo (n = 34), PTH (1-34; teriparatide) 20 micrograms, or 40 micrograms within ten days of fracture. The 20 microgram dose is the currently marketed and well-tolerated dose for treatment of osteoporosis. The results showed that the estimated median time from fracture to first radiographic evidence of complete cortical bridging in three of four cortices was 9.1, 7.4, and 8.8 weeks for placebo, teriparatide 20 micrograms, and teriparatide 40 micrograms, respectively ($p=0.015$). There was no statistically significant difference between the higher and the lower doses of teriparatide nor between the higher dose and the placebo group. The time to healing was shorter in the teriparatide 20 microgram group than in placebo ($p=0.006$). The investigators concluded that the shortened time to healing may suggest that fracture repair can be accelerated by teriparatide but that these results require further investigation.

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OTA BASIC SCIENCE FORUM *Growth Factors: Beyond BMP's*

Gene Therapy for Growth Factor Delivery: Does it have a Role in Orthopaedic Trauma

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Introduction

Traumatic bone defects and non-union represent a significant source of morbidity and socioeconomic burden in trauma patients. The treatment of these conditions is currently hampered by inadequate therapies. This has prompted a new era of investigation into biologic therapies for augmenting fracture healing. Within this body of research, gene therapy has arisen as a novel and effective method of delivering therapeutic proteins at a site of desired bone regeneration. Gene therapy has shown tremendous potential in pre-clinical studies of fracture healing, but to date no clinical trials have occurred. This session reviews the scientific basis for gene therapy in fracture healing, briefly describes results from important pre-clinical studies which have been performed to date, and discusses the current barriers and future directions of gene therapy as it applies to fracture healing and orthopaedic trauma.¹

Defining Gene Therapy

Gene therapy can be defined as the therapeutic transfer of genetic information to cells.² While originally conceived as method for replacing a defective gene in patients with single-gene genetic disorders (e.g. the cystic fibrosis transmembrane regulator, or CFTR, gene in patients with cystic fibrosis), the concept and application of gene therapy has evolved significantly over the past decade. Current thinking now posits that the major role of gene therapy is to genetically manipulate cells to produce a therapeutic effect in complex or acquired diseases. More specifically, gene therapy is being used as a tool for the delivery of therapeutic proteins (**growth factors**) to specific tissues or cells. This is especially relevant to orthopaedic trauma given the recent body of literature identifying the importance of numerous growth factors in the processes of fracture healing and bone regeneration.^{3,4} Gene therapy in this form involves the transfer of a complementary deoxyribonucleic acid sequence (cDNA) encoding for a therapeutic protein to target cells. The cells acquire the therapeutic cDNA and subsequently serve as protein factories, producing the desired protein locally in a prolonged manner.⁵

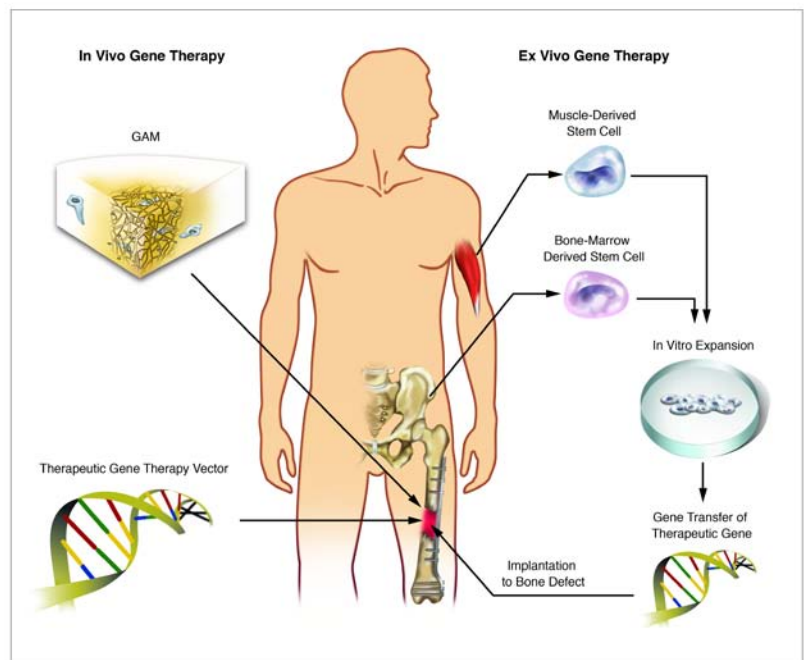
Advantages of Gene Therapy Over Conventional Growth Factor Delivery

The current application of growth factors to orthopaedic trauma mainly involves the use of BMP's. Current clinical applications of BMP's include open tibial fractures, segmental bone defects, and non-unions. In these applications, BMP is delivered in a one-time, supraphysiologic dose to a site of desired bone healing. Gene therapy offers several potential advantages over this strategy:

- Gene therapy allows sustained production of a growth factor over a duration that is compatible with fracture healing. This allows the growth factors to be delivered at more physiologic levels over a sustained period.
- Growth factors delivered by gene therapy undergo authentic post-translational processing within local cells, allowing a more physiologic presentation.
- Gene therapy can be used to deliver both osteoinductive and angiogenic growth factors, either alone, or in combination.
- Gene therapy can be combined with cell-based methods to allow delivery of growth factors and osteogenic or angiogenic progenitor cells at the same time.

Ex Vivo versus In Vivo

Two fundamental strategies of gene therapy exist: “in vivo” and “ex vivo” methods. In vivo gene transfer involves the direct administration of a therapeutic gene within the host using a vector that is administered either systemically or locally at a desired site. Ex vivo gene transfer requires the harvest of cells from a patient (usually by bone marrow aspiration), culture expansion of the cells, gene transfer in tissue culture, and then subsequent reimplantation of the genetically modified cells at a desired site (see figure). Each strategy has inherent advantages and limitations, and both have been shown to be effective in pre-clinical studies. However, neither strategy has been shown to be clearly superior to the other.



Use of a Vector

The amount and duration of protein expression is dependent on the type of vector used and the gene-transfer technique employed. Vectors are used to facilitate the transfer of cDNA sequences into target cells. Both viral and non-viral vectors are available and have been assessed in pre-clinical studies of fracture healing. The term “transduction” is used to denote gene transfer using a viral vector. Viral vectors currently represent the most efficient method of transferring genetic information to cells; as a result, most gene therapy studies to date, both in and outside of orthopaedics, have used a transduction approach.^{6,7} The use of non-viral methods of gene transfer is termed “transfection”. Several forms of transfection exist, including exposure of the cell to naked DNA, electroporation, complexing DNA with liposomes or polymeric complexes, and the use of a gene gun.

Conclusions

Gene therapy represents a promising approach for growth factor delivery in orthopaedic trauma. While gene therapy has shown impressive results in pre-clinical studies of fracture healing, no clinical trials have occurred to date, and ongoing concerns exist about the safety and cost of a gene therapy approach. Continued research is necessary for gene therapy to become a clinical reality in orthopaedic trauma.

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