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Systemic administration of pharmacological agents and bone repair: What can we expect

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ABSTRACT

Pharmacologic agents that modulate bone formation and bone remodelling are in broad use and development for the treatment of osteoporosis and other disorders of bone fragility. There is significant interest into the effect these agents may have on bone repair and fracture healing and whether these agents may be beneficial or detrimental to bone repair. Bisphosphonates delay callus remodelling, but increased callus volume seen during endochondral bone repair with bisphosphonate use allows for equivalent biomechanical properties for the fractured bone. Teripartide stimulates bone formation and in bone repair appears to have the potential to accelerate fracture callus formation and remodelling, potentially accelerating fracture healing. Animal models of fracture healing have demonstrated accelerated healing with larger callus volume, more rapid remodelling to mature bone, and improved biomechanical properties of the fractured bone. Clinical data with teriparatide has shown mixed results for its ability to stimulate fracture healing. Wnt signalling is one of the major pathways through which cartilage and bone formation is regulated during development. This same pathway has been identified as one of the ways that teriparatide stimulates bone formation. Antibodies to downstream proteins in this pathway, Dkk-1 and sclerostin, show significant promise of accelerating even normal fracture healing in preclinical animal models.

Introduction

Systemic agents influencing the remodelling of bone have been in broad use for the treatment of osteoporosis and the prevention of fragility fractures for many years. These agents have been shown to either reduce bone remodelling or increase bone formation in order to improve bone biomechanics and decrease fracture risk. Interest into the effects of these bone modulating agents on fracture repair seeks to confirm that these agent do not interfere with normal bone fracture healing and to see if in any way these agents might improve or accelerate fracture healing and fracture bone biomechanics. Three classes of agents are under investigation for their effects on fracture prevention as well as fracture healing: antiresorptive agents (including bisphosphonates and RANK ligand inhibitors), parathyroid hormone analogues, and Wnt signalling modulators. This review looks at the preclinical and clinical evidence of how these agents may affect bone repair.

Antiresorptive agents

Bisphosphonates are widely used for the treatment of osteoporosis and prevention of fragility fractures. Systemically administered bisphosphonates avidly bond to the hydroxyapatite crystals of bone and inhibit bone resorption by osteoclasts, leading to their apoptosis. This decreases the rate of bone remodelling. Several preclinical models of fracture in bisphosphonate treated animals have demonstrated that for fractures healing by endochondral ossification, bisphosphonate treatment increases callus volume, trabecular bone volume, and bone mineral content, but delays the maturation and remodelling of the callus.[24,29] Biomechanical properties of this larger but less histologically mature callus are equivalent to those of normal callus formed by placebo treated animals. The initiation of callus formation appears unaffected by bisphosphonate therapy. Once a calcified cartilage callus is formed both its remodelling to woven bone and the subsequent remodelling to mature lamellar bone are delayed. This delay can persist even after bisphosphonate administration is stopped. If functional recovery and pain are controlled once a calcified cartilage callus has formed at a fracture site, this remodelling delay has not been considered to be clinically significant, but clinical evidence is sparse. Fracture healing in children with osteogenesis imperfecta (OI) on pamidronate

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therapy with a long bone fracture was not delayed in a large case series, but a smaller case series did demonstrate a delay in 30% of patients at early healing timepoints.[31] A significant delay in the expected time to healing was noted for OI patients on pamidronate undergoing long bone osteotomy.[31] Two studies of patients on bisphosphonates with distal radius fracture did not demonstrate a significant delay in healing. [1,38]

Animal models of spine fusion have shown a delay in remodelling of the fusion mass.[16,41] One study showed a 50% decrease in fusion rates in bisphosphonate treated animals.[16] No significant clinical data exist for spine fusion patients on bisphosphonate, but these animal studies prompt some practitioners to stop bisphosphonate therapy until a spine fusion is considered healed. Timing of bisphosphonate treatment relative to fracture has also been questioned. Treatment of hip fracture patients with IV bisphosphonate within 2 weeks negated the mortality and fracture reduction seen for patients who received drug between 3 and 12 weeks post fracture.[12] The cause of this finding is unclear but it has prompted a recommendation to delay bisphosphonate administration for 6 weeks after a hip fracture. This may not be relevant for oral bisphosphonates which are given in divided doses rather than the single annual dose of IV treatment. Less is known about stress fracture repair and bisphosphonates. Stress fractures heal through direct primary bone remodelling, and bisphosphonate therapy does appear to affect this healing. Bisphosphonates were unable to prevent the deterioration of mechanical properties of rat bone subject to repeated cyclic loading, and did not prevent stress fractures in military recruits during basic training.[9,30] No other direct clinical data are available.

Bisphosphonate use for osteoporosis treatment does produce some modest gains in bone mineral density (BMD) potentially by allowing unremodelled bone to increase its bone mineral content. One study of bone marrow stromal cells demonstrated an increase in osteoblast proliferation and differentiation with bisphosphonate treatment, potentially through a BMP-2 mediated pathway.[17] A rat critical defect healing model combined local BMP-7 treatment with systemic bisphosphonate therapy. The combined therapy demonstrated increased bone mineral content, callus volume, and bone strength compared to BMP-7 treatment alone.[27] In a small study of limb lengthening patients, treatment with bisphosphonates after regenerate had formed produced rapid and sustained improvement in regenerate BMD.[20] Further research is needed to determine whether clinically significant anabolic effects of bisphosphonates can be seen using current dosing parameters.

Denosumab is a newly approved antiresorptive treatment for osteoporosis that works by inhibiting osteoclast recruitment, formation, and activity. This antibody serves as a decoy receptor in the RANK ligand signalling pathway, preventing osteoblast produced RANK ligand from reaching osteoclast RANK receptors. Denosumab does not deposit into bone, but produces a rapid reduction in bone remodelling after subcutaneous injection that is lost after approximately 6 months. It does not appear to have any direct effect upon osteoblasts, but osteoporosis treatment trials demonstrate increases in BMD with treatment. In animal models of fracture healing, denosumab did not show any significant effects on fracture union rates. Similar to bisphosphonates, fractures healing by endochondral bone formation demonstrated increased callus volume and delayed remodelling. Denosumab treated animals also demonstrated increased BMD in callus tissue whilst bisphosphonate demonstrated only increased bone mineral content (BMC). Mechanical properties were not compromised.[14] No clinical data regarding fracture healing in denosumab treated patients has been published. Fractures were observed in patients treated with denosumab in the osteoporosis and cancer patient clinical trials, but no significant adverse effect on fracture healing in denosumab treated patients has been reported.

Parthyroid hormone (PTH)

A human derivative of parathyroid hormone, 1-34 PTH or teriparatide, has been in clinical use for a decade now as an anabolic agent to treat patients with severe osteoporosis who are at high risk for fragility fracture. Once daily injection of 1-34 PTH increases bone formation on all bone surfaces including trabeculae, endosteal bone, and periosteal bone.[13] It has also been shown to increase trabecular connectivity and cortical bone thickness. These changes improve bone microarchitecture and biomechanical properties. Treatment with teriparatide expands osteoblast and osteoblast precursor populations contributing to its bone anabolic effects. Native parathyroid hormone also stimulates expansion of mesenchymal stem cell populations that contribute to osteogenic and chondrogenic cell populations and there is considerable interest into whether teriparatide may have similar stem cell effects. This combined anabolic effect on bone and potential to stimulate mesenchymal stem cell precursors for bone formation has made teriparatide a target for potential therapuetic intervention in fracture healing.

Several animal studies using rats, mice, rabbits and monkeys have investigated the potential of PTH on fracture healing and spine fusion.[3–5,21,28,32] Supraphysiologic doses of PTH demonstrate increased fracture site strength and callus quantity in treated animals. A larger and more mature callus forms at the fracture site and mineralises more rapidly than callus in control animals. Marked increases in callus volume, biomechanical properties (stiffness, tortional strength), and density are noted with PTH treatment. Spine fusion models showed increased rates of fusion and increased fusion mass size with PTH treatment.[32] Teriparatide treatment also increased the amount of cartilage formed in fracture callus and spine fusion sites, indicating that it may influence both osteogenic and chondrogenic cell line development and activity.[18] A study of human mesenchymal stem cell cultures treated with teriparatide showed a decrease in adipose cells and an increase in alkaline phosphatase activity, suggesting a shift in cell differentiation towards the osteogenic lineage.[23] Activation of Wnt signalling pathways by PTH treatment has been noted in animal models of fracture healing and may account for a portion of the anabolic and fracture healing enhancement effects of PTH on bone.[18,37]

Clinical studies of 1-34 PTH have demonstrated mixed success. The supraphysiologic doses used in preclinical animal models (5–200 mcg/kg/dose) lead to concerns that the dose used safely in human (20–40 mcg total/dose) may not have the same effects on fracture healing, although these doses demonstrate anabolic effects in the treatment of osteoporosis. Only one randomised, double-blind, placebo controlled study of teriparatide in fracture healing has been published and it failed to meet its primary endpoint.[6] This study looked at fracture healing in distal radius fractures sustained by postmenopausal women. The primary endpoint of accelerated healing with the 40 mcg daily dose was not met, but accelerated healing in the 20 mcg dose group was noted ($p = 0.006$).[7] Multiple case reports of healing of fractures of long bones (fresh and nonunion) and of spinal vertebrae have been reported using teriparatide alone with no surgical intervention.[11,34,36,39] Two cases series have been presented showing improvements in pain and radiographic bone formation with teriparatide treatment in both fractures with delayed healing and fractures not amenable to operative treatment (such a pelvic fragility fractures).[10,35] Observations in these case series demonstrated most significant improvement in pain and fracture bridging for fractures in predominantly trabecular bone, an observation

consistent with the observation that in osteoporosis treatment, teriparatide has its greatest anabolic effect on trabecular bone. A randomised clinical trial of acute pelvic fragility fractures and teriparatide treatment is currently enrolling.

Wnt signalling proteins

Wnt signalling is known to be an important pathway during skeletal development, playing a role in chondrogenesis and chondrocyte maturation during bone formation.[19] Wnt signalling also appears to play a role in the regulation of both bone remodelling and bone modelling. Bone modelling describes the independent formation and deposition of bone by osteoblasts without prior local osteoclastic activity. Wnt signalling appears to be important in the development of osteoblasts from pluripotent mesenchymal stem cells. With the recognition that anabolic agents such as PTH produce their major anabolic effect on bone through the Wnt signalling pathway, attention has turned to downstream components within the pathway that have the potential to produce anabolic effects on bone. Binding of Wnt ligands to cell surface receptors LRP5/LRP 6 (low density lipoprotein receptor related proteins 5/6) allows translocation of β-catenin into the nucleus which stimulates transcription of Wnt responsive genes. The potential for this signalling pathway to play a role in fracture healing was first identified when LRP5 mRNA was noted to be upregulated in rat fracture callus in proliferating chondrocytes, osteoblasts, and osteoblast precursor stem cells.[42] Wnt signalling appears to be important in both endochondral and intramembranous bone formation.

Several regulators of Wnt signalling have been identified including the secreted proteins Dickkopf 1 (Dkk1) and sclerostin. These secreted proteins bind to LRP 5/6 and inhibit Wnt signalling. Antibodies to both Dkk1 and sclerostin have been developed and appear to have the potential to induce bone formation by allowing Wnt signal transduction. Treatment of mice and rats with Dkk1 antibody increased both cortical and trabecular bone formation.[15] Dkk1 has been noted to be upregulated in unloaded bone and thought to potentially contribute to the bone mass loss seen with loss of mechanical cycling of bone. A rat model treated animals with Dkk1 antibody looked at changes in metaphyseal bone of the proximal tibia.[2] Changes in pullout strength for screws placed in that region were measured in animals that were allowed mechanical loading as well as animals whose limbs were paralysed. Screw pullout force was increased by 100% and bone volume increased by 50% in animals who were allowed to mechanically load the treatment limb. For animals whose treated limb was paralysed, screw pullout force was equivalent to placebo animals, but bone volume increased by 233%. Cortical bone in the treated rat metaphyses also thickened compared to control animals. A murine closed femur fracture model demonstrated that Dkk1 antibody administered on the day of fracture increased callus volume, biomechanical strength, BMD, and BMC.[22] The unfractured limb also saw gains in BMC and BMD. Animals treated with Dkk1 antibody at day 4 did not demonstrate these gains, and in fact showed a 10% reduction in BMD and BMC in the uninjured leg and at 13% reduction in ultimate force biomechanical testing of the fractured leg. These findings demonstrate that timing of Dkk1 antibody administration after fracture may be very important in order to obtain fracture healing gains from treatment. The same study looked at LRP5 knockout mice and demonstrated a significant delay in the restoration of biomechanical properties of a fractured limb, reinforcing the concept that Wnt signalling is important in fracture healing. A study of stromal cells derived from human fracture nonunions demonstrated that these cells had significantly reduced capacity to differentiate into osteoblasts and to induce bone mineralization when compared to normal human bone marrow mesenchymal stromal cells.[8] Cultures of these stromal cells demonstrated increased levels of Dkk1, and treatment of normal stromal cell cultures with conditioned media from these nonunion stromal cells decreased the ability of these normal stromal cells to differentiate into osteoblasts. These findings suggest that fracture nonunion may be influenced by a local increase in Dkk1 secretion that interferes with osteoblast differentiation and function and opens the possibility that timely Dkk1 antibody administration may be able to influence the pathogenesis of a nonunion.

Sclerostin antibody shows similar capacity to increase bone formation and bone mass in both fractured and unfractured animal models. Rat models of postmenopausal osteoporosis and aged male rats both demonstrate increased bone formation at both cortical and trabecular sites, increased bone mass, and increased bone strength with sclerostin antibody treatment.[25,26] A closed rat femur fracture model and a closed cynomolgus monkey fibular osteotomy model both demonstrated increased bone mass and increased bone strength at the fracture site and more advanced remodelling of the callus compared to control animals with less cartilage callus and fibrovascular tissue, and more bone formation with smaller fracture gaps at the fracture site.[33] Sclerostin antibody also demonstrated increases in trabecular bone volume and thickness, bone formation rates, and mineralizing bone surfaces in a rat model of hindlimb immobilization and compared to normal controls.[40] Similar to Dkk1, sclerostin antibody may represent a treatment to prevent bone loss during immobilization and a therapy during fracture healing to improve bone formation and accelerated biomechanical stabilization of the fracture site. Two phase II clinical trials with a sclerostin antibody administered to patients with acute tibial diaphyseal fractures or displaced intertrochanteric hip fractures are currently enrolling. Similar trials are in development with Dkk1 antibody treatments.

Conclusion

One of the great potential advances for orthopaedic care in the next two decades is the development of systemic therapeutic molecules that will allow enhanced the fracture healing responses in both operative and nonoperative fracture care. Currently agents such as BMP2 and BMP7 as well as a variety of bone graft techniques and bone graft substitutes allow improvements in fracture healing, but require a surgical intervention with placement of the material directly at the fracture site. As we begin to understand the molecular pathways involved in bone development and fracture repair, systemic agents allow us the possibility to both prevent fractures as well as potentially accelerate and improve fracture repair rates. Careful clinical trials must occur with these agents in order for us to optimise bone repair benefits without causing problems that will interfere with bone repair or the overall structural integrity of the remainder of the skeleton. Advances in our understanding of bisphosphonates, parathyroid hormone derivatives, and Wnt signalling modulators are already allowing us to use these systemic agents in a clinical setting.

Conflict of interest statement

Consulting: Eli Lilly, Amgen. *Speaker's Bureau*: Eli Lilly, Amgen, Novartis. *Research support*: NIH, OREF.

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