

MEETING REPORTS

Pre-Clinical Fracture Repair Investigations: Meeting Report from the 30th Annual Meeting of the American Society for Bone and Mineral Research

September 12-16, 2008 in Montréal, Québec, Canada

Michelle M. McDonald and David G. Little

Orthopaedic Research and Biotechnology, The Children's Hospital at Westmead, Westmead, Australia

Fracture repair is a complex process involving many stages of cellular recruitment, differentiation and proliferation, followed by extensive matrix production and remodeling. The mechanisms underlying situations of impaired bone healing are often complex and can affect many of these stages. As pre-clinical models of the many different healing situations emerge, our understanding of how and when this complex process can be manipulated has improved, leading to advances in options for therapeutic intervention, particularly in cases of poor repair.

At the recent ASBMR Annual Meeting, a number of pre-clinical investigations expanded our knowledge of the mechanism behind the effects seen when currently used clinical agents are administered during periods of bone healing. Moreover, new agents aimed at promoting cellular recruitment, proliferation and matrix production during bone repair were unveiled, while new techniques were employed to examine cellular contributions to this complex process.

Parathyroid hormone (PTH), currently one of the most commonly used clinical anabolic agents, has been thoroughly examined in numerous animal models of bone healing. Its application enhances greatly callus size and strength, through stimulation of new bone formation (1). New developments in the use of PTH to enhance bone repair and the mechanisms behind its anabolic potential were presented at the meeting. In healthy rats, a new PTH analogue (cPTH 1-

31) was compared to PTH 1-34 in a closed fracture model (2). Equivalent increases in bone volume, content and strength of the early healing callus were documented with both these analogues. However, during the later remodeling phase, cPTH 1-31 showed a tendency towards enhancements in these parameters compared to PTH 1-34, although this was not significant. In addition, in a cortical bone defect model in ovariectomized rats, PTH 1-38 enhanced new woven bone formation both within intramedullary spaces and on endocortical surfaces, translating to enhanced mechanical strength of the limb (3).

While PTH treatment clearly enhances a robust repair response in normal situations, its effects may be somewhat attenuated in a more challenged environment. In closed fractures performed in ovariectomized rats, both strontium ranelate and PTH 1-34 increased callus bone volume at 28 days post fracture compared to vehicle-treated controls (4). Whereas strontium increased the torsional strength of the callus, PTH 1-34 failed to do so in this model. This difference was attributed by the authors to the slightly larger calluses formed in the strontium-treated group.

Glucocorticoid (GC) treatment has been associated with impaired bone healing, with delayed union and reduced callus size and strength (5). In GC-treated mice, daily PTH 1-34 preserved bone mass and mechanical integrity in intact limbs compared to vehicle-treated GC-dosed mice (6). In the fracture callus however, in contrast to the 80%

increase in callus force with PTH treatment in non-GC-treated mice, although slight increases in callus size were documented with PTH treatment in GC-treated mice, these did not translate to increased mechanical strength of the callus. Interestingly the increased bone formation noted with PTH treatment in calluses in non-GC-treated mice was not noted in GC-treated mice. Moreover, the delay in endochondral repair noted with GC treatment was not improved with PTH therapy. The authors concluded that previous GC treatment may attenuate the anabolic effect of PTH during bone healing.

Taken together, these new results confirm those that previously demonstrated a strong anabolic potential for PTH in situations of normal bone healing in healthy rats or small bony defects with robust repair. However when an additional systemic challenge such as estrogen deficiency or glucocorticoid treatment leads to impaired healing, the effects of PTH may be somewhat attenuated. This presents a challenge for research into PTH effects in clinical trials of impaired bone healing.

New developments in the mechanisms underlying the anabolic potential of PTH were also presented at the meeting, supporting previous suggestions that PTH attenuates fracture repair by activating Wnt signaling pathways (7). Both canonical and non-canonical Wnt pathways have been directly associated with PTH administration, with a large number of studies presented at the meeting exploring this mechanism (8-12). As the role of Wnt signaling during bone repair is becoming more apparent (13), emerging new agents that manipulate the Wnt pathway, such as inhibitors of sclerostin or Dickkopf-1, which were presented in detail ((14;15) and 13th Annual Meeting of the *In Vivo* Working Group), may offer new avenues to explore the pharmaceutical enhancement of bone repair.

New alternatives to promote bone repair are under development and have been examined in pre-clinical models. Both transdermal application (16) and local delivery using nano-particles (17) of

lovastatin have been shown recently to enhance fracture repair. In a follow-up investigation, administration of lovastatin locally using micro-particles was delayed until 1 week after closed fracture in mice and compared to dosing 2 hours post fracture (18). Delayed dosing produced further increases in callus mechanical strength and improved bridging of the fracture gap. Lovastatin delivery 1 week post fracture led to an extension of the period of peak BMP-2 expression usually observed post fracture. The authors suggested this may be the mechanism behind the enhanced response. Supporting this theory was an *in vitro* study showing that lovastatin applied to pre-osteoblast cell cultures produced a more rapid increase in BMP-2 mRNA expression compared to controls (19). Therefore, in contrast to PTH stimulation of the ubiquitous Wnt signaling pathway, statins may enhance anabolism through BMP signaling, a more robust specific osteogenic pathway with documented success in promoting healing in non-union situations through stimulating specific anabolic responses (20;21).

Bone repair with reduced stability is commonly achieved through the replacement of an initial soft cartilaginous callus with woven bone through endochondral ossification. It would be advantageous to advance this process and thus hasten the rate of union. Inhibition of the enzyme activity of 5-lipoxygenase (5-LO), a regulator of inflammation, was shown to accelerate endochondral ossification in a rat fracture model (22). Compared to treatment with a COX-2 inhibitor (celecoxib), 5-LO inhibition with AA-861 led to an earlier peak in cell proliferation, with celecoxib delaying this peak. AA-861 also produced increased collagen X expression in the early callus, suggesting increased levels of hypertrophic chondrocytes. On the other hand, celecoxib treatment enhanced collagen II expression, hence delaying differentiation of chondrocytes towards the hypertrophic stage and delaying endochondral repair. 5-LO inhibition therefore enhanced differentiation of the cartilaginous callus, advancing endochondral repair in this rat fracture model and offering a new agent for potential

applications in orthopedics. Limitations to the application of this agent may exist when one considers its lack of specificity to sites of bone healing and deficient anabolic response in situations where endochondral bone healing is impaired or absent.

Addressing a situation commonly associated with impaired bone healing, diabetes, locally delivered recombinant human platelet derived growth factor (rhPDGF) was explored in a diabetic closed femoral rat fracture model (23). The diabetic rats demonstrated reduced early cellular proliferation and decreased biomechanical properties of the callus compared to normal rats. rhPDGF treatment enhanced early cell proliferation, and led to a significant increase in callus load in torsion at 8 weeks post fracture. By 12 weeks, mineralized callus area was increased along with histological union rates in rhPDGF groups. rhPDGF enhanced cellular proliferation in the initial phase of the healing response, manifesting to slight improvements in union rates and mechanical strength at later stages. The lack of a specific osteogenic stimulus from PDGF however may limit the application of this agent to situations where an endogenous bone-specific anabolic response is active.

The contribution of specific cells to bone repair from both the local environment but also the surrounding tissue has become an area of important investigation. A pool of mesenchymal stem cells (MSCs) is localized to the fracture site in the early stages of the repair process. These cells differentiate into either chondrocytes or fibroblasts in response to the local signals, eventually taking part in the cascade of events leading to bone union (24). Transplantation of MSCs into mice after production of fractures led to enhanced callus tissue volume, and when cells were selected based on their expression of surface receptor CRX4 this was further enhanced, producing a stronger callus (25). A specific osteogenic stimulus, however, is deficient using this method, hence in challenged healing environments, application of MSCs may require an exogenous anabolic stimulus, such as addition of a BMP, to promote repair.

The source of multi-potential mesenchymal cell populations requires further exploration. Although the bone marrow and periosteum are proven contributors to this cell population, other sources are being considered. A study utilizing GFP reporters to assess cell lineages demonstrated the relative contributions of different cell types to bone healing (26). Using a Col3.6 GFP reporter for pre-osteoblasts-early osteoblasts, these cells were shown to contribute mainly to the periosteal-driven bone formation forming the woven bone callus from the external edges towards the center. By combining this with the smooth muscle actin GFP reporter (SMMA), early progenitor cells with multi-lineage potential towards bone and fat were identified. These cells appeared at the leading front of the cellular invasion into the fracture site and were associated with blood vessels. They were then followed by cells positive for both SMMA and Col3.6 and then cells only positive for Col3.6, suggesting the SMMA positive cells differentiated into an osteogenic lineage. In addition, this technique was used to examine the progression of cells from a chondrocyte (Col2A1 promoter) to osteogenic (Col3.6) lineage. As demonstrated by this study, this novel technique of tracking cell lineages may not only provide new insights into the cell contribution and biology of bone repair, but also provide mechanisms for impaired healing in certain situations.

Finally, utilizing available therapeutic agents, an examination of the role that osteoclasts and matrix metalloproteinases (MMPs) play during endochondral bone repair was performed (27). In the absence of osteoclast function, using bisphosphonates, and with complete deficiency of osteoclast differentiation, using osteoprotegerin (OPG), the progression of endochondral bone union proceeded normally, with all calluses achieving bony union and removal of soft callus at the same rate in rat closed fractures. However, when an inhibitor of MMPs was administered, fracture union and removal of soft callus was greatly impeded. This study demonstrated for the first time that MMP function is in fact more vital to the

achievement of fracture union than osteoclast function.

The topics raised by pre-clinical investigations into fracture repair at the meeting covered a broad range of issues and interventions highly applicable to the clinical setting. While those agents currently used clinically have been thoroughly explored in bone repair, their applications appear limited, calling for novel interventions. Statins, rhPDGF and 5-LO inhibitors appear to be options with potential for this indication, along with a number of cell-based therapies. New agents at the forefront of osteoporosis treatment, such as denosumab and sclerostin inhibitors, offer new directions for enhancing fracture repair, studies of which have not yet been presented. However, the development of alternatives such as inhibitors of Wnt pathway antagonists may direct the future of pre-clinical and eventually clinical studies examining options to enhance bone repair.

Conflict of Interest: Dr. Little reports having received research funding, and patent licensing fees, from Novartis. Dr. McDonald reports no conflicts of interest.

References

1. Barnes GL, Kakar S, Vora S, Morgan EF, Gerstenfeld LC, Einhorn TA. Stimulation of fracture-healing with systemic intermittent parathyroid hormone treatment. *J Bone Joint Surg Am.* 2008 Feb;90(Suppl 1):120-7.
2. Young ML, Fontaine R, Mason Z, Bishop G, Gerstenfeld LC, Morgan EF, Einhorn TA. Comparison of cyclic parathyroid hormone (cPTH) 1-31 to PTH 1-34 in the enhancement of experimental fracture healing. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S413. [\[Abstract\]](#)
3. Komatsu DE, Brune KA, Schmidt AL, Liu H, Han B, Lu Y, Westmore MS, Wolos JA, Zeng QQ, Ma YL, Sato M. In vivo longitudinal QCT analysis of PTH efficacy in a rat cortical defect model. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S424. [\[Abstract\]](#)
4. Habermann B, Olender G, Eberhardt C, Augat P, Kurth AA. Strontium ranelate and teriparatid (PTH 1-34) enhance fracture healing in osteoporotic Sprague Dawley rats. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S206. [\[Abstract\]](#)
5. Waters RV, Gamradt SC, Asnis P, Vickery BH, Avnur Z, Hill E, Bostrom M. Systemic corticosteroids inhibit bone healing in a rabbit ulnar osteotomy model. *Acta Orthop Scand.* 2000 Jun;71(3):316-21.
6. Li J, Doyon AR, Ferries IK. Effects of parathyroid hormone on fracture repair in glucocorticoid treated mice. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S19. [\[Abstract\]](#)
7. Kakar S, Einhorn TA, Vora S, Miara LJ, Hon G, Wigner NA, Toben D, Jacobsen KA, Al-Sebaei MO, Song M, Trackman PC, Morgan EF, Gerstenfeld LC, Barnes GL. Enhanced chondrogenesis and Wnt signaling in PTH-treated fractures. *J Bone Miner Res.* 2007 Dec;22(12):1903-12.
8. O'Brien CA, Galli C, Plotkin L, Vyas K, Cazer P, Goellner JJ, Berryhill S, Webb W, Robling A, Bouxsein M, Schipani E, Turner CH, Weinstein RS, Jilka RL, Manolagas SC, Bellido TM. PTH receptor signaling in osteocytes increases bone mass and the rate of bone remodeling through Wnt/LRP5-dependent and -independent mechanisms, respectively. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S12. [\[Abstract\]](#)
9. Wan M, Yang C, Wu X, Li J, Yuan H, He X, Nie S, Chang C, Cao X. PTH signaling through LRP5/6 in osteoblasts. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S5. [\[Abstract\]](#)
10. Terauchi M, Nanes MS, Weitzmann M, Zayafoon M, Weitzmann M, Lane TF, Pacifici R. T cells amplify the anabolic action of PTH through Wnt10b signaling. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S56. [\[Abstract\]](#)

11. Yao W, Cheng Z, Busse C, Pham A, Shahnazari M, Lane N. Overexpression of secreted Frizzled-related protein 1 inhibits bone formation and attenuates PTH bone anabolic effects. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S29. [\[Abstract\]](#)
12. Bergenstock MK, Tamasi J, Partridge NC. WNT-4 acts through the non-canonical pathways to stimulate osteoblast and bone marrow stromal cell differentiation. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S90.
13. Chen Y, Whetstone HC, Lin AC, Nadesan P, Wei Q, Poon R, Alman BA. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. *PLoS Med.* 2007 Jul 31;4(7):e249.
14. Glantschnig H, Hampton R, Wei N, Scott K, Nantermet P, Zhao J, Chen F, Fisher J, Su Q, Pennypacker B, Cusick T, Sandhu P, Reszka A, Strohl W, Flores O, Wang F, Kimmel D, An Z. Fully human anti-DKK1 antibodies increase bone formation and resolve osteopenia in mouse models of estrogen-deficiency induced bone loss. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S60-S61. [\[Abstract\]](#)
15. Jee WS, Li X, Tian XY, Paszty C, Ke HZ. Anti-sclerostin antibody increases bone mass by stimulating bone formation and inhibiting bone resorption in a hindlimb-immobilization rat model. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S40. [\[Abstract\]](#)
16. Gutierrez GE, Edwards JR, Garrett IR, Nyman JS, McCluskey B, Rossini G, Flores A, Neidre DB, Mundy GR. Transdermal lovastatin enhances fracture repair in rats. *J Bone Miner Res.* 2008 Nov;23(11):1722-30.
17. Garrett IR, Gutierrez GE, Rossini G, Nyman J, McCluskey B, Flores A, Mundy GR. Locally delivered lovastatin nanoparticles enhance fracture healing in rats. *J Orthop Res.* 2007 Oct;25(10):1351-7.
18. Gutierrez GE, Nyman JS, Munoz SA, Jadhav SB, Mundy GR. Lovastatin best enhances fracture repair when administered one week post-fracture. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S84-S85. [\[Abstract\]](#)
19. Moore MM, Edwards JR, Gutierrez GE, Mundy GR. Effects of statins on eNOS and BMP2 in osteoblasts. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S338. [\[Abstract\]](#)
20. Hak DJ, Makino T, Niikura T, Hazelwood SJ, Curtiss S, Reddi AH. Recombinant human BMP-7 effectively prevents non-union in both young and old rats. *J Orthop Res.* 2006 Jan;24(1):11-20.
21. Little DG, McDonald M, Bransford R, Godfrey CB, Amanat N. Manipulation of the anabolic and catabolic responses with OP-1 and zoledronic acid in a rat critical defect model. *J Bone Miner Res.* 2005 Nov;20(11):2044-52.
22. Cottrell JA, O'Connor JP. Chondrocyte differentiation is differentially affected by cyclooxygenase-2 and 5-lipoxygenase during fracture healing. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S272. [\[Abstract\]](#)
23. Al-Zube L, Breitbart EA, Simon AM, O'Connor JP, Young CS, Bradica G, Hart CE, Perrien DS, Lin S. Stimulation of fracture healing by recombinant human platelet-derived growth factor BB (rhPDGF-BB) combined with β -tricalcium phosphate/collagen matrix in a diabetic rat fracture model. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S40. [\[Abstract\]](#)
24. Granero-Molto F, Weis JA, Longobardi L, Spagnoli A. Role of mesenchymal stem cells in regenerative medicine: application to bone and cartilage repair. *Expert Opin Biol Ther.* 2008 Mar;8(3):255-68.

25. Granero-Molto F, Weis JA, Landis B, Longobardi L, Miga MI, Spagnoli A. Mesenchymal stem cells enhance fracture healing: essential role for cytokines in homing and anti-inflammatory response. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S166-S167. [\[Abstract\]](#)
26. Ushiku C, Xi J, Wang L, Adams DJ, Rowe DW. Use of GFP reporters to assess cell lineage in a murine model of tibial fracture repair. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S392-S393. [\[Abstract\]](#)
27. McDonald MM, Mikulec K, Peacock L, Lah T, Little DG. Inhibition of MMP activity delays bone repair whereas inhibition of osteoclast function and formation does not. *J Bone Miner Res.* 2008 Sep;23(Suppl 1):S149. [\[Abstract\]](#)