Current Status and Future Needs of Pediatric Orthopaedic Pharmacology

(Organized by the Pediatric Society of North America (POSNA) and ORS)

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Pediatric Bone Degeneration: Current Concepts and Therapies: Lessons Learned from Osteogenesis Imperfecta and Disuse Osteopenia

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Osteogenesis imperfecta (OI) is a genetic disorder caused by mutations in collagen and related proteins which lead to increased bone fragility and low bone mass. Brittle bones are the hallmark of OI and fracture rate is highest during childhood and decreases at adolescence and adulthood. Another low bone mass state in children, disuse osteopenia is common in diseases such as spastic quadriplegic cerebral palsy and spina bifida. Here disuse is a large contributor to low bone mass and increased fracture risk. In both of these low bone mass states identifying interventions to prevent fractures is of particular interest. This section will primarily focus on antiresorptive bisphosphonates and anabolic sclerostin antibody as used in young (growing) murine models of OI, human trials in OI, and human trials in osteopenia to treat these low bone mass diseases in children.

Clinically, antiresorptive bisphosphonates (BP) are used in an attempt to increase bone mass and reduce fracture risk in OI. A number of studies have demonstrated decreased rate of compression fractures of the spine but an unclear decrease in long bone fractures. However, bisphosphonates reside in the skeleton for many years and long-term administration may impact bone material quality. Acutely, there is concern about risk of non-union of fractures that occur near the time of bisphosphonate administration. This study investigated the effect of alendronate, a potent aminobisphosphonate, on fracture healing. Results suggest a history of bisphosphonate therapy has minimal effect on cortical bone repair when treatment is halted at the time of fracture, but calluses from Brtl/+ mice treated with alendronate during healing had a decreased mineral-to-matrix ratio, decreased crystallinity and increased carbonate-to-phosphate ratio. (Meganck et al. Bone, 2013).

Sclerostin antibody (Scl-Ab) therapy is potently anabolic in the skeleton by stimulating osteoblasts via the canonical wnt signaling pathway, and may be beneficial for treating OI. Scl-Ab therapy was investigated in 3 week old WT and Brtl/+ mice (Gly→Cys substitution in col1a1). Two weeks of Scl-Ab successfully stimulated osteoblast bone formation, which improved bone mass and reduced long-bone fragility. Image-guided nanoindentation revealed no alteration in local tissue mineralization dynamics with Scl-Ab. These results contrast with previous findings of antiresorptive efficacy in OI both in mechanism and potency of effects on fragility. (Sinder et al. JBMR, 2013).

Differing mechanisms of improving bone mass suggest that orchestrating precisely timed combination therapy could have synergistic improvement in bone. We evaluate whether the concurrent use of BP and SclAb confers structural and functional gains in bone mass in Brtl/+ model of OI. Femur reveals increased trab number with increasing PAM doses while scl ab mainly thickens existing trabeculae. Top panel PBS, bottom panel Scl-Ab (Olvera et al. ASBMR 2016)

Studies of BPs in disuse osteopenia in children (Bianchi et al, Arthritis Rheum 2000), (Henderson et al Pediatr 2002), (Sholas et al JPO 2005) have shown safety in use, increased BMD, but variable effects on fracture rates in patients. Identifying how disuse affects bone mass during growth, and how BP therapy affects bone formation and resorption will be important for the management of pediatric osteopenia.

These studies of antiresorptives and anabolics administered separately and in combination suggest potential new therapies to improve bone mass and reduce fractures in pediatric OI and disuse osteopenia.
Neurofibromatosis as a model for identifying new therapies to improve bone health in children

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Patients with neurofibromatosis type 1 (NF1) have shorter than expected bones, with low bone mineral density. In addition, they can develop pseudoarthrosis either after trauma, or sometimes spontaneously. NF1 encodes the protein neurofibromin, which is a negative regulator of the ras signal transduction pathway. Interestingly, it is expressed in many of the cells in tissues affected by the disease, such as oligodendrocytes and Schwann cells surrounding neurons. Investigations into how NF1 mutations alter bone and bone healing have revealed possible new therapeutic targets for bone problems in children with NF1

Florent Elefteriou’s research group (De la Croix Ndong J, Makowski AJ, Uppuganti S, Vignaux G, Ono K, Perrien DS, Joubert S, Baglio SR, Granchi D, Stevenson DA, Rios JJ, Nyman JS, Elefteriou F. Asfotase-α improves bone growth, mineralization and strength in mouse models of neurofibromatosis type-1. Nat Med. 2014 Aug;20(8):904-10) studied a mouse model of the disorder. They noticed that the mice had Mice lacking Nf1 in bone have more osteoid compared to control mice. Bones from these mice produced high levels of pyrophosphate, which is essentially two phosphate molecules joined together. Phosphate by itself promotes bone mineralization, but pyrophosphate inhibits the process. The mice also expressed very low levels of alkaline phosphate (ALP). One function of ALP is to split pyrophosphate into separate phosphate molecules. They then treated Nf1 mutant mice with asfotase-α, an engineered form of alkaline phosphatase. Asfotase-α is currently in clinical trials for hypophosphatasia. This treatment both improved bone mass and mechanical properties in the mice.

Data from our lab showed that tissue from the fracture site of patients with Neurofibromatosis type 1 and from mice deficient in the Nf1 gene both show elevated levels of β-catenin protein and activation of β-catenin mediated signaling. Constitutively elevated β-catenin leads to a delayed and fibrotic fracture repair process. Genetically Inhibiting β-catenin in Nf1 mutant mice improved fracture repair in these animals (Ghadakzadeh S, Kannu P, Whetstone H, Howard A, Alman BA. β-Catenin modulation in neurofibromatosis type 1 bone repair: therapeutic implications FASEB J. 2016 Sep;30(9):3227-37). (RS)-5-methyl-1-phenyl-1,3,4,6-tetrahydro-2,5-benzoxazocine (Nefopam, a centrally-acting, non-narcotic analgesic agent used in patients in several countries in Europe, Asia,
and the Middle East) was found to inhibit β-catenin mediated signaling during skin wound repair. When mice deficient in Nf1 were treated with Nefopam, their fracture sites showed increased the bone and cartilage content and decreased the fibrotic tissue.

These studies show that understanding the pathophysiology of bone disorders identifies possible new therapeutic approaches, using agents already in use for patient care. As such, they can be rapidly translated to the clinical setting.

**Treatment with Nefopam reverses the shortcomings in fracture repair seen for Nf1-/- mice 21 days after injury.** Mice expressing a floxed Nf1 gene were investigated for fracture repair potential. Mice were treated with Ad-GFP (Nf1<sup>fl/fl</sup>) or Ad-GFP+Cre (Nf1<sup>fl/fl</sup>, Cre). A subset of these mice were treated with Nefopam (Nef). Fracture calluses were isolated 21-days post fracture, fixed, and investigated for tissue regeneration. Calluses were observed using radiographs (left), radio-intensity maps (middle), and histological analysis using Safranin-O/Fast Green stain (right). There is improved healing with more bone at the fracture site with Nefopam treatment in Nf1-/- mice.
Significance: The process of ossification (Figure 1A) provides bone which is essential for support and protection. The formation and maintenance of bone is promoted by calcium and phosphate concentrations in the extracellular space near their saturation point. While these concentrations of calcium and phosphate are ideal for maintaining bone integrity, they can aberrantly aggregate in soft tissue, especially following injury, a process referred to as dystrophic (or pathologic) calcification. Dystrophic calcification is injurious imposing chronic inflammation and potentially loss of the afflicted organ’s function. It is involved in a myriad of disease processes, such as Alzheimer’s disease, renal calcinosis and breast cancer (Figure 1B). Dystrophic calcification also commonly occurs in muscle following injury secondary to exceptionally high concentrations of calcium and phosphate required for muscle function. Following, calcification of smooth muscle in the vascular wall is a pathophysiologic component of atherosclerosis. Calcification of skeletal muscle is a pathophysiologic consequence of a disease commonly referred to as heterotopic ossification (HO) which occurs following severe injuries such as burn, blast or spinal cord injury. Additionally, patients with mutations of the BMP signaling pathway (Fibrodyplasia Ossificans Progressiva) or pyrophosphate regulation (Pseudoxanthoma Elasticum) are susceptible to calcification of musculoskeletal tissue. The overall objective of this seminar is to discuss the pathophysiology of soft tissue calcification: highlighting the normal processes by which tissue protect themselves from, and the drugs used to increase protection from dystrophic calcification. These processes will be highlighted in the working model of muscle calcification following injury.

Skeletal muscle calcification: Normally, when muscle is injured, resident mesenchymal stem cells perform myogenesis resulting in functionally competent muscle. In certain circumstances injured muscle can develop dystrophic calcification. Dystrophic calcification results in chronic inflammation and failed muscle regeneration causing pain and a variable loss of limb function. Additionally, dystrophic calcification provides a microenvironment in which resident mesenchymal stem cells differentiate into osteoblasts instead of myocytes and develop heterotopic ossification (HO). HO results in complete loss of muscle function, and if surrounding a joint, loss of that limb function. Together, muscle dystrophic calcification and HO are a major source of morbidity and health care expenditure following severe burn, blast, or neurologic injuries as well as certain orthopaedic procedures. Although estimates vary, between 10-80% of patients suffer from various forms of HO after traumatic injury and trauma induced muscle calcification has complicated more than 60% of severe wartime extremity orthopaedic injuries during the Afghanistan and Iraqi conflicts. The molecular pathogenesis of trauma-induced muscle calcification, both dystrophic calcification and HO, is uncertain. Severe traumatic injury appears to alter the tissue microenvironment in injured skeletal muscle so as to promote osteogenesis over myogenesis, independent of any known genetic predisposition in bone morphogenetic protein (BMP) signaling. Surgical excision is extensive, morbid and often incomplete.

Until recently, current and proposed treatments for skeletal muscle calcification all have the undesirable adverse effect of disrupting systemic bone homeostasis and inhibiting fracture repair. Recent breakthroughs should allow for novel therapeutics that protect against pathologic muscle calcification while promoting normal bone biology. REFs: 1) Plasmin Prevents Dystrophic Calcification After Muscle Injury. J Bone Miner Res. PMID: 27530373. 2) Bisphosphonates: from softening water to treating PXE Cell Cycle: PMID: 25894689