Current Osteoporosis Reports (2019) 17:36–47
https://doi.org/10.1007/s11914-019-00501-5

EPIDEMIOLOGY AND PATHOPHYSIOLOGY (F COSMAN AND D SHOBACK, SECTION EDITORS)

Mechanisms Underlying Normal Fracture Healing and Risk Factors for Delayed Healing

Cheng Cheng [1,2] · Dolores Shoback [1,2]

Published online: 22 January 2019

Abstract

Purpose of Review Substantial advances have been made in understanding the biological basis of fracture healing. Yet, it is unclear whether the presence of osteoporosis or prior or current osteoporosis therapy influences the healing process or is associated with impaired healing. This review discusses the normal process of fracture healing and the role of osteoporosis and patient-specific factors in relation to fracture repair.

Recent Findings The definitive association of osteoporosis to impaired fracture healing remains inconclusive because of limited evidence addressing this point. eStudies testing anabolic agents in preclinical models of ovariectomized animals with induced fractures have produced mostly positive findings showing enhanced fracture repair. Prospective human clinical trials, although few in number and limited in design and to testing only one anabolic agent, have similarly yielded modestly favorable results.

Summary Interest is high for exploring currently available osteoporosis therapies for efficacy in fracture repair. Definitive data supporting their efficacy are essential in achieving approval for this indication.

Keywords Fracture healing · Risk factors · Osteoporosis · Bisphosphonates · Denosumab · Estrogen · Raloxifene · Parathyroid hormone (PTH) · PTH-related peptide · Atypical femoral fracture · Sclerostin · Dickkopf-1

Introduction

Impaired fracture healing has serious medical consequences for patients and the health care system. It is estimated that 10–15% of all fractures develop the complications of impaired healing, such as delayed union or non-union [1], although rates vary by anatomic location of the fracture [2]. Osteoporosis, characterized by low bone mass and weakened bone microarchitecture and strength, is known to increase the risk of fragility fractures, but the role of the disease itself in disrupting the healing process is less clear. Over two million osteoporotic fractures occurred in 2005, corresponding to a financial burden of ~ $17 million [3]. Moreover, the incidence of osteoporotic fractures in the US is predicted to rise to over three million by the year 2025 [3], in parallel with similar trends in Europe and even higher incidence in Asian countries. Overall, ~ 40% of women and ~ 14% of men over age 50 are projected to suffer from an osteoporotic fracture in their remaining lifetime [4]. Adding to these alarming statistics is the notion that osteoporosis also contributes to the rates of high impact fractures, where the extent of causative injury results from falls from greater than a standing height of the individual to road and motor vehicle accidents, implying that osteoporosis is potentially responsible for the majority of fractures [5].

As a health care issue, the problem of fracture healing is a critical one. The consequences that impaired fracture healing, particularly non-unions, impose on patients and the health care system of additional medical costs for more treatments, must be added to the indirect financial costs of lost work productivity [6]. Two US studies that analyzed the median healthcare cost per tibial fracture non-union have estimated it to be $11,333 and $25,556 for years 1997 and 2006, respectively (doubling in less than 10 years) [7, 8]. A European

This article is part of the Topical Collection on *Epidemiology and Pathophysiology*

✉ Dolores Shoback
dolores.shoback@ucsf.edu

[1] Endocrine Research Unit, Department of Medicine, San Francisco Veterans Affairs Medical Center, 1700 Owens Street, Room 369, San Francisco, CA 94158, USA

[2] Division of Endocrinology and Metabolism, Department of Medicine, University of California, San Francisco, USA

study across three countries that specifically examined the prospective productivity loss, in addition to healthcare cost, found that there was a saved cost of €7380 to €8145 associated with faster healing time of 221 days in open tibial fracture patients who received adjunct therapy with bone morphogenic protein 2 (BMP-2), as opposed to 266 days without this treatment [9].

Mechanisms of Normal and Impaired Fracture Healing

Definition of Impaired Fracture Healing

Although the time to healing differs with the specific fracture type and severity, most long bone fractures heal in 6–8 weeks, and 8–10 weeks is the usual healing time for vertebral fractures. The majority of fracture experiments consider the formation of bony callus with bridging as the end-point of fracture healing [10]. Impaired fracture healing beyond these expected time frames typically manifest as either delayed union or non-union [11, 12]. There is no consensus on a standardized definition of delayed union, but non-union is defined as failure to heal by 9 months or the lack of progressive signs of healing by 3 months, according to the US Food and Drug Administration (FDA) [13]. Fracture non-unions are further divided, based on Weber's classification, into hypervascular and avascular non-unions [14]. Whereas hypervascular or hypertrophic non-unions appear to be consequences of instability at the fracture fixation site that present as a large cartilagenous callus lacking a bony bridge, avascular (also called atrophic) non-unions demonstrate minimal to absent callus formation [15]. Etiologies of avascular non-unions are multiple, often involving critical damage to intrinsic factors local to the fracture site, resulting in disruption of the fundamental healing process. Optimization of fixation to enhance stability as the treatment of choice for hypervascular non-unions where the biological support for healing remains intact is unlikely to heal avascular non-unions without the addition of some form of adjunctive therapy [15].

Risk Factors for Delayed Fracture Healing

Introduction

Multiple risk factors have been identified that affect fracture healing [16]. Extrinsic factors include medical comorbidities and use of certain medications. Even social habits like smoking and alcohol consumption, in particular, can contribute to impaired healing. Intrinsic factors, such as injury to periosteum or endosteum and poor vascularization at the injury site, also directly influence the healing process.

This review will provide a concise background on fracture healing including the pathophysiology, risk factors, and medical treatment. The interested reader is referred to excellent recent reviews on the topic [6, 16, 17]. We will highlight current evidence on the relationship of osteoporosis to fracture healing and separately discuss the distinctive biology of osteoporotic fracture healing, the risk factors for impaired healing, and the effects of osteoporosis treatments on fracture healing. The latter topic is one confronted daily in the offices of clinicians and on the hospital wards where patients with disabling fractures are admitted and treated. The scope of therapeutic agents we present in this review is limited to systemically administered osteoporosis medications. There is considerable work on other approaches to augment healing, ranging from stem cell implantation to the local delivery of biological factors and biomechanical devices that has been published [17••].

Biology of Fracture Healing

Most fractures heal via a process of secondary healing, in which intramembranous ossification precedes and overlaps with endochondral ossification and ultimately results in bony bridging of the fracture gap. This is opposed to primary healing, during which direct bone formation occurs under condition of absolute stability, achieved with rigid fixation following perfect reduction with minimal to absent callus development [6, 16]. The formation of a hematoma and inflammation at the fracture site initiate the process. The release of cytokines and various immunogenic factors promotes recruitment and migration of mesenchymal stem cells from the periosteum and endosteum to the site [6]. This is accompanied by an increase in local vascular perfusion at the fracture site. The process is modulated by multiple signaling molecules that include fibroblast growth factor (FGF) 2, BMPs, insulin like growth factors (IGFs), and parathyroid hormone related protein (PTHrP) at distinct steps. Osteoblastogenesis is preferentially induced at the periosteum, which begins the process of intramembranous ossification. That process involves osteoblasts directly depositing osteoid that later becomes mineralized bone [6, 16]. Endochondral ossification, on the other hand, involves sequential steps of chondrogenesis through cell differentiation, the formation of a cartilagenous callus that bridges the bony ends, mineralization and expansion of the immature callus, and remodeling of the callus by osteoblasts and osteoclasts that replace woven bone with mechanically stronger lamellar bone [6]. The process may be divided into an early anabolic phase, where the callus forms via intramembranous or endochondral ossification, followed by a catabolic phase that includes remodeling of the formed callus into a mature mineralized callus [16]. Risk factors for avascular non-union include the following: [1] perturbation of local factors; [2] periosteal stripping; [3] loss or disruption of the hematoma; and [4] morphology of the fracture site

itself, such as a large fracture gap, bone fragment comminution and displacement, or an open fracture with segmental loss that critically damages the integrity of the scaffold on which the fracture should heal [16, 18]. Furthermore, these injury factors may account for the higher susceptibility of certain skeletal sites to development of non-union following a fracture, such as the tibia, related to its thin surrounding soft tissue coverage predisposing to comminuted and open fractures that are associated with devitalization of ends of the bones and vascular damage [19].

Special Aspects of Healing in Osteoporotic Fractures

The argument that fractures in individuals with osteoporosis are intrinsically different from fractures that occur in individuals with normal bone mass derives largely from orthopedic studies reporting greater implant fixation failures in individuals with osteoporosis who have fractured. These failures are ascribed to altered underlying bone quality, rather than hardware failure [20]. These failures are attributed to reduced bone mass and compromised biomechanical properties of the bone associated with osteoporosis [20, 21•]. Specifically, thinned cortices with increased porosity [21•], altered orientation of trabeculae with respect to direction of loading [21•, 22], and as changes in the composition of bone matrix [23] have all been linked to poor bone quality and lower mechanical performance that associates with up to 10% complication rates of non-unions following surgical repair raising the possibility of healing of osteoporotic fractures being a process distinctive from non-osteoporotic fractures [24].

Animal models that incorporate ovariectomy in combination with a low calcium diet, according to the FDA [25], best mimic the state of post-menopausal osteoporotic bone. It is additionally felt that achievement of complete bone discontinuity by complete osteotomies better characterize clinical fractures over simplified methods, such as drill hole defects or partial osteotomies that heal differently [26].

Studies in such models have revealed disruption in either the anabolic or catabolic phases of fracture healing that overall are associated with significantly lower maximum load but prolonged fracture healing time, shown in a systemic review of 26 animal trials specifically examining fracture biomechanical characteristics [27]. Defects associated with the anabolic or early phase of healing have been reported in several studies, as exemplified in both a sheep tibial osteotomy and a rat open femoral fracture model [28, 29] that yielded reduction in callus area and bone mineral density (BMD) in as early as 3 weeks post-fracture as well as a 2-week delay in bending stiffness in osteoporotic groups. Other investigations focusing on catabolic phase of healing, including a rat tibial and rat femoral fracture model [30, 31], found evidence of delay in endochondral ossification resulting in decreased callus failure load and stress. Again, majority of fracture experiments consider formation of bony callus with bridging as the endpoint of fracture healing, whereas evaluation of the remodeling phase is rarely performed [10].

While these findings seem to support an influence of osteoporosis on fracture healing, they remain debatable, as the experimental parameters are inconsistent, with wide variation in fracture type or animal age studied [21•].

Recent progress has been made in designing experiments that more precisely simulate the clinical scenarios of osteoporotic fractures, which commonly occur at the metaphyseal regions [32], rather than the diaphyseal locations characteristic of most lab-generated fractures. Although very few in number, results from these more recent studies support findings from diaphyseal fracture models that the healing of osteoporotic bone eventually produces a bone of significantly less mechanical strength, evidenced by both lower yield point and lower bending rigidity on biomechanical testing [33, 34]. One study additionally revealed abnormal bony bridging of the formed callus with higher unmineralized content relative to control [34].

Risk Factors for Altered Fracture Healing

Smoking as a Risk Factor

One meta-analysis of both intrinsic and extrinsic risk factors linked to impaired fracture healing revealed that smoking was as significant a risk factor as certain fracture site-specific factors conventionally associated with greater contributions to impaired healing [35]. Animal studies have demonstrated the negative impact of smoking on fracture healing related to nicotine, which causes vasoconstriction leading to decreased perfusion and is thought to play a role in lowering mechanical strength of the formed callus [36–38]. Multiple studies and a systematic review have validated smoking as a major risk factor for non-unions [35, 39]. Comparing smokers to non-smokers, the former have statistically significantly longer healing times as well as higher infection rates [39]. Other studies have suggested that the healing time and union of fracture are improved by smoking cessation [40, 41]. The impact of alcohol consumption on fracture healing is less well studied in comparison, with most of the evidence derived from animal studies that have shown impairment of biological healing at multiple steps include induced osteoblast dysfunction [42, 43], increased oxidative stress indirectly inhibiting activation of Wnt pathway [44, 45], and suppression of mesenchymal stem cell migration to the fracture site [46]. Both higher fracture risk and association with non-unions have been observed with alcohol abuse in few orthopedic studies [47, 48].

Diabetes as a Risk Factor

Increased fracture risk is established for patients with type 1 or 2 diabetes mellitus [49]. As importantly, however, are the elevated rates of impaired fracture healing in patients with diabetes [50, 51].These rates are reported to be as high as a 3.4-fold increased risk of delayed or non-unions, compared to those without diabetes [50]. The pathogenesis of impaired healing in patients with diabetes, based on findings from experimental studies, is noted to be multifactorial, involving poor recruitment and differentiation of mesenchymal stem cells at the fracture site [52], imbalance on a cellular level between osteoblastic and osteoclastic activity [53], as well as altered type I collagen synthesis from non-enzymatic glycation [54, 55]. Clinical studies have further delineated that more diabetics with complications, such as peripheral neuropathy and vasculopathy after ankle surgeries, for example, suffer from non-unions compared to patients with diabetes without complications [56]. Another study of risk factors for poor healing in patients with diabetes undergoing arthrodesis, osteotomy, or fracture reduction found a significant association of peripheral neuropathy and hemoglobin A1c > 7% with the ultimate development of non-union, delayed union, and malunion [57].

Medications as Risk Factor

Concerns regarding the effects of bisphosphonates on fracture healing have been raised because of their long half-life in bone and their incorporation into the matrix itself, but there have been few clinical studies. Preclinical data from animal models have not shown significantly delayed healing [58, 59]. One study showed that there was a delay in remodeling of woven to lamellar bone leading to larger but mechanically intact calluses [60]. A meta-analysis of 16 clinical studies addressed bisphosphonate use in fracture healing and included three randomized trials as well as seven retrospective cohorts [61]. The study evaluated differences in healing time and non-union rates in the context of chronic use before the occurrence of fracture as well as the initiation following fracture [61]. This analysis largely corroborated findings from preclinical studies that there was no significant correlation between the duration of bisphosphonate use or its introduction with healing times or non-union rates [61]. One conclusion from the meta-analysis was, however, that there were significantly longer healing times in patients already on bisphosphonate before sustaining distal radius fractures [61]. Additional studies on the healing of upper extremity fractures have produced conflicting results [62–64]. Some have reported an increase in non-union rates but no difference in healing time [62], while the opposite was described by the others [63, 64].Taken altogether, there is insufficient evidence to support or refute the notion that bisphosphonates inhibit fracture healing.

Like bisphosphonate, denosumab yielded larger, mechanically robust calluses accompanied by delay in callus remodeling but exhibited greater gain in bone volume and BMD by micro-computed tomography analysis in a comparison animal fracture model with alendronate. Neither drug was found to impact healing negatively [65].

Several classes of medications that conventionally affect fracture healing including corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) [66]. Some [67, 68] but not all studies [69–71] in animal models have shown that glucocorticoids can decrease callus formation [67], retard healing [68], and alter callus mineralization. Studies with NSAIDs in animal models have shown variable effects on fracture healing [72–74]. Data on fracture healing in patients taking glucocorticoids or NSAIDs are sparse [66]. In general, there are higher rates of non-union with higher doses and with prolonged post-operative use of NSAIDs [75–77]. NSAIDs, however, have also been linked to reduced heterotopic bone formation after hip surgery [78]. Other pharmacologic agents with evidence for detrimental effects on fracture healing include certain chemotherapeutic agents, anticoagulants, and antibiotics [66].

Malnutrition as a Risk Factor

Calcium and vitamin D deficiencies as risk factors for altered fracture healing stem from animal models and epidemiologic studies. Results from animal studies are conflicting [79–82]. Only one study, which compared several types of diet including one deficient in calcium, phosphorus, and vitamin D and another supplemented with these minerals, marginally supported impaired fracture healing [79], while others that examined calcium- and phosphorus-deficient, vitamin D-deficient, or calcium- and vitamin D-deficient diets failed to show any effects [80–82]. Clinical studies, which are mostly observational in nature, have been inconclusive. One case series reported that > 80% of 37 patients with non-unions were vitamin D-deficient. Another study that compared patients with non-unions to those with normal healing failed to detect a significant difference with respect to vitamin D deficiency [83, 84]. Other nutritional derangements that have been linked to impaired fracture healing include vitamin C and B6 deficiencies, as well as hypoalbuminemia from low dietary protein intake, even though the levels of evidence for all of them are weak [85–87].

Medications and Strategies to Accelerate Fracture Healing (See Table 1)

Most research into pharmacologic therapies for fracture healing have focused on optimizing the local fracture microenvironment, either to promote bone regeneration through direct implantation of biomaterials (e.g., bone stem cell graft), to

stimulate the fracture scaffold with a biomechanical device

Table 1 Effects of osteoporosis medications on fracture healing

Agent	Evidence	Mechanism of action
Bisphosphonates [60–66, 78–83] (anti-resorptive)	Preclinical [60–62] Clinical (weak) [63–66, 78–83]	• Animal studies show larger callus, delayed callus remodeling but stronger mechanical strength • No definitive evidence to suggest inhibition of healing, but delayed healing has been rarely reported with prolonged use and unclear if there are benefits in augmenting healing
Denosumab [85–88] (anti-resorptive)	Preclinical [85, 86] Clinical (weak) [87]	• Animal studies show larger callus and delayed callus remodeling but with stronger mechanical strength • Unclear if benefits in enhancing healing
Estrogen/raloxifene [89–92] (anti-resorptive)	Preclinical [30, 91, 92]	• Limited animal studies show improved callus strength
PTH 1–34, PTH 1–84 [93–108] (anabolic)	Preclinical [94–96, 97••, 98–102] Clinical (moderate) [103–112]	• Animal models, especially post-ovariectomy fracture models, show increased callus size and with mineralized content, better mechanical strength • Accelerated healing of long bone and spine fractures and re-enforce implant stabilization • Some evidence for improved healing of delayed unions, non-unions and atypical femoral fractures
PTHrP [113–118] (anabolic)	Preclinical [113–117]	• Various animal models including post-ovariectomy, glucocorticoid-induced, and diabetic fracture models show better callus formation and enhanced union
Sclerostin inhibitor [119–133], DKK1 inhibitor [134–137] (Wnt/β catenin activators)	Preclinical [123–137]	• Animal studies show shorter healing time, increase in bone mass at fracture site, and better mechanical strength • Co-inhibition of DKK1 and sclerostin (vs inhibition of one molecule) demonstrated superiority in enhancing healing in recent animal model

(e.g., low-intensity pulsed ultrasound), or to enhance osteogenesis indirectly via the local supplementation of biological factors, such as BMPs that are involved in the molecular healing cascade [16]. And often, modification of surgical fixation methods, such as preferential selection of locked plates or locked intramedullary nailing in osteoporotic fractures, is needed to reduce rates of complications.

Systemic agents, currently in use for treatment of osteoporosis that mechanistically target steps in the molecular pathway of fracture healing, have garnered strong interest and are under ongoing investigation in fracture repair studies. Despite the lack of approved drugs for fracture healing, some of these pharmacologic agents have been shown to have potential benefits in the healing process in both preclinical and clinical studies (summarized in Table 1). Furthermore, the putative effects on healing in patients taking them are significant clinical issues.

Bisphosphonates

There is a paucity of evidence on the effects of bisphosphonates either to accelerate fracture healing or rescue impaired healing [58]. Clinical studies examining the outcomes of bisphosphonate use in internal fixation of peritrochanteric osteoporotic fractures and prosthetic knee replacements showed improved fixation stability, although it is unclear if this occurred in association with faster healing [88–90]. Timing of bisphosphonate administration in the post-fracture period may be key. The highest grade clinical evidence is illustrated by the HORIZON RFT (Health Outcomes and Reduced Incidence with Zoledronic Acid Once Yearly Recurrent Fracture Trial) that demonstrated reduced fracture risk and improved mortality, despite a lack of significant difference in time to heal or evidence of delayed healing based on serial clinical and radiographic assessment [91], in the cohort that received zoledronic acid from beyond 2 to up to 12 weeks after hip fracture [92, 93]. There is no consensus on the role of bisphosphonates in fracture healing, and stronger clinical data are needed to clarify this important clinical issue.

Denosumab

As described earlier, rare lab-based studies have been performed with denosumab, an anti-resorptive agent and monoclonal antibody directed against the receptor for nuclear factor-kappaB ligand (RANKL) [94], to evaluate its effect on fracture healing and have produced neutral findings [65, 95]. The only clinical evidence regarding denosumab in

fracture healing was generated from a subgroup analysis of the FREEDOM (Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months), a double-blind, placebo-controlled trial in post-menopausal women investigating fracture risk reduction by denosumabvsplacebo [96]. In the 199 women who sustained non-vertebral fractures, there was no evidence of impaired or delayed healing observed in those who had been treated with denosumab [96, 97••].

Estrogen and Selective Estrogen Receptor Modulators

Estrogen and the selective estrogen receptor modulator (SERM) raloxifene are both approved agents for the treatment of osteoporosis in post-menopausal women [94, 98]. Regarding fracture healing, a study compared both agents to alendronate in rats post-ovariectomy that underwent femoral osteotomy. Healing in both the estrogen- and raloxifene-treated groups was similar to controls [99]. In contrast, in diaphyseal and metaphyseal fracture models, there was improved fracture healing (due to raloxifene or estrogen) in either the early or all phases of the biological healing sequence, with the callus formed possessing increased resistance and elasticity [34, 100, 101]. Estrogen specifically was shown to enhance endosteal bone formation, whereas raloxifene induced callus formation that yielded increased mineralization and trabecular thickness in one study [100, 101]. Clinical trials have not tested either estrogen or raloxifene as agents to promote fracture healing, perhaps due to the pro-coagulant effects that both have, likely to be exacerbated in the post-fracture setting.

Teriparatide

Studies in a number of animal models have shown that teriparatide or recombinant human parathyroid hormone [PTH 1–34] enhances early endochondral responses in the healing cascade likely by stimulating Wnt signaling leading to recruitment followed by proliferation and differentiation of chondrogenic and osteogenic precursors [102]. Findings of increased callus volume, mineralization, and mechanical strength were uniformly observedin both osteotomy and closed fracture models across species ranging from rats [103–105] and rabbits [106] to monkeys [107], with wide variances in dosing of PTH (1–34) (0.75–200 μg/kg)and in duration of treatment of mostly 21–28 days [108]. In the largest study of rats with induced closed femoral fractures, groups receiving intermittent treatment with PTH (1–34) (5 or 30 μg/kg/day) compared to vehicle alone for 5 weeks experienced substantial gains gain in callus morphometric and mechanical parameters, while earlier improvement at 3 weeks was seen with the higher dose [138]. There was no impact on osteoclastic activity, in contrast to the pro-osteoclastogenic activity described in an earlier fracture study with PTH (1–34) (at a dose of 10 μg/kg/day) [103].

In animal models of osteoporotic fracture (post-ovariectomy), intermittent administration of PTH (1–34) produced positive results supportive of improved repair [108]. One study that investigated healing of metaphyseal defects in ovariectomized rats by micro-computed tomography suggested an overall anabolic effect of PTH (1–34) that was reflected in increased bone formation over the area of the defect accompanied by gain in total bone volume, and trabecular thickness and number [139]. A different study tested full-length recombinant human PTH (1–84), intermittently administered over 30 days, compared to 17-beta estradiol and vehicle, in the healing of bilateral tibial fractures in ovariectomized rats stabilized via intramedullary nailing. There was both enhanced callus formation and strength in animals treated with PTH (1–84) compared to vehicle or 17-beta estradiol, similar to the findings with PTH (1–34) [140].

A meta-analysis identified five clinical studies of either upper or lower extremity fractures examining the effect of synthetic PTH peptides on healing. This meta-analysis corroborated the animal data in support of the ability of PTH analogues to shorten healing time [109]. The two randomized clinical trials included in the meta-analysis were conducted in post-menopausal women who suffered acute distal radius or pelvic fractures. Subjects in these trials received PTH (1–34) (20 or 40 μg/kg/day) or PTH (1–84) (100 μg/kg/day) to assist with fracture healing, which was monitored by serial imaging [110, 111]. In these trials, PTH (1–34) was administered to patients with radius fractures, and PTH (1–84) was given to patients with pelvic fractures. These treatments resulted in significantly shorter healing times compared to controls, 7.4 vs 9.1 weeks with PTH (1–34) or 7.8 vs 12.6 weeks with PTH (1–84), but there was a lack of statistical significance in the PTH (1–34) dose of 40 μg/kg/day (8.8 vs 9.1 weeks) [110, 111], which remains unexplained. Evidence that PTH analogues improve clinically documented impaired fracture healing, on the other hand, is limited to case reports. The application of PTH [1–34] in a variety of reported delayed or non-union fractures ranging from fractures in the upper to lower extremities mostly stated favorable outcomes, although rigorous placebo controls were lacking [112, 113, 141].

Atypical Femoral Fractures: Use of PTH Peptides

In consideration of the positive preclinical data on fracture healing with recombinant PTH in animal models summarized above, interest has recently focused on the use of teriparatide for the treatment of atypical femoral fractures (AFFs). AFFs are defined by their specific location between the distal lesser trochanter and proximal to the supracondylar flare in the femur, their typically non-comminuted appearance, and their occurrence in the setting of minimal to no prior trauma [114,

115]. They are exceedingly low in incidence (0.35% of all femoral fractures) and are associated with long-term bisphosphonate or denosumab use [116]. According to the American Society of Bone and Mineral Research (ASBMR) Task Force Report on AFFs, PTH (1–34) therapy can be considered for AFFs that fail to heal with conventional therapy [114, 115]. Improved union was reported in two retrospective studies. One assessed the introduction of PTH (1–34) after surgical repair in patients with AFFs and found higher rates of union in the treated group compared to the non-treated group [117]. The other was a retrospective cohort study that included complete and incomplete AFFs, either surgically or conservatively managed, and compared those that received daily PTH (1–34) injection vs alfacalcidol or menatetrenone [118]. A caveat about the findings of the retrospective cohort study was the lack of a significant difference in the conservatively managed, incomplete AFFs upon subgroup analysis [118]. Two prospective non-randomized trials that examined bone turnover, in addition to fracture healing in AFFs, both observed increased remodeling, evidenced by changes in bone turnover markers [119, 120]. This was hypothesized as PTH (1–34) promoting turnover of the bisphosphonate residing in bone, but still in these cases there were inconsistent rates of union [119, 120].

PTH-Related Peptide and Analogues

PTH-related peptide (PTHrP) acts through the same receptor as PTH (the PTH/PTHrP receptor 1 or PTH/PTHrP-R1) and plays an important role in endochondral ossification, via its interaction with Indian hedgehog and other signaling molecules [121]. PTHrP enhances chondrocyte proliferation, while inhibiting differentiation, to ensure progression toward callus development [121]. In several animal models including PTHrP haploinsufficient rats, studies have shown that targeting the PTH/PTHrP-R1 with PTHrP enhances fracture healing [122–124]. These findings were also from studies in glucocorticoid-treated and diabetic animal models [125, 126]. Abaloparatide, the recently FDA-approved PTHrP analog, has gathered much attention for its greater gains in BMD compared to PTH (1–34) as demonstrated in the ACTIVE (Abaloparatide Comparator Trial In Vertebral Endpoints) trial [127]. The testing of this medication to promote healing of fractures and delayed unions remains unexplored at this time.

Investigational Therapies (See Table 1)

Targeting Sclerostin

Inhibition of endogenous antagonists of the Wnt/β catenin signaling pathway, sclerostin, and Dickkopf-1 (DKK1) has emerged as a possible approach to achieve bone anabolism for osteoporosis treatment and for bone repair and regeneration [15]. Functioning as an endogenous antagonist, sclerostin exerts its inhibitory actions on the binding of the Wnt ligand to LRP5/6, the low-density lipoprotein receptor-related protein, which serves as a co-receptor for Wnt ligands, along with the frizzled protein. Interference with Wnt signaling blocks the ability of β-catenin, a molecule downstream in the Wnt pathway, to activate osteoblastic gene transcription [128]. Activation of Wnt signaling (or relief of Wnt antagonism) furthermore diverts the differentiation of pluripotent mesenchymal stem cells toward the osteoblastic lineage and suppresses chondrogenesis [15]. Treatment with a neutralizing antibody to sclerostin upregulates Wnt signaling leading to enhanced osteoblastogenesis and bone formation. Several preclinical studies and clinical trials have validated this approach to raise BMD and reduce fractures using antibodies that inhibit sclerostin or models of sclerostin gene knockout [129–131].

From a fracture healing perspective, studies in animal fracture models using sclerostin neutralizing antibodies have largely shown improved parameters of bone mass, especially in the diabetic and osteoporotic models [132, 133, 142]. Studies in which rats with femoral defects were treated with the anti-sclerostin antibody at both early and late time points post-fracture [134, 135] have uniformly demonstrated faster healing time and increased BMD at fractured sites, relative to control sites, although complete union rates were unimpressive, even in the anti-sclerostin antibody groups [136, 137, 143]. In two open osteotomy rat models [137, 143], animals given sclerostin antibody at 3 and 6 weeks or 3, 6, and 9 weeks demonstrated more highly mineralized calluses and greater BMD at the fractured sites. These findings replicated those from the earlier rat and monkey closed fracture models [137, 143]. These studies, in addition, revealed increased callus angiogenesis. Application of sclerostin antibody, given twice weekly for either 3 or 8 weeks, in two recent models of ovariectomized rats with fractures showed healing of the fractures in these osteoporotic rodents. These studies further and importantly showed near restoration of bone mass in the ovariectomized osteoporotic rats, similar to the levels of sham-operated saline control rats [135, 136]. Notably, while there are some data from sclerostin gene knockout models that suggested earlier fibrocartilage callus removal during fracture healing, an increase in callus bone volume accompanied by enhanced strength was reported by moststudies [144, 145]. Regarding the clinical evidence for targeting sclerostin to promote fracture healing in humans, two multicenter trials investigating the use of the sclerostin antibody romosozumab in the healing of acute tibial shaft fractures following intramedullary nailing and of acute unilateral hip fracture post-surgical fixation have recently been completed, but the data are not yet published [15].

DKK1

Like sclerostin, DKK1 exerts its inhibitory effect on the Wnt/β catenin pathway through interaction with LRP5/6 [146]. Antagonizing actions of DKK1 in fracture healing was achieved in early animal experiments through over-expression of DKK1 via delivery of adenovirus containing DNA encoding DKK1 to both young and adult rats [147, 148]. A tibial fracture rat model demonstrated down-regulation of β catenin protein expression in the formed calluses and subsequent failure to heal with substantial reduction in bone and cartilage volume by 3 weeks post-fracture [147]. While these findings were corroborated in another study that deployed adenovirus-mediated over-expression of DKK1 in 7-month-old adult non-fractured rats and showed significant decreases in lumbar and whole leg BMD, 4-week treatment with anti-DKK1 antibody in a femoral fracture model showed significant gains in bone mass in the wild-type, gonad-intact, young, and adult rats, but not in the wild-type, ovariectomized adult rats [148]. Reversal of blockage of the Wnt/β catenin pathway via antibody-mediated inhibition of DKK1in tibial and femoral fracture models in mice, likewise, showed repressed healing in the β catenin null allele/conditional knockout or LRP5 knockout mice [147, 149]. In these studies, greater BMD and enhanced mechanical strength of the healing calluses were observed in the wild-typemice [147, 149].

A recent animal study suggested that there is a negative feedback relationship between sclerostin and DKK1in bone healing. Florio et al. used a bispecific antibody to mediate dual inhibition of both sclerostin and DKK1 in a fracture healing model [150]. The investigators first illustrated, in sclerostin knockout rats and in sclerostin antibody-treated ovariectomized mice, that sclerostin inhibition is accompanied by a compensatory increase in DKK1 expression, indicative of the inhibitory redundancy of DKK1 in the negative regulation of sclerostin. These investigators confirmed in subsequent experiments, comparing treatment with sclerostin antibody, DDK1 antibody, or the bispecific antibody for 7 weeks in a closed femoral fracture rat model, that the bispecific antibody was superior in inducing bridging and produced the largest callus mass compared to either monotherapy [150]. Data demonstrating DKK1 inhibition as a viable means to enhance fracture healing in humans are lacking at present, but positive results from preclinical studies hold great promise for future testing in clinical trials.

Conclusion

The process of fracture healing is highly orchestrated and subject to influence by multiple intrinsic as well as extrinsic factors. While distinctive properties of osteoporotic fractures in the repair process have been suggested by some of the experimental studies, further exploration is needed to better understand if and exactly how osteoporosis retards healing. Since osteoporotic fractures account for a large proportion of all fractures, anabolic treatments for osteoporosis specifically have sparked great interest for their prospects in fracture repair. High quality clinical data on the long-term use of osteoporosis treatments for the indication of augmentation of fracture repair remain limited, although several anabolic agents have yielded positive results in ovariectomized osteoporotic animal fracture models. Intensive investigations are ongoing in an effort not only to address the current gaps in clinical evidence but also to look into the application of new systemic agents.

Compliance with Ethical Standards

Conflict of Interest Dolores Shoback reports personal fees from Radius Pharamaceutical, outside the submitted work. Cheng Cheng declares that there is no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Bahney CS, Hu DP, Miclau T, Marcucio RS. The multifaceted role of the vasculature in endochondral fracture repair. Front Endocrinol. 2015;6:4.
2. Tzioupis C, Giannoudis PV. Prevalence of long-bone non-unions. Injury. 2007;38(Suppl 2):S3–9.
3. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005–2025. J Bone Miner Res. 2007;22(3):465–75.
4. Eastell R, Lambert H. Strategies for skeletal health in the elderly. Proc Nutr Soc. 2002;61(2):173–80.
5. Sanders KM, Pasco JA, Ugoni AM, Nicholson GC, Seeman E, Martin TJ, et al. The exclusion of high trauma fractures may underestimate the prevalence of bone fragility fractures in the community: the Geelong Osteoporosis Study. J Bone Miner Res. 1998;13(8):1337–42.
6. Hak DJ, Fitzpatrick D, Bishop JA, Marsh JL, Tilp S, Schnettler R, et al. Delayed union and non-unions: epidemiology, clinical issues, and financial aspects. Injury. 2014;45(Suppl 2):S3–7.
7. Beaver R, Brinker MR, Barrack RL. An analysis of the actual cost of tibial non-unions. J La State Med Soc. 1997;149:200–6.
8. Antonova E, Le TK, Burge R, Mershon J. Tibia shaft fractures: costly burden of non-unions. BMC Musculoskelet Disord. 2013;14:42.

9. Alt V, Donell ST, Chhabra A, Bentley A, Eicher A, Schnettler R. A health economic analysis of the use of rhBMP-2 in Gustilo-Anderson grade III open tibial fractures for the UK, Germany, and France. Injury. 2009;40(12):1269–75.
10. Tulipan J, Jones CM, Ilyas AM. The effect of osteoporosis on healing of distal radius fragility fractures. Orthop Clin North Am. 2015;46(4):541–9.
11. Gómez-Barrena E, Rosset P, Lozano D, Stanovici J, Ermthaller C, Gerbhard F. Bone fracture healing: cell therapy in delayed unions and non-unions. Bone. 2015;70:93–101.
12. Azar FM, Beaty JH, Canale ST. Fractures, dislocations, and fracture-dislocations of the spine. In: Williams KD, editor. Campbell's operative orthopaedics. 13th ed. Philadelphia, PA: Elsevier; 2017.
13. United States Food and Drug Administration (USFDA), Office of Device Evaluation, Guidance Document for Industry and CDRH Staff for the Preparation of Investigational Device Exemptions and Premarket Approval Application for Bone Growth Stimulator Devices. 1988.
14. Weber BG, Cech O. Pseudarthrosis, pathotogy, biomechanics, therapy, results. Can Med Assoc J. 1977;117(4):337–8.
15. Morshed S. Current options for determining fracture union. Adv Med. 2014;2014:708574.
16. Kostenuik P, Mirza FM. Fracture healing physiology and the quest for therapies for delayed healing and non-union. J Orthop Res. 2017;35(2):213–23.
17.•• Einhorn TA, Gerstenfeld LC. Fracture healing: mechanisms and interventions. Nat Rev Rheumatol. 2015;11(1):45–54 **This paper provides a comprehensive review of all aspects of fracture healing with a particularly thorough description of the biological process of healing**.
18. Copuroglu C, Calori GM, Giannoudis PV. Fracture non-union: who is at risk? Injury. 2013;44(11):1379–82.
19. Ferreira N, Marais LC, Aldous C. The pathogenesis of tibial non-union. SA Orthop. J. 2016;15(1):51–59.
20. Féron JM, Mauprivez R. Fracture repair: general aspects and influence of osteoporosis and anti-osteoporosis treatment. Injury. 2016;47(Suppl 1):S10–4.
21.• Giannoudis P, Tzioupis C, Almalki T, Buckley R. Fracture healing in osteoporotic fractures: is it really different? A basic science perspective. Injury. 2007;38(Suppl 1):S90–9 **This is the most comprehensive review in the literature that summarizes special aspects of healing in osteoporotic fractures based on findings of current animal fracture models**.
22. Hagiwara H, Inoue N, Matsuzaki H. Relationship between structural anisotropy of the vertebral body and bone mineral density. Trans Orthop Res Soc. 2000;25:738.
23. Carden A, Morris MD. Application of vibrational spectroscopy to the study of mineralized tissues (review). J Biomed Opt. 2000;5:259–68.
24. Khan AZ, Rames RD, Miller AN. Clinical management of osteoporotic fractures. Curr Osteoporos Rep. 2018;16(3):299–311.
25. Thompson DD, Simmons HA, Pirie CM, Ke HZ. FDA guidelines and animal models for osteoporosis. Bone. 1995;17:125S–33S.
26. Wong RMY, Choy MHV, Li MCM, Leung KS, K-H Chow S, Cheung WH, et al. A systematic review of current osteoporotic metaphyseal fracture animal models. Bone Joint Res. 2018;7(1):6–11.
27. Chen L, Yang L, Yao M, Cui XJ, Xue CC, Wang YJ, et al. Biomechanical characteristics of osteoporotic fracture healing in ovariectomized rats: a systematic review. PLoS One. 2016;11(4):e0153120.
28. Lill CA, Fluegel AK, Schneider E. Sheep model for fracture treatment in osteoporotic bone: a pilot study about different induction regimens. J Orthop Trauma. 2000;14(8):559–65 **discussion 565-6**.
29. Namkung-Matthai H, Appleyard R, Jansen J, Hao Lin J, Maastricht S, Swain M, et al. Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. Bone. 2001;28(1):80–6.
30. Wang JW, Li W, Xu SW, Yang DS, Wang Y, Lin M, et al. Osteoporosis influences the middle and late periods of fracture healing in a rat osteoporotic model. Chin J Traumatol. 2005;8:111–6.
31. Qiao L, Xu KH, Liu HW, Liu HQ. Effects of ovariectomy on fracture healing in female rats. Sichuan Da Xue Xue Bao Yi Xue Ban. 2005;36(1):108–11.
32. Cheung WH, Miclau T, Chow SK, Yang FF, Alt V. Fracture healing in osteoporotic bone. Injury. 2016;47(Suppl 2):S21–6.
33. Alt V, Thormann U, Ray S, Zahner D, Dürselen L, Lips K, et al. A new metaphyseal bone defect model in osteoporotic rats to study biomaterials for the enhancement of bone healing in osteoporotic fractures. Acta Biomater. 2013;9:7035–42.
34. Stuermer EK, Sehmisch S, Rack T, Wenda E, Seidlova-Wuttke D, Tezval M, et al. Estrogen and raloxifene improve metaphyseal fracture healing in the early phase of osteoporosis. A new fracture-healing model at the tibia in rat. Langenbeck's Arch Surg. 2010;395:163–72.
35. Santolini E, West R, Giannoudis PV. Risk factors for long bone fracture non-union: a stratification approach based on the level of the existing scientific evidence. Injury. 2015;46(Suppl 8):S8–S19.
36. Calori GM, Albisetti W, Agus A, Iori S, Tagliabue L. Risk factors contributing to fracture non-unions. Injury. 2007;38S:S11–8.
37. Donigan JA, Fredericks DC, Nepola JV, Smucker JD. The effect of transdermal nicotine on fracture healing in a rabbit model. J Orthop Trauma. 2012;26(12):724–7.
38. Forrest CR, Pang CY, Lindsay WK. Pathogenesis of ischemic necrosis in random pattern skin flaps induced by long-term low-dose nicotine treatment in the rat. Plast Reconstr Surg. 1991;87(3):518–28.
39. Adams CI, Keating JF, Court-Brown CM. Cigarette smoking and open tibial fractures. Injury. 2001 Jan;32(1):61–5.
40. Cook SD, Ryaby JP, McCabe J, Frey JJ, Heckman JD, Kristiansen TK. Acceleration of tibia and distal radius fracture healing in patients who smoke. Clin Orthop Relat Res. 1997;337:198–207.
41. Kyrö A, Usenius JP, Aarnio M, Kunnamo I, Avikainen V. Are smokers a risk group for delayed healing of tibial shaft fractures? Ann Chir Gynaecol. 1993;82(4):254–62.
42. Elmali N, Ertem K, Ozen S, Inan M, Baysal T, Güner G, et al. Fracture healing and bone mass in rats fed on liquid diet containing ethanol. Alcohol Clin Exp Res. 2002;26(4):509–13.
43. Chakkalakal DA, Novak JR, Fritz ED, Mollner TJ, McVicker DL, Garvin KL, et al. Inhibition of bone repair in a rat model for chronic and excessive alcohol consumption. Alcohol. 2005;36(3):201–14.
44. Almeida M, Han L, Martin-Millan M, O'Brien CA, Manolagas SC. Oxidative stress antagonizes Wnt signaling in osteoblast precursors by diverting beta-catenin from T cell factor- to forkhead box O-mediated transcription. J Biol Chem. 2007;282(37):27298–305.
45. Shin SY, Kim CG, Jho EH, Rho MS, Kim YS, Kim YH, et al. Hydrogen peroxide negatively modulates Wnt signaling through downregulation of beta-catenin. Cancer Lett. 2004;212(2):225–31.
46. Obermeyer TS, Yonick D, Lauing K, Stock SR, Nauer R, Strotman P, et al. Mesenchymal stem cells facilitate fracture repair in an alcohol-induced impaired healing model. J Orthop Trauma. 2012;26(12):712–8.
47. Levy RS, Hebert CK, Munn BG, Barrack RL. Drug and alcohol use in orthopedic trauma patients: a prospective study. J Orthop Trauma. 1996;10(1):21–7.

48. Duckworth AD, Bennet SJ, Aderinto J, Keating JF. Fixation of intracapsular fractures of the femoral neck in young patients: risk factors for failure. J Bone Joint Surg Br. 2011;93(6):811–6.
49. Janghorbani M, Feskanich D, Willett WC, Hu F. Prospective study of diabetes and risk of hip fracture: the nurses' health study. Diabetes Care. 2006;29(7):1573–8.
50. Folk JW, Starr AJ, Early JS. Early wound complications of operative treatment of calcaneus fractures: analysis of 190 fractures. J Orthop Trauma. 1999;13(5):369–72.
51. Retzepi M, Donos N. The effect of diabetes mellitus on osseous healing. Clin Oral Implants Res. 2010;21(7):673–81.
52. Dede AD, Tournis S, Dontas I, Trovas G. Type 2 diabetes mellitus and fracture risk. Metabolism. 2014;63(12):1480–90.
53. Foulke BA, Kendal AR, Murray DW, Pandit H. Fracture healing in the elderly: a review. Maturitas. 2016;92:49–55.
54. Tang SY, Zeenath U, Vashishth D. Effects of non-enzymatic glycation on cancellous bone fragility. Bone. 2007;40(4):1144–51.
55. Vashishth D, Gibson GJ, Khoury JI, Schaffler MB, Kimura J, Fyhrie DP. Influence of nonenzymatic glycation on biomechanical properties of cortical bone. Bone. 2001;28(2):195–201.
56. Jones KB, Maiers-Yelden KA, Marsh JL, Zimmerman MB, Estin M, Saltzman CL. Ankle fractures in patients with diabetes mellitus. J Bone Joint Surg Br. 2005;87(4):489–95.
57. Shibuya N, Humphers JM, Fluhman BL, Jupiter DC. Factors associated with non-union, delayed union, and malunion in foot and ankle surgery in diabetic patients. J Foot Ankle Surg. 2013;52(2):207–11.
58. Kates SL, Ackert-Bicknell CL. How do bisphosphonates affect fracture healing? Injury. 2016;47(Suppl 1):S65–8.
59. Feher A, Koivunemi A, Koivunemi M, Fuchs RK, Burr DB, Phipps RJ, et al. Bisphosphonates do not inhibit periosteal bone formation in estrogen deficient animals and allow enhanced bone modeling in response to mechanical loading. Bone. 2010;46(1):203–7.
60. Fu LJ, Tang TT, Hao YQ, Dai KR. Long-term effects of alendronate on fracture healing and bone remodeling of femoral shaft in ovariectomized rats. Acta Pharmacol Sin. 2013;34(3):387–92.
61. Molvik H, Khan W. Bisphosphonates and their influence on fracture healing: a systematic review. Osteoporos Int. 2015;26(4):1251–60.
62. Solomon DH, Hochberg MC, Mogun H, Schneeweiss S. The relation between bisphosphonate use and non-union of fractures of the humerus in older adults. Osteoporos Int. 2009;20:895–901.
63. Tang ZH, Kumar VP. Alendronate-associated ulnar and tibial fractures: a case report. J Orthop Surg (Hong Kong). 2011;19:370–2.
64. Rozental TD, Vazquez MA, Chacko AT, Ayogu N, Bouxsein ML. Comparison of radiographic fracture healing in the distal radius for patients on and off bisphosphonate therapy. J Hand Surg [Am]. 2009;34:595–602.
65. Gerstenfeld LC, Sacks DJ, Pelis M, Mason ZD, Graves DT, Barrero M, et al. Comparison of effects of the bisphosphonate alendronate versus the RANKL inhibitor denosumab on murine fracture healing. J Bone Miner Res. 2009;24(2):196–208.
66. Pountos I, Georgouli T, Blokhuis TJ, Pape HC, Giannoudis PV. Pharmacological agents and impairment of fracture healing: what is the evidence? Injury. 2008;39(4):384–94.
67. Blunt JW, Plotz CM, Lattes R, Howes EL, Meyer K, Ragan C. Effect of cortisone on experimental fractures in the rabbit. Proc Soc Exp Biol Med. 1950;73:678–81.
68. Sisson HA, Hadfield GJ. The influence of cortisone on the repair of experimental fracture in rabbit. Br J Surg. 1951;38:172–8.
69. Sato S, Kim T, Arai T, et al. Comparison between the effects of dexamethasone and indomethacin on bone wound healing. Jpn J Pharmacol. 1986;42:71–8.
70. Key JA, Odell RT, Taylor LW. Failure of cortisone to delay or to prevent the healing of fractures in rats. J Bone Joint Surg Am. 1952;24:665–75.
71. Weiss R, Ickowich M. The influence of cortisone on the healing of experimental fractures in rats. Acta Anat (Basel). 1964;59:163–81.
72. Reikeraas O, Engebretsen L. Effects of ketoralac tromethamine and indomethacin on primary and secondary bone healing. An experimental study in rats. Arch Orthop Trauma Surg. 1998;118:50–2.
73. Brown KM, Saunders MM, Kirsch T, Donahue HJ, Reid JS. Effect of COX-2-specific inhibition on fracture-healing in the rat femur. J Bone Joint Surg Am. 2004;86-A:116–23.
74. Gerstenfeld LC, Thiede M, Seibert K, Mielke C, Phippard D, Svagr B, et al. Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs. J Orthop Res. 2003;21:670–5.
75. Deguchi M, Rapoff AJ, Zdeblick TA. Posterolateral fusion for isthmic spondylolisthesis in adults: analysis of fusion rate and clinical results. J Spinal Disord. 1998;11:459–64.
76. Glassman SD, Rose SM, Dimar JR, Puno RM, Campbell MJ, Johnson JR. The effect of postoperative nonsteroidal anti-inflammatory drug administration on spinal fusion. Spine. 1998;23:834–8.
77. Reuben SS, Ablett D, Kaye R. High dose nonsteroidal antiinflammatory drugs compromise spinal fusion. Can J Anaesth. 2005;52:506–12.
78. Ritter MA, Vaughan RB. Ectopic ossification after total hip arthroplasty. Predisposing factors, frequency, and effect on results. J Bone Joint Surg Am. 1977;59:345–51.
79. Einhorn TA, Bonnarens F, Burstein AH. The contributions of dietary protein and mineral to the healing of experimental fractures. A biomechanical study. J Bone Joint Surg Am. 1986;68(9):1389–95.
80. Doepfner W. Consequences of calcium and-or phosphorus defcient diets on various parameters of callus formation and on growth rate in young rats. Br J Pharmacol. 1970;39(1):188–9.
81. Melhus G, Solberg LB, Dimmen S, Madsen JE, Nordsletten L, Reinholt FP. Experimental osteoporosis induced by ovariectomy and vitamin D deficiency does not markedly affect fracture healing in rats. Acta Orthop. 2007;78(3):393–403.
82. Fischer V, Haffner-Luntzer M, Prystaz K, Scheidt AV, Busse B, Schinke T, et al. Calcium and vitamin-D deficiency marginally impairs fracture healing but aggravates posttraumatic bone loss in osteoporotic mice. Sci Rep. 2017;7(1):7223.
83. Brinker MR, O'Connor DP, Monla YT, Earthman TP. Metabolic and endocrine abnormalities in patients with non-unions. J Orthop Trauma. 2007;21(8):557–70.
84. Boszczyk AM, Zakrzewski P, Pomianowski S. Vitamin D concentration in patients with normal and impaired bone union. Pol Orthop Traumatol. 2013;78:1–3.
85. Dodds RA, Catterall A, Bitensky L, Chayen J. Abnormalities in fracture healing induced by vitamin B6-deficiency in rats. Bone. 1986;7:489–95.
86. Mohan S, Kapoor A, Singgih A, Zhang Z, Taylor T, Yu H, et al. Spontaneous fractures in the mouse mutant sfx are caused by deletion of the gulonolactone oxidase gene, causing vitamin C deficiency. J Bone Miner Res. 2005;20:1597–610.
87. Koval KJ, Maurer SG, Su ET, Aharonoff GB, Zuckerman JD. The effects of nutritional status on outcome after hip fracture. J Orthop Trauma. 1999;13:164–9.
88. Aspenberg P. Bisphosphonates and implants: an overview. Acta Orthop. 2009;80(1):119–23.
89. Moroni A, Faldini C, Hoang-Kim A, Pegreffi F, Giannini S. Alendronate improves screw fixation in osteoporotic bone. J Bone Joint Surg Am. 2007;89(1):96–101.

90. Hilding M, Aspenberg P. Local peroperative treatment with a bisphosphonate improves the fixation of total knee prostheses: a randomized, double-blind radiostereometric study of 50 patients. Acta Orthop. 2007;78(6):795–9.
91. Lyles KW, Colón-Emeric CS, Magaziner JS, Adachi JD, Pieper CF, Mautalen C, et al. Zoledronic acid and clinical fractures and mortality after hip fracture. N Engl J Med. 2007;357(18):1799–809.
92. Colón-Emeric C, Nordsletten L, Olson S, Major N, Boonen S, Haentjens P, et al. Association between timing of zoledronic acid infusion and hip fracture healing. Osteoporos Int. 2011;22(8):2329–36.
93. Eriksen EF, Lyles KW, Colón-Emeric CS, Pieper CF, Magaziner JS, Adachi JD, et al. Antifracture efficacy and reduction of mortality in relation to timing of the first dose of zoledronic acid after hip fracture. J Bone Miner Res. 2009;24(7):1308–13.
94. Chapurlat RD, Genant HK. Osteoporosis: treatment. In: Jameson JL, DeGroot LJ, editors. Endocrinology adult and pediatric. 7th ed. Philadelphia: Saunders Elsevier; 2016.
95. Bernhardsson M, Sandberg O, Aspenberg P. Anti-RANKL treatment improves screw fixation in cancellous bone in rats. Injury. 2015;46(6):990–5.
96. Adami S, Libanati C, Boonen S, Cummings SR, Ho PR, Wang A, et al. Denosumab administration is not associated with fracture healing complications in postmenopausal women with osteoporosis: results from the FREEDOM trial. J Bone Joint Surg Am. 2012;94(23):2113–9.
97.•• Goldhahn J, Féron JM, Kanis J, Papapoulos S, Reginster JY, Rizzoli R, et al. Implications for fracture healing of current and new osteoporosis treatments: an ESCEO consensus paper. Calcif Tissue Int. 2012;90(5):343–53 **This paper provides both good bench and clinical evidence of all pharmacologic osteoporosis agents in fracture healing**.
98. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321–33.
99. Cao Y, Mori S, Mashiba T, Westmore MS, Ma L, Sato M, et al. Raloxifene, estrogen, and alendronate affect the processes of fracture repair differently in ovariectomized rats. J Bone Miner Res. 2002;17(12):2237–46.
100. Spiro AS, Khadem S, Jeschke A, Marshall RP, Pogoda P, Ignatius A, et al. The SERM raloxifene improves diaphyseal fracture healing in mice. J Bone Miner Metab. 2013;31:629–36.
101. Spiro AS, Khadem S, Jeschke A, Marshall RP, Pogoda P, Ignatius A, et al. Effects of estrogen on fracture healing in mice. J Bone Miner Metab. 2013;31(6):629–36.
102. Holzer G, Majeska RJ, Lundy MW, Hartke JR, Einhorn TA. Parathyroid hormone enhances fracture healing: a preliminary report. Clin Orthop Relat Res. 1999;366:258–63.
103. Andreassen TT, Fledelius C, Ejersted C, Oxlund H. Increases in callus formation and mechanical strength of healing fractures in old rats treated with parathyroid hormone. Acta Orthop Scand. 2001;72(3):304–7.
104. Nakajima A, Shimoji N, Shiomi K, Shimizu S, Moriya H, Einhorn TA, et al. Mechanisms for the enhancement of fracture healing in rats treated with intermittent low-dose human parathyroid hormone (1–34). J Bone Miner Res. 2002;17(11):2038–47.
105. Nakazawa T, Nakajima A, Shiomi K, Moriya H, Einhorn TA, Yamazaki M. Effects of low-dose, intermittent treatment with recombinant human parathyroid hormone (1–34) on chondrogenesis in a model of experimental fracture healing. Bone. 2005;37(5):711–9.
106. Morgan EF, Mason ZD, Bishop G, Davis AD, Wigner NA, Gerstenfeld LC, et al. Combined effects of recombinant human BMP-7 (rhBMP-7) and parathyroid hormone (1–34) in metaphyseal bone healing. Bone. 2001;28(5):538–47.
107. Manabe T, Mori S, Mashiba T, Kaji Y, Iwata K, Komatsubara S, et al. Human parathyroid hormone (1–34) accelerates natural fracture healing process in the femoral osteotomy model of cynomolgus monkeys. Bone. 2007;40(6):1475–82.
108. Hegde V, Jo JE, Andreopoulou P, Lane JM. Effect of osteoporosis medications on fracture healing. Osteoporos Int. 2016;27(3):861–71.
109. Lou S, Lv H, Wang G, Zhang L, Li M, Li Z, et al. The effect of teriparatide on fracture healing of osteoporotic patients: a meta-analysis of randomized controlled trials. Biomed Res Int. 2016;2016:6040379.
110. Aspenberg P, Genant HK, Johansson T, Nino AJ, See K, Krohn K, et al. Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. J Bone Miner Res. 2010;25:404–14.
111. Peichl P, Holzer LA, Maier R, Holzer G. Parathyroid hormone 1–84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. J Bone Joint Surg. 2011;93:1583–7.
112. Resmini G, Iolascon G. 79-Year-old post-menopausal woman with humerus fracture during teriparatide treatment. Aging Clin Exp Res. 2007;19:30–1.
113. Rubery PT, Bukata SV. Teriparatide may accelerate healing in delayed unions of type III odontoid fractures: a report of 3 cases. J Spinal Disord Tech. 2010;23:151–5.
114. Shane E, Burr D, Abrahamsen B, Adler R, Brown T, Cheung A, et al. Atypical subtrochanteric and diaphyseal femoral fractures: second report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2014;29(1):1–23.
115. Shane E, Burr D, Ebeling PR, Abrahamsen B, Adler RA, Brown TD, et al. Atypical subtrochanteric and diaphyseal femoral fractures: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2010;25(11):2267–94.
116. Park-Wyllie LY, Mamdani MM, Juurlink DN, Hawker GA, Gunraj N, Austin PC, et al. Bisphosphonate use and the risk of subtrochanteric or femoral shaft fractures in older women. JAMA. 2011;305(8):783–9.
117. Yeh WL, Su CY, Chang CW, Chen CH, Fu TS, Chen LH, et al. Surgical outcome of atypical subtrochanteric and femoral fracture related to bisphosphonates use in osteoporotic patients with or without teriparatide treatment. BMC Musculoskelet Disord. 2017;18(1):527.
118. Miyakoshi N, Aizawa T, Sasaki S, Ando S, Maekawa S, Aonuma H, et al. Healing of bisphosphonate-associated atypical femoral fractures in patients with osteoporosis: a comparison between treatment with and without teriparatide. J Bone Miner Metab. 2015;33(5):553–9.
119. Watts NB, Aggers D, McCarthy EF, Savage T, Martinez S, Patterson R, et al. Responses to treatment with teriparatide in patients with atypical femur fractures previously treated with bisphosphonates. J Bone Miner Res. 2017;32(5):1027–33.
120. Chiang CY, Zebaze RM, Ghasem-Zadeh A, Iuliano-Burns S, Hardidge A, Seeman E. Teriparatide improves bone quality and healing of atypical femoral fractures associated with bisphosphonate therapy. Bone. 2013;52(1):360–5.
121. Maes C, Kronenberg HM. Bone development and remodeling. In: Jameson JL, DeGroot LJ, editors. Endocrinology adult and pediatric. 7th ed. Philadelphia: Saunders Elsevier; 2016.
122. Wang YH, Qiu Y, Han XD, Xiong J, Chen YX, Shi HF, et al. Haploinsufficiency of endogenous parathyroid hormone-related peptide impairs bone fracture healing. Clin Exp Pharmacol Physiol. 2013;40(11):715–23.

123. Wang M, Nasiri AR, Broadus AE, Tommasini SM. Periosteal PTHrP regulates cortical bone remodeling during fracture healing. Bone. 2015;81:104–11.
124. Wang Y, Fang X, Wang C, Ding C, Lin H, Liu A, et al. Exogenous PTHrP repairs the damaged fracture healing of PTHrP+/− mice and accelerates fracture healing of wild mice. Int J Mol Sci. 2017;6:18 (2).
125. Bostrom MP, Gamradt SC, Asnis P, et al. Parathyroid hormone-related protein analog RS-66271 is an effective therapy for impaired bone healing in rabbits on corticosteroid therapy. Bone. 2000;26:437–42.
126. Lozano D, de Castro LF, Dapia S, et al. Role of parathyroid hormone-related protein in the decreased osteoblast function in diabetes-related osteopenia. Endocrinology. 2009;150:2027–35.
127. Miller PD, Hattersley G, Riis BJ, Williams GC, Lau E, Russo LA, et al. Effect of abaloparatide vs placebo on new vertebral fractures in postmenopausal women with osteoporosis: a randomized clinical trial. JAMA. 2016;316(7):722–33.
128. Gamie Z, Korres N, Leonidou A, Gray AC, Tsiridis E. Sclerostin monoclonal antibodies on bone metabolism and fracture healing. Expert Opin Investig Drugs. 2012;21(10):1523–34.
129. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab treatment in postmenopausal women with osteoporosis. N Engl J Med. 2016;375(16):1532–43.
130. Kramer I, Loots GG, Studer A, Keller H, Kneissel M. Parathyroid hormone (PTH)-induced bone gain is blunted in SOST overexpressing and deficient mice. J Bone Miner Res. 2010;25(2):178–89.
131. Li X, Ominsky MS, Niu QT, Sun N, Daugherty B, D'Agostin D, et al. Targeted deletion of the sclerostin gene in mice results in increased bone formation and bone strength. J Bone Miner Res. 2008;23(6):860–9.
132. McDonald MM, Morse A, Mikulec K, Peacock L, Yu N, Baldock PA, et al. Inhibition of sclerostin by systemic treatment with sclerostin antibody enhances healing of proximal tibial defects in ovariectomized rats. J Orthop Res. 2012;30(10):1541–8.
133. Liu Y, Rui Y, Cheng TY, Huang S, Xu L, Meng F, et al. Effects of sclerostin antibody on the healing of femoral fractures in ovariectomised rats. Calcif Tissue Int. 2016;98(3):263–74.
134. Alaee F, Virk MS, Tang H, Sugiyama O, Adams DJ, Stolina M, et al. Evaluation of the effects of systemic treatment with a sclerostin neutralizing antibody on bone repair in a rat femoral defect model. J Orthop Res. 2014;32(2):197–203.
135. Virk MS, Alaee F, Tang H, Ominsky MS, Ke HZ, Lieberman JR. Systemic administration of sclerostin antibody enhances bone repair in a critical-sized femoral defect in a rat model. J Bone Joint Surg Am. 2013;95(8):694–701.
136. Jawad MU, Fritton KE, Ma T, Ren PG, Goodman SB, Ke HZ, et al. Effects of sclerostin antibody on healing of a non-critical size femoral bone defect. J Orthop Res. 2013;31(1):155–63.
137. Suen PK, He YX, Chow DH, Huang L, Li C, Ke HZ, et al. Sclerostin monoclonal antibody enhanced bone fracture healing in an open osteotomy model in rats. J Orthop Res. 2014;32(8):997–1005.
138. Alkhiary YM, Gerstenfeld LC, Krall E, Westmore M, Sato M, Mitlak BH, et al. Enhancement of experimental fracture-healing by systemic administration of recombinant human parathyroid hormone (PTH 1–34). J Bone Joint Surg Am. 2005;87(4):731–41.
139. Komrakova M, Stuermer EK, Werner C, Wicke M, Kolios L, Sehmisch S, et al. Effect of human parathyroid hormone hPTH (1–34) applied at different regimes on fracture healing and muscle in ovariectomized and healthy rats. Bone. 2010;47(3):480–92.
140. Kim HW, Jahng JS. Effect of intermittent administration of parathyroid hormone on fracture healing in ovariectomized rats. Iowa Orthop J. 1999;19:71–7.
141. Chintamaneni S, Finzel K, Gruber BL. Successful treatment of sternal fracture non-union with teriparatide. Osteoporos Int. 2010;21:1059–63.
142. Yee CS, Xie L, Hatsell S, Hum N, Murugesh D, Economides AN, et al. Sclerostin antibody treatment improves fracture outcomes in a type I diabetic mouse model. Bone. 2016;82:122–34.
143. Suen PK, Zhu TY, Chow DH, Huang L, Zheng LZ, Qin L. Sclerostin antibody treatment increases bone formation, bone mass, and bone strength of intact bones in adult male rats. Sci Rep. 2015;5:15632.
144. Morse A, Yu NY, Peacock L, Mikulec K, Kramer I, Kneissel M, et al. Endochondral fracture healing with external fixation in the Sost knockout mouse results in earlier fibrocartilage callus removal and increased bone volume fraction and strength. Bone. 2015;71:155–63.
145. Ominsky MS, Li C, Li X, Tan HL, Lee E, Barrero M, et al. Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. J Bone Miner Res. 2011;26(5):1012–21.
146. Ke HZ, Richards WG, Li X, Ominsky MS. Sclerostin and Dickkopf-1 as therapeutic targets in bone diseases. Endocr Rev. 2012;33(5):747–83.
147. Li X, Grisanti M, Fan W, Asuncion FJ, Tan HL, Dwyer D, et al. Dickkopf-1 regulates bone formation in young growing rodents and upon traumatic injury. J Bone Miner Res. 2011;26:2610–21.
148. Chen Y, Whetstone HC, Lin AC, Nadesan P, Wei Q, Poon R, et al. Beta-catenin signaling plays a disparate role in different phases of fracture repair: implications for therapy to improve bone healing. PLoS Med. 2007;4(7):e249.
149. Komatsu DE, Mary MN, Schroeder RJ, Robling AG, Turner CH, Warden SJ. Modulation of Wnt signaling influences fracture repair. J Orthop Res. 2010 Jul;28(7):928–36.
150. Florio M, Gunasekaran K, Stolina M, Li X, Liu L, Tipton B, et al. A bispecific antibody targeting sclerostin and DKK-1 promotes bone mass accrual and fracture repair. Nat Commun. 2016;7:11505.