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Pharmacological approaches to enhance bone healing

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To the kind and loving people around me that gave me time and space to fail, learn and grow. I am eternally grateful for all the love and support

Introduction

Bone fracture has a major economic and social impact on people's lives, causing individual disability and societal productivity loss. With the evolution of medicine and technology, there was an increase in the number of traumas related to driving accidents and age-related fractures [1].

The direct and indirect cost of this injury generates a total expense of billions of dollars in the health care industry [1].

Besides efforts in optimizing fracture care, 5-10% of fractures end up in non-union or delayed fracture healing.

Therefore, ways to enhance bone regeneration it's a relevant topic not only to improve the quality of the provided care but also to diminish the total costs of this injury.

Bone healing is a complex physiological process that involves temporal and spatial coordination of different cellular and mechanosensitive processes.

Usually, it's divided into three main stages: the inflammatory stage, the reparative stage, and the remodeling stage.

Because of the overlapping of these processes, any alteration in the pathways can lead to bone healing complications. Factors such as age, estrogen deficiency, diabetes, use of tobacco/alcohol, and glucocorticoid exposure can lead to attenuated osteogenic abilities. Additionally, fracture site factors such as wide fracture gap, excess fragmentary movement or infected local soft tissues. [2] have also been considered risk factors for delayed- and non-union fracture. This topic gains even more relevance knowing that these risk factors are more frequent in elderly people which leads to more complications, more healing time and overall disability. This topic gains even more relevance knowing that these risk factors are more frequent in elderly people which leads to more complications, increased healing time and overall disability.

This article reviews the available knowledge regarding the possible use of systemic drugs in the enhancement of fracture healing, starting from drugs used in osteoporosis, drugs with possible off-label use such as statins and phosphodiesterase-5 inhibitors, and finishing with new delivery methods that target to the bone.

1. Biphosphonates

Bisphosphonates (BPs) are antiresorptive agents used in the treatment of osteoporosis thanks to their capability to improve BMD and decrease risk fractures [3].

This class of drugs has the capability to inhibit osteoclastic activity, increase osteoclastic apoptosis and decrease bone resorption [3]. Since bone remodeling is important to the progression from callus formation to new bone tissue, concerns about the influence of BPs in fracture healing were raised [4].

In fact, relevant clinical impairment was found in preclinical studies using repair via direct healing processes, such as very rigidly fixed fractures or a stress fracture [5].

The different approaches to enhance the treatment with BPs were analyzed in preclinical studies: the continuous dosing regimen leads to delayed callus remodeling [6] and worse material properties of the callus, while systemic single dose of zoledronic acid produces a larger and stronger callus [7], also a delayed application (after the initial anabolic fracture response) [3] results in a higher bone volume and stiffness [7].

Clinical studies didn't find delayed bone healing when given alendronate within 14 days of DRT (distal radius fracture) [8], in fact, two studies found that post fracture BPs lead to an increase in BMD in fracture site [9, 10]. A study evaluating the effect of zoledronic acid given at least 2 weeks after surgical repair of hip fracture shows an increase in hip BMD, a reduction in clinical vertebral and non-vertebral fractures, along with a decrease in all-cause mortality [11].

Supporting the concerns about the negative influence of BPs, clinical trials found that in patients with an intertrochanteric fracture previously treated with BPs had an increased risk of delayed fracture when treated with BPs within 3 months of the fracture [12], the same risk wasn't seen in non-previous users of BPs [13].

Similarly, in a large study in elderly patients with humerus fracture, the group that was treated with BPs before and after the fracture had a doubled risk of non-union after therapy with BPs. [14]

Furthermore, Ha et al. [15] showed that patients with osteoporotic vertebral fracture treated with BPs developed intervertebral clefts, what could also indicate impaired healing.

Having in consideration the overwhelming benefits of BPs and the incongruent results of this studies, the use of BPs should only be interrupted in fractures treated with very rigid fixation until the achievement of fracture union [5].

2. Estrogen and Raloxifene

Estrogen is an endogenous hormone that has protective effects on the bone. Given its decrease in menopause, SERMs (Selective Estrogen Receptor Modulators) such as Raloxifene began being used as estrogen receptor agonist in the bone [16], inhibiting bone resorption, maintaining osteoblastic activity while attenuating osteoclastic activity [17].

Preclinical studies made with ovariectomized rats supplemented with either estrogen or raloxifene show that both drugs increased trabecular density and improved callus biomechanics, raloxifene increased total callus formation and estrogen was responsible for endosteal bone formation [18].

They also have a similar action in fracture healing, both increasing chondrogenesis in its early stage and callus remodeling in later stages [3].

Studies also indicate that raloxifene has a superior capacity for the treatment of bone fractures relative to estrogen and could have a pharmacological application in enhancing fracture healing in women [19], but this possible use hasn't been confirmed in clinical trials.

3. Calcitonin

Calcitonin is a calciotropic hormone used as an anti-osteoporotic second-line agent [20]. This hormone inhibits bone resorption by decreasing the number and activity of osteoclasts [21], and at the same time, it transiently increases bone formation potentially due to osteoblast stimulation [3].

Clinical studies in postmenopausal women treated with calcitonin for osteoporosis show a reduction in the risk of vertebral fracture by 33% and a stabilizing or increasing in BMD [22].

Aware of the publication bias, most of the preclinical studies with this hormone show an enhancement in callus formation and earlier callus maturation, concluding that calcitonin stimulates early endochondral ossification, causing increased cartilage formation, while it also enhances the biomechanical properties of the healing bone, what can contribute to earlier weight-bearing and mobilization [23] [24].

Clinical studies in elderly hip fracture patients treated with calcitonin showed a statistically significant difference in radiographic fracture fusion after 3 months, when it was observed in 84% of the patients treated with calcitonin compared to 63% in the placebo group. This same study, however, did not find a significant difference in mortality, length of hospital stay, or functional recovery between the two groups [21].

Another double-blind placebo-controlled trial in patients with osteoporotic vertebral crush fractures posited that the treatment with calcitonin, and by way of its analgesic effect, has led to decreased pain, earlier mobilization and faster restoration of locomotor function [24].

Further studies are needed to prove the potential beneficial effect of calcitonin in fracture healing.

4. PTH

Due to the known importance of PTH in calcium homeostasis and bone health, recombinant PTH analogues have been used in the treatment of osteoporosis. This agent when in continuous administration lead to an increase in osteoclastic activity inducing bone resorption, when given intermittently it induces osteoblast activation and increases bone formation [25] and it was also proved in a preclinical study the action of teriparatide in increasing chondrocyte recruitment and differentiation, enhancing endochondral ossification. [26]

Preclinical data in osteoporotic and in normal bone fractures proved the effect of intermittent therapy with teriparatide in enhancing fracture healing by accelerating callus remodeling, improving callus mineralization and increasing the mechanical strength of the callus [27-29]. These positive results are amplified in bones exposed to weight-bearing and mechanical stress [30].

Clinical trials with post-menopausal women proved the positive effect of PTH analogues in fractures, namely pelvic, where PTH (1-84) accelerated the time of fracture healing (approximately 5 less weeks), improved the functional outcome and decreased pain [31].

In a controlled multicenter trial with 102 post-menopausal women with distal radius fracture (DRT) treated nonoperatively, teriparatide (PTH 1-34) significantly decreased the time of healing compared with the control [32]. These results go along with Huang et al. that showed that the use of teriparatide in osteoporotic patients with intertrochanteric hip fractures leads to accelerated fracture healing, improved functional recovery, and it markedly reduced complications and mortality rates [33].

Besides the positive results in case reports and small studies there is still necessity of large clinical trials to clarify the efficacy of teriparatide and the possible “off-label” use of this drug to enhance fracture repair, particularly in patients with poor bone metabolism [33] or with impaired fracture healing [34].

5. Strontium Ranelate

Strontium Ranelate (SR) is an antiresorptive agent capable of inhibiting osteoclast differentiation and promoting their apoptosis [25], the anabolic effect of this agent is due to its capability to activate pre-osteoblasts and replace calcium for strontium in the bone what increases BMD [35].

Clinical studies in osteoporotic women with SR therapy showed an increase in lumbar spine BMD by 14,4% [36] and a reduction in the risk of vertebral fracture by 41% [36], non-vertebral fracture by 15% and hip fracture by 43%. [37]

In fact, this drug is approved in Europe as a treatment for severe osteoporosis both in men and women but it isn't approved by the FDA.

There is a lack of studies regarding the capability of this drug in enhancing bone fracture, but preclinical studies show that SRs didn't improve fracture healing in the normal bone [38], but its capable to enhance fracture healing in the osteoporotic bone [39]. In fact, in ovariectomized rats there was a significant increase in callus maturity, fracture stiffness, and mechanical strength compared with the control group [39].

Regarding the capability of this drug in the enhancement of bone fractures in non-osteoporotic patients, some clinical cases support [40] [41] the healing effect of SRs on delayed fracture healing or non-union, even in non-osteoporotic patients. However [42], the largest clinical trial evaluating the effect of early administration of SR in wrist fractures, didn't show any evidence of enhancement or improvement of bone healing.

So far and due to the inconsistent results regarding the use of SRs to improve bone healing, more extensive/comprehensive clinical trials need to take place.

6. Denosumab

Denosumab is a monoclonal antibody that binds to the receptor activator of nuclear factor-κβ ligand (RANKL), preventing the osteoblast-produced RANKL to bind with the RANK receptors in the surface of the osteoclast and osteoclast precursors [3]. This leads to an inhibition of osteoclast formation, function and survival which results in a decrease in bone resorption [43].

Denosumab is being used as a potent antiresorptive treatment used in osteoporosis, but when exploring its effect in bone healing, pre-clinical studies show that animals treated with denosumab display an increase in the volume of callus and a rise in bone mineral density [44, 45].

When under mechanical evaluation (42 days after the fracture), only 29% of the animals treated with denosumab had a refracture versus 57% of the animals under alendronate and 87% of the control group. That calls into question the true effect of denosumab in bone remodeling since it doesn't show a delay in bone formation or in bone healing and in animal studies it presents an improvement in mechanical properties [44].

The largest clinical study about denosumab named FREEDOM (Fracture Reduction Evaluation of Denosumab in Osteoporosis) supports the administration of denosumab in menopausal women since it was demonstrated that patients taking this medication did not have bone repair complications after non-vertebral fractures and that discontinuing the administration of denosumab would provide resolution to any negative effects [46].

7. Sclerostin antibodies (Scl-Ab)

Sclerostin (SOST) is one key regulator of osteoblast activity. Expressed by osteocytes this peptide inhibits Wnt/β-catenin signals resulting in a decrease of osteoblastogenesis and bone formation, as well an increase in bone resorption [47, 48]

Pathologies as sclerosteosis and van Buchem's syndrome display increased bone mass with excellent biomechanical stability as a consequence of mutation affecting sclerostin expression and a secondary inefficiency to inhibit Wnt pathways. [49, 50]

Knowing this mechanism and trying to apply it in a way to increase bone formation and decrease bone resorption, several sclerostin antibodies were created. Preclinical trials in rats and nonhuman primates succeed showing an increase in callus formation, bone mineral density, enhanced neovascularization and accelerate fracture repair [51-54], resulting in increased bone formation, bone mass and strength.

These encouraging results led to the development of Romosozumab, a humanized monoclonal antibody, highly specific against sclerostin that is administrated subcutaneously every month [47].

Clinical trials in women with postmenopausal osteoporosis showed an increase in BMD at lumbar spine and hip, and a decrease in risk of vertebral, non-vertebral and clinical fracture, compared with control group [55], and even compared with the risk of fracture in woman with severe osteoporosis taking bisphosphonate alendronate (ARCH study) [56]. Nevertheless, the incidence of cardiovascular events was higher in patients taking Romosozumab in comparison with the group treated with alendronate.

The proved double action of this antibody [57], stimulation of bone formation and inhibition of bone resorption, resulted in the recent approval of the drug in Europe [58], also in others countries can now be used in the treatment of severe osteoporosis in patients without cardiovascular pathologies.

Further clinical studies are necessary to confirm the applicability of Romosozumab and others sclerostin antibodies in the enhancement of bone fracture.

8. Statins

Statins are a lipid-lowering drug used in the treatment of dyslipidemia by inhibiting cholesterol synthesis [59]. Besides this main use, studies report the propitious effect of statins in the bone, promoting osteogenesis, inhibiting osteoblasts apoptosis, and decreasing bone resorption by inhibition of osteoclastic differentiation and activity [60] [61].

The proposed mechanism by which statins stimulate bone formation involves an increase in osteogenetic gene expression and synthesis of vascular endothelial growth factor (VEGF), bone morphogenetic protein 2 (BMP2), and core-binding factor alpha 1 (CBFα1). [62]

Skoglund et al. [63] in a preclinical study showed improvement in biomechanical parameters, using continuous systemic and local delivery methods, with an increase in force at failure of 60 and 70%, respectively.

Aiming the application of these benefits in the healing of the bone fracture, multiple studies have been trying to find a suitable delivery method to overcome the low bioavailability of this drug and the liver first-pass effect, trying to find a method to localize and sustainably release statin in fracture sites [35].

This is of concern because an overall analysis of studies comparing a systemic and local approach revealed a higher failure rate of the systemic therapy in bone healing and osteoporosis [60, 61] and there is also the risk that systemic routes of administration may expose the patients to high doses of statins and all the possible adverse effects that may occur, such as liver failure or rhabdomyolysis [60].

Local delivery of statins via carriers have been proved effective in preclinical trials, in the use of polyethylene glycol-based trimmers to locally deliver simvastatin into mouse osteotomy models [64] and in the local administration of gelatin hydrogels conjugated with low-doses of simvastatin [65], resulting in an increase in callus formation and a decrease in the total dose necessary for bone repair [61].

Clinical studies in postmenopausal women with systemic administration of statins show an increase in the BMD at the spine and hip of 7-8 % [66] and a reduction in the hip fracture risk [67].

Nevertheless, the lack of clinical trials and mixed results regarding the effects of different statins are an obstacle to address [68].

Given the safety profile of statins and the results obtained in preclinical studies, if clinical trials confirm these anabolic and antiresorptive proprieties on the bone, statins may have an additional clinical applicability, namely as an anti-osteoporotic drug, or lead to a new recommendation of statins' dosage in hyperlipidemic patients to decrease the risk of fragility fractures [68, 69].

9. Phosphodiesterase-5 inhibitors

Phosphodiesterase-5 inhibitors (PDE5 inhibitors) such as sildenafil, vardenafil, and tadalafil are used in the treatment of pathologies such as erectile dysfunction and pulmonary hypertension.

Since angiogenesis plays an essential role in bone healing and PDE5 inhibitors are capable of up-regulating pro-angiogenic factors, it was questioned the possible positive effect of these drugs in fracture healing.

Preclinical trials with sildenafil showed significant differences in fracture healing scores, with increased bone density [70] and increased biomechanical stiffness [71].

Moreover, besides a higher expression of pro-angiogenic factors, an increase in osteogenic cysteine-rich protein (CYR) 61 was found, what confirms that PDE5 inhibitors can accelerate bone healing by increasing bone formation [71].

Up to this date there is only preclinical evidence regarding this topic, but if confirmed by clinical trials, PDE5 inhibitors can be used as a supporting factor to enhance bone healing.

10. Salvianic Acid A (SAA)

Salvianic Acid A, also known as Danshensu or SAA, is a water-soluble active compound from salviae miltiorrhizae (Danshen).

Studies show that SAA and its analogs, through direct or indirect regulation of distinct signaling pathways, are capable of stimulating osteogenesis.

However, the clinical application of this potent bone anabolic agent SAA is hampered by the short serum half-life and the lack of osteotropicity.

To resolve SAA's poor bioavailability to the skeleton Liu Y et al [2], developed a bone-targeting liposome (BTL) using biodegradable pyrophosphate as the targeting moiety. In this way assuring the effective delivery to all major skeletal tissues and associated cells [72] and the local retention of this payload up to 20 days after local administration.

Employing this formula with a minimally invasive local injection in a mouse model with delayed fracture union induce by prednisone has shown significant improvement in the formation of fracture callus and an accelerated mineralization rate resulting in a reduction of 22 days in terms of healing time, as well as an improvement in mechanical properties (with improved stiffness, yield stress, and flexural modulus) when comparing SAA-BTL treatment with the dose equivalent non-targeting SAA liposome (SAA-NTL), only SAA or no treatment [2].

The preclinical study of Liu Y et al validates the potential use of SAA-BTL as a safe, effective and affordable intervention in the clinical management of delayed fracture union, and the possible clinical application of BTL with other bone anabolic agents.

11. Dasatinib

Dasatinib is an oral available short-acting inhibitor of multiple tyrosine kinases, as ABL and Src, normally used in the treatment of chronic myeloid leukemia.

Researches show the potential effect of dasatinib in the bone formation since the inhibition of Src kinase results in an increase in the number and activity of osteoblasts, an increase in matrix production and mineralization, as well as improvement in bone formation, revealing the crucial role of Src kinase in bone formation and turnover [73]. The rate alteration of bone resorption due to the inhibition of Src kinase has as main adverse effect osteopetrosis [74].

In an attempt to circumvent this adverse effect and improve the bioavailability and reach of dasatinib to the area of bone fracture, Wang, M., et al. [75] linked to this drug a decapeptide of aspartic acid, exploring its affinity to the hydroxyapatite present in fracture surfaces as a way to increase the concentration of dasatinib at the fracture site [76]. This new approach showed an increase in the density of the bone target with dasatinib in mice, an increase in the total bone volume by 82% and the recovery of mechanical strength occurs twice as fast. This reduction of 60% in the healing time didn't affect the normal healing of the bone since the final repaired bone was similar between treated and control groups.

This enhancement of bone regeneration occurs without interfering with the homeostasis of healthy bone and using a dose of dasatinib below the one normally used in the treatment of CML and using it only every other day, what should cause significantly fewer adverse effects.

There are still no clinical trials employing dasatinib with this new approach and testing its possible applicability.

Conclusion

Fracture healing is a complex process with overlapping different variables. The difficulty to access the fracture area to deliver the drug and the lack of auxiliary diagnostic tools to follow the healing process makes it difficult to understand the real impact of the drugs in fracture healing.

Another fact to have in mind is the different kinds of ossification, such as endochondral or intramembranous, and the huge number of factors that can influence bone regeneration, making it difficult to get a group of people with the same fracture.

Properties such BMD, callus size, mechanical strength and radiological healing time were some of the outcome variables that we used to evaluate the enhancement capabilities of the drugs.

Impairment of these properties in patients with very rigid fixation taking bisphosphonates is enough evidence to support the suspension of bisphosphonates until the achievement of fracture union [5].

There isn't enough evidence to support most of the reviewed drugs because some of these possible applications were only explored in pre-clinical trials.

Although the results in clinical trials were positive in most of the analyzed drugs, it is essential to highlight the lack of clinical studies and the lack of diversity in these, since most of them were performed in patients with osteoporosis, menopausal women and/or older patients.

Given the publication bias, and the presence of inconsistent results, at this time, the cost/benefit ratio of auxiliary drug treatment is still not well understood.

However, the appearance of new delivery methods and new drugs may have an impact on the future treatment of bone fractures, namely as an additional aid for patients with impaired bone healing and patients with delayed union.

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