Pharmacological agents and bone healing

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Summary

Osteoporosis is the most common alteration of bone metabolism. It derives from an increase in bone resorption with respect to bone formation and is characterized by microarchitectural alterations, decreased bone mass and increased risk of fracture. The coupling between bone formation and resorption is a fundamental concept in skeletal metabolism, and it explains how a certain amount of removed tissue can be replaced by the same amount of new bone. Various substances used to treat osteoporosis may also be used for orthopaedic conditions such as fracture healing, implant fixation, bone grafts and osteonecrosis. Fracture healing consists in the replacement of the lost bone by a tissue that has the same biomechanical properties as those preceding the fracture. The repair process is triggered by the local response to the tissue injury that damaged the continuity of bone. The duration of each phase of the healing process can vary significantly, depending on the site and characteristics of the fracture, on patient related factors and on the treatment choice. While most of the fractures heal with conventional treatment, they can also cause permanent damage and complications, especially in a certain kind of patients. Osteoporosis and old age may contribute in delaying or impairing the reparative process. In animal models the healing process is slower in older and/or ovariectomized animals. Biomechanical tests have also shown that bone strength is compromised in human osteoporotic cadaver bone. The same problems were highlighted in the surgical treatment of fractures in osteoporotic patients. Mainly in the treatment of hip fractures there is an increased risk of cut-out, re-fractures and implant failure in patients with osteoporosis. Preclinical studies have shown that certain pharmacological agents (bisphosphonates, strontium ranelate, teriparatide) may enhance osseointegration and stimulate reparative processes. They may be administered systemically and/or used locally at the fracture site on the implant surface. The aim of fracture treatment is to restore bone biomechanical properties and to allow restoring normal function at the affected site. If the new pharmacological approaches could be translated into clinical benefit and offered to patients with osteoporosis or other factors that put at risk the process of healing (subjects with severe loss of substance or fractures at high risk of complications), they could represent a valuable aid in the treatment of fractures.

KEY WORDS: fracture healing, bone remodeling, osteoporosis treatment.

Introduction

Osteoporosis is a major public health problem. It is characterized by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased susceptibility to fractures of the hip, spine, and wrist. Poor bone quality in patients with osteoporosis challenges the surgeon with difficult treatment decisions. Bone healing is characterized by a complex series of physiological events that lead to fracture union and complete regain of bone mechanical strength. The primary goal of fracture healing is to re-establish the structural integrity of the injured bone, restoring function of the affected limb. Fracture repair has different pathways and combinations of bone formation mechanisms, which depend also on the fracture fixation which should be chosen in order to achieve the needed stability. Bone repair is conventionally divided into four stages, each of them characterized by a specific set of cellular and molecular events (1, 2). The four-stage model originated as the result of histological observations of healing fractures in both human patients and animal models. However, research of the past several decades has explored both the cellular and molecular forces that drive the underlying processes. The duration of each of the healing phases can vary significantly, depending on the site and characteristics of the fracture, patient-related factors, and the chosen treatment (3, 4).

Bone healing process

A fracture is typically associated with disruption of the local soft tissue integrity, interruption to normal vascular function, and a distortion of the marrow architecture. This damage leads to activation of non-specific wound healing pathways that accompany non-skeletal injuries. Bone repair starts with the formation of an inflammatory haematoma due to disruption of capillaries at the fracture site. Thus cells are released from the blood stream and start to locally secrete pro-inflammatory cytokines (TGF-beta, IL-1, IL-6, PDGF, VEGF, FGF-2). The subsequent soft callus formation by chondroblasts and fibroblasts is stimulated by growth factors such as TGF-beta2, FGF-1 and BMPs. Neoangiogenesis provides the newly formed callus with blood vessels. In 4-5 weeks, osteoblasts from the periosteum increase their own osteogenetic activity, secreting bone matrix which is gradually mineralized, leading to substitution of soft callus with hard callus. Massive angiogenesis provides differentiating osteoblasts with O2 from the blood stream. This process is known as endochondral ossification and the resulting callus is made of so-called woven bone, i.e. bone without precise architecture. This stage is
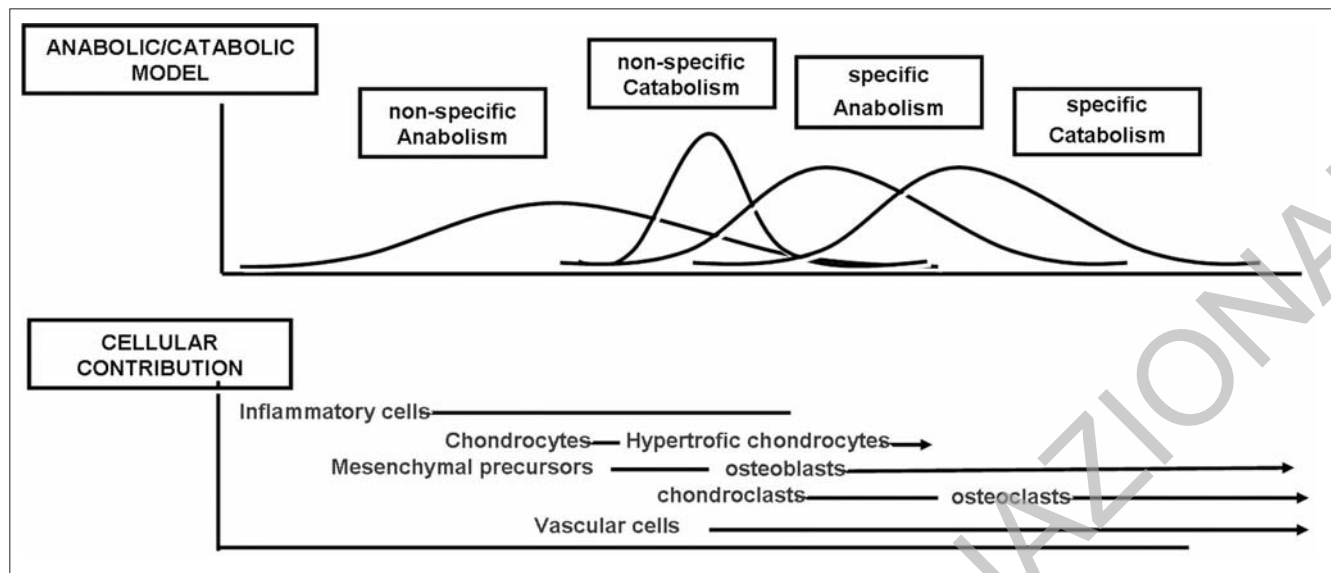
Figure 1 - Models of fracture repair and the cellular participants. Adapted from Schindeler A et al. 2008 (8).

dominated on a cellular level by chondrocytes and fibroblasts, although the relative proportions of the different cell types can vary between fractures. These cells produce a semi-rigid soft callus that is able to provide mechanical support to the fracture, as well as act as a template for the bony callus that will later supersede it. Cartilage callus is principally non-vascular, although its subsequent replacement with woven bone involves vascular invasion. Constant remodelling allows new bone achieving its original lamellar and trabecular structure. During remodelling osteoblasts produce cytokines which regulate both the resorption/formation process (RANK-L) and osteoclasts differentiation (M-CSF). The vasculature is known to be critical for hard callus formation, with increased local oxygen tension necessary for osteoblast differentiation. Stimulation of vessel formation using angiogenic factors can augment bone formation and fracture healing in model systems (5). However, recent studies have shown that delayed union can occur even after the vasculature has been re-established, indicating that the blood supply alone is not the only determinant of fracture healing success (6). The final stage of fracture repair encompasses the remodelling of the woven bone into the original cortical and/or trabecular bone configuration. This phase can also be referred to as secondary bone formation (1). Initially, this involves converting the irregular woven bone callus into lamellar bone, although the standard cortical structure is eventually restored. The remodelling process is driven by a coupled process of orderly bone resorption followed by the formation of lamellar bone. Osteoblast-like cells may also play a minor role in proteolysis of osteoid elements, prior to new bone formation (7). Although the sequential four-stage model describes the fundamental events that occur over the timeline of a healing fracture, there are often significant overlaps between stages.

The classic model of bone healing through well defined phases has been recently re-interpreted based on an anabolic/catabolic model, in which the initial part of the process is seen as a-specific anabolism and catabolism, intended to quickly re-establish primary bone continuity and resistance. Applying the concepts of anabolism and catabolism may provide a useful alternative system for understanding the fracture repair process. In this system, the outcome of the fracture repair process exists as a balance between the anabolic (bone forming) and catabolic (bone reabsorbing) responses. Subsequent specific

remodelling tends to re-create original spatial organization thus leading to restitution of pre-fracture state (Figure 1).

It has been speculated that the speed of fracture healing may be determined by the processes of non-specific anabolism and catabolism (recruiting cells, revascularisation), while the strength of repair relates to the mechanically driven balance between bone-specific anabolism and catabolism. Alterations of these processes could lead to several complications, such as vicious consolidation and fracture non-union (8) (Figure 2).
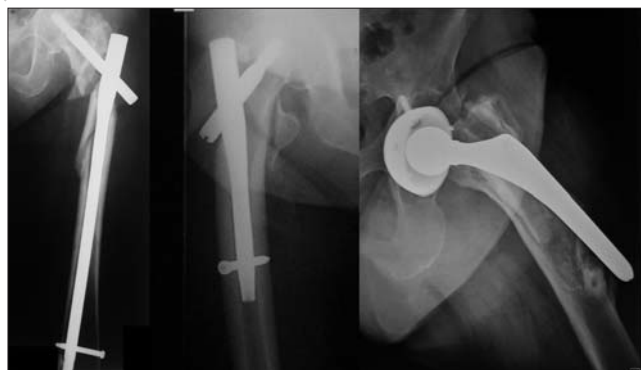

Figure 2 - Examples of implant failure in elderly patients with severe osteoporosis.

Factors which impair bone healing

Several conditions can influence bone healing. These include extrinsic factors as smoking and alcohol abuse. Smoking decreases cell proliferation and angiogenesis, in turn leading to reduced local availability of O2 and anti-oxidant factors. Alcohol abuse inhibits cell proliferation and osteoblastic activity. Even comorbidities can impair bone healing, e.g. diabetes, anaemia, malnutrition, peripheral vasculopathies and hypothyroidism. Pharmacological agents can also influence bone healing. The anti-proliferating and cytotoxic effects of chemotherapy negatively influence angiogenesis, bone formation and bone matrix mineralization. Corticosteroids inhibit osteoblastogenesis and soft and hard callus formation, while inducing osteoblast and ostecyte apoptosis. This effects are directly relat-

ed to dosing and duration of treatment. Some antibiotics have negative effects on bone repair. Floroquinolones induce retardation of bone healing in animals. Ciprofloxacin inhibits cell proliferation, reduces callus stiffness and torsional strength in rats. Anticoagulants, despite non-discussed advantages in terms of embolism prevention in fractured patients, seem to delay fracture healing and to decrease bone formation while increasing bone resorption in animal models (9). It has been supposed that bone active agents used for the treatment of osteoporosis could affect bone healing processes. Anabolic agents have a proved potential in improving bone repair, especially when bone quality is compromised (10). Moreover, both bisphosphonates and anabolic agents can increase bone implant stability and osseointegration. Increased bone resorption during the bone repair cascade can impair bone callus mechanical strength. The anti-resorptive properties of bisphosphonates, together with the slight inhibitory action on osteoblasts, could explain the positive influence on bone formation and osseointegration (11). However, for this to occur, widely accepted guidelines are important, in order to encourage the conduction of studies that evaluate bioactive substances, drugs, and new agents that may promote fracture union and subsequent return to normal function.

Pharmacological agents improving bone healing

In the elderly there are important alterations in the response to injury. During fracture healing there are lowered bone formation, delayed periosteal reaction and cell differentiation and impaired bone remodeling. Moreover mesenchymal stem cells are decreased in number and have less divisional capacity. The responsiveness to signaling molecules is reduced, so as angiogenesis and extracellular matrix osteoconductive capacity. At a molecular level there is an increase in oxidative damage, cell senescence, apoptosis and local metabolic alterations (12). This is why there is an increased risk of complications in old people, also due to reduced bone quality and osteoporosis. So it's not only difficult to treat a fracture, but also to maintain reduction of the bone fragments. A weak bone is susceptible of implant failure and vicious consolidation (Figure 2). Bone active agents that can improve bone metabolism could be used to stimulate bone healing in elderly patient and in younger patients in which bone regeneration is compromised for other reasons. It is difficult to investigate the effects of pharmacologic agents on bone healing in humans, due to scarce reproducibility and standardization of results. So these agents have been tested in animal studies. Here we report some of the studies conducted.

To examine the effect of alendronate on fracture repair, the drug was given to mature beagle dogs at 2 mg/kg/day for 9 weeks preceding fracture, 16 weeks after fracture, or both before and after fracture (25 weeks). A transverse mid-diaphyseal fracture of the right radius was surgically induced and was stabilized by external coaptation splinting. Fracture healing and bone remodeling were evaluated by radiography, gross and histological examination, and bone histomorphometry. In dogs that received alendronate during the fracture healing period, at 16 weeks the calluses were approximately 2-3 times larger than those in dogs that received a placebo during the healing period.

Mechanical testing showed that the ultimate load at failure and the flexural rigidity of both the fractured and contralateral intact bone were unaffected by treatment with alendronate (13). Recently, an experimental study was conducted using a porcine model to evaluate the influence of systemic administration of alendronate on bone-pedicle screw interface fixation in posterior lateral vertebral fusions with instrument fixation. The pigs in the treatment group received alendronate 10 mg/day orally, which began on the second postoperative day and lasted for three months. Alendronate treatment in this study resulted in increased biomechanical anchor strength at the bone and pedicle screw interface with borderline significance. The control group showed a lower maximum torque and lower initial angular stiffness. Based on the results of histological evaluations, the treatment group had a higher percentage of bone growth on the pedicle screw surface. The bone volume within the area between the screw threads was also high in the alendronate-treatment group (14). An experiment explored the hypothesis that a temporary delay in remodeling using a single systemic dose of N-BP can increase bone mineral content and accelerate the restoration of strength in an open fracture model in rats at the time of initial union. An open femoral rat osteotomy model with stabilization via Kirschner wires (K-wires) was used. Forty 12-week old male Wistar rats were randomly assigned to one of the four treatment groups. Two groups were either treated with 0.9% saline (control group) or 3 mg/kg pamidronate S.C. at the time of surgery. In the remaining two groups rats were treated either with a low dose of 0.1 mg of pamidronate or a high dose of 1.0 mg of pamidronate locally delivered on a coated K-wire. A bolus subcutaneous injection of pamidronate at the time of surgery was shown to increase the BMC, volume, and mechanical strength of the fracture callus. With the administration of pamidronate, as a single dose at the time of surgery, the strength of the operated femurs was 111% higher than that of the non-operated control femurs. Significant increases in callus BMC and volume of the bolus systemic dose group were found compared to the saline control. Further, the strength of the systemic dose callus was increased by 60% in the systemic group. Local treatment did not result in increased strength (15). Another work studied the effects of Risedronate and Calcarea phosphorica 6CH (homeopathic medicine) on the repair of bone lesions in male rats with osteoporosis induced by castration. Eighty-four three-month-old rats were used divided into four groups of twenty-one animals each. Three groups where castrated and one group was submitted to Sham surgery. All animals were operated to create a 3 mm cortical lesion on the medial face of the tibial proximal extremity. By the 28th day of the repair process The castrated Risedronate group had the thickest callus with a great amount of trabecular bone filling the defect and extending into the medullary space (16). It was also investigated a possible benefit in treatment of fractures by local application of zoledronic acid released by a biodegradable poly(D,L-lactide) (PDLLA) coating of intramedullary implants. Standardized midshaft fractures of the right tibia of 5-month-old rats were stabilized either with uncoated, PDLLA-coated, or Zoledronate-coated implants. Maximum load and torsional stiffness were highest in the group treated with Zoledronate. 84 days after fracture, the torsional stiffness of the Zoledronate-treated group remained higher than that of the uncoated group whereas the maximum load for the control groups reached the results for the Zoledronate-coated group. So local application of Zoledronate from PDLLA coating appears to accelerate the achievement of mechanical stability in fractures (17). Exogenous PTH could affect chondrogenesis and fracture healing. In an animal study unilateral femoral fractures were produced in 2-month-old Sprague-Dawley rats. Daily subcutaneous injections of 10 µg/kg of recombinant human PTH(1-34) [rhPTH(1-34)] were administered over a 28-day period of fracture healing. Control animals were injected with vehicle solution (normal saline) alone. The results showed that, on day 14 after fracture, cartilage area in the PTH-treated group was significantly increased (1.4-fold) compared with the controls, but this increase was not observed at days 21 and 28. After 14 days, there were no significant differences between groups in either cell proliferation or the expression levels of cartilage differentiation-related

genes. These results suggest that intermittent treatment with low-dose rhPTH(1–34) induces a larger cartilaginous callus but does not delay chondrocyte differentiation during fracture healing (18). The effects of Teriparatide and Strontium ranelate on femoral fracture healing were compared in osteoporotic Sprague Dawley rats by micro-CT and torsional tests and the results seem to show that Strontium ranelate can improve the torsional strength in the femurs of treated rats with respect to both the placebo and Teriparatide groups (19). Pharmacological agents may also enhance osseointegration, which is defined as the contact which intervenes, without interposition of non-bone tissue, between normal remodelled bone and an implant which can bear the distribution of load from the implant to and inside bone tissue. Bone ingrowth is defined as the formation of bone tissue inside the porous surface of an implant (20). The long-term durability of total joint replacements is critically dependent on adequate peri-implant bone stock, which can be compromised by wear debris-mediated osteolysis. This study investigated the effects of bisphosphonates on enhancing peri-implant bone in the presence of clinically relevant ultra-high molecular weight polyethylene (UHMWPE) wear debris. Fiber-mesh coated titaniumalloy plugs were implanted bilaterally in the femoral condyles of 36 New Zealand white rabbits. Implants in the left femora were covered with submicron UHMWPE particles during surgery. Rabbits were administered either no drug, subcutaneous alendronate weekly (1.0 mg/kg/week) or a single dose of intravenous zoledronate (0.015 mg/kg). Radiographically, both bisphosphonates significantly increased periprosthetic cortical thickness at 6 weeks and at 12 weeks. Histomorphometrically, alendronate and zoledronate raised peri-implant bone volume (BV/TV) up to 2-fold after 6 weeks without added wear debris and more than 3-fold when wear debris was present. Furthermore a 6-week bisphosphonate treatment increased osteoid thickness in the absence of wear debris and in the presence of wear debris. In summary, alendronate and zoledronate treatment increased periprosthetic bone stock in a rabbit femoral model, particularly in the presence of UHMWPE wear debris (11). Also Teriparatide (60 mcg/kg/die) has enhanced implant osseointegration in animal models, increasing screw fixation by 2,5 fold after 2 weeks and screw torsional strength by 3,5 fold after 4 weeks in rats (21). Alendronate was also tested in osteoporotic human patients with a pertrochanteric fracture. Fractures were fixed using a thochanteric fixator and four hydroxyapatite-coated pins. Two pins were implanted in the femoral head and two were placed in the femoral diaphysis. In the patients who received an oral dose of 70 mg of alendronate per week the extraction torque increased twofold with the pins implanted in cancellous bone (22). In our experience, we tested the clinical outcome in patients with osteoporosis and fragility fractures and/or with a great bone gap or displaced and comminuted fractures, treated with daily rhPTH (1-34) for a minimum of 3 months to a maximum of 18 months. The results were generally good, and we observed faster regain of normal function and recorded no complications, including implant failure and refracture (23).
In experimental studies, various pharmacological substances have proven effective in enhancing bone healing. Clinical trials are needed, directed to patients with osteoporosis or wide bone gaps. In human subjects the evaluable results are essentially clinical, such as an acceleration of the healing process, a faster return to normal function and a reduction in the complication rate (24).

Conclusions

Continuous improvement of knowledge concerning bone tissue pathophysiology is leading to a more and more appropriate uti-

lization of pharmacological substances in order to optimize bone quality while reducing fracture risk. Many animal studies have demonstrated that the drugs commonly used against osteoporosis can positively influence fracture repair and implant osseointegration. The possibility of modulating anabolic and cathabolic phenomena happening in the skeleton both locally and systemically opens a new horizon about enhancing bone healing, especially when bone tissue is qualitatively and/or quantitatively compromised. The orthopaedic surgeon must evaluate the patient globally, in order to identify the conditions leading to a bone healing deficiency, which can compromise the therapeutical act, either surgical or not. An accurate study of the host bone quality and metabolism can facilitate not only the implant choice, but also the use of substances that can "guide" the repairing process. The choice must in fact be tailored on the patient's characteristics and on his own bone. It is also necessary to define more and more specific criteria in order to conduct human studies which are reproducible and reliable. Further research is needed about improving bone healing in humans using pharmacological agents which can shorten or optimize fracture repair and, more generally, bone response to injury, both in younger and elderly patients.

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