Osteoporotic fractures – the biological perspective

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Editorial

Osteoporotic fractures – the biological perspective

“Osteoporosis is a silent disease but its impact is not: in the United States, an estimated 2 million osteoporotic fractures occur each year, resulting in more than half a million hospitalizations, more than 800,000 emergency room encounters, more than 2,600,000 physician office visits, and the placement of nearly 180,000 individuals into nursing homes, a situation that most of the participants in a study compared unfavorably to death.” is the leading statement in a recent analysis on the burden of illness of osteoporotic fractures [1–3].

Current strategies to improve the clinical outcome of osteoporotic fractures include addressing “biomechanical” and “biological” considerations. For better mechanical fixation of these fractures, angular stable implants have become popular, if not state of the art, in the Western world [4]. On the “biological” front, systemic anti-osteoporotic treatment is the cornerstone for avoiding secondary fractures [5], and better strategies for enhancing fracture healing in elderly patients by local or systemic therapies continue to be developed. Ongoing debates on the risks and benefits of systemic anti-osteoporotic medications for the treatment of atypical fractures relative to their potential effects on bone healing in an acute osteoporotic fracture continue. Literature lacks evidence-based recommendations for the stimulation of healing in osteoporotic fractures with the use of biomaterials or systemic therapies, such as anti-sclerostin antibodies or PTH, and clinical research with randomized control trials remains challenging [6]. Much of the information available for clinical decision-making, therefore, comes from basic research studies using animal models. The relevance of these models to the clinical condition is a critical issue, and the development of clinically relevant models, including metaphyseal fracture models, remains a challenge [7].

The Orthopaedic Trauma Care (OTC) Foundation as a global network of surgeons and scientists, dedicated to the advancement of osteosynthesis and trauma care, has recently sponsored several workshops on hot topics in the field and published proceedings of presentations from these meetings [8]. This current supplement edition is based on a workshop entitled “Osteoporotic Fractures – The Biological Perspective” held in London in November 2013. This symposium focused on key issues and questions in osteoporotic fractures, including the following topics: (1) experimental approaches – animal models for fracture healing and osteoporosis; (2) systemic osteoporosis treatment and its effects on bone metabolism and fracture healing; (3) local treatment for the enhancement of osteoporotic fracture healing; and (4) the surgeon’s role and fragility fracture programs.

Animal models should recreate clinical conditions as closely as possible, including those for osteoporosis and osteoporotic fractures. But can we really mimic osteoporotic bone conditions in animals as osteoporosis, which is an age-related disease in humans that does not occur in animals? If so, which models are most appropriate for studying fracture healing and should metaphyseal models be used instead of diaphyseal models? These critical questions are addressed in the first section of this supplement by Oheim et al. and Simpson and Murray that review the currently available small and large animal models for osteoporosis. The latter article focuses further on the selection of the most appropriate model for a given research question.

The second major topic in this supplement is dedicated to the current standards of medical anti-osteoporosis treatment and the effects of therapeutics on bone metabolism and fracture healing. This section is introduced by two articles on bone mineral density in the femoral neck of hip fracture patients by Guerado et al. and an overview work of fracture healing in osteoporosis and the role of anti-osteoporosis medication by Feron and Mauprivez. Subsequent articles by Kates and Ackert-Bicknell, Larsson, and Collinge address the efficacy of bisphosphonates, anti-sclerostin antibodies and PTH therapies for osteoporotic fracture patients. Per Aspberg provides a critical and “personal” overview of atypical fractures.

The third section targets treatment strategies to locally enhance osteoporotic fracture healing. Lieshout and Alt review the literature on the use of clinically available biomaterials, including bone morphogenetic proteins, and identify a significant lack of evidence-based recommendations for the use of these materials for osteoporotic fractures. Cheung et al. and Watanebe et al. present and discuss alternative technologies for osteoporotic fracture treatment that include low intensity pulsed ultrasound for local stimulation of bone healing and mesenchymal stem cell-derived chondrocytes (MSC-DCs) for large bone defects. One article from Ray et al. looks at the results of a strontium-enriched scaffold to simulate fracture healing in an animal model.

Finally, Rosenwasser and Cuellar describe the role of the orthopedic surgeon in the peri-operative management of osteoporotic fracture, including screening, diagnosis, medical treatment, and follow-up to reduce the risk of secondary fractures and to improve overall outcome. S. Kates reviews the performance of Fragility (or Geriatric) Fracture Programs with dedicated treatment pathways on the outcome of osteoporotic fracture patients, including reduced length of stay, reduced in-hospital mortality rates, and reduced complications.

We hope that this supplement helps stimulate further investigation on the treatment and outcome of osteoporotic fracture patients.

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Foundation (OTC) and the grantor Stryker® for the sponsorship of the workshop in London 2013 and of this supplement in Injury.

Conflict of interest

Volker Alt serves as a research member of the Osteosynthesis and Trauma Care Foundation, which is supported by the Stryker Corporation. He is a paid consultant for Medtronic and has been involved in speakers bureau/paid presentations for aap Implantate and Heraeus. Theodore Miclau serves as the chairman of the research committee of the Osteosynthesis and Trauma Care Foundation, which is supported by the Stryker Corporation. He is a paid consultant for Acelity and Amgen Corporations, receives research support from Baxter Corporation and DePuy Synthes, and serves on the Board of Directors for the Orthopaedic Trauma Association, Inman Abbott Society, and San Francisco General Hospital. Foundation.

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Can we induce osteoporosis in animals comparable to the human situation?

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Introduction

Osteoporosis is a chronic disease characterized by systemic deterioration of bone mass and microarchitecture leading to skeletal fragility associated with an increased risk of fractures. This socially and economically dramatic health problem of the developed world is going to be even more critical as a result of the on-going demographic change [1,2]. Chrischilles et al. calculated that every second white woman above 50 years of age would suffer from an osteoporotic fracture during her lifetime – leading to disability, increased mortality, and financial burden [3]. Ross et al. reported that also every third man would suffer from osteoporotic fractures during his lifetime [4]. Today in Europe not only 22 million women but also 5.6 million men suffer from osteoporosis and the calculated health burden is within the range of other widespread chronic diseases [5].

Till today the underlying regulatory mechanisms of bone metabolism leading to progressive loss of bone mass and structural integrity are not fully understood and surgical as well as non-surgical treatment options are yet not satisfactorily resolved. This is why massive efforts are undertaken to further investigate this critical illness.

With this review article we aimed at giving an overview of some established animal models for osteoporosis focusing on important general characteristics of suitable models. Furthermore, ethical concerns changed dramatically in society and research community during the past decades, which is why there is nowadays a need for a much more critical view on all established animal models.

General comments

For the on-going osteoporosis research animal models are of great value and still essential at this time. But “if a disease or condition is not fully understood, how can one design a good animal model of the disease?” This is the “animal model paradox” [6]. Not surprisingly, there is up-to-date no ideal animal model for osteoporosis – and probably never will be – because osteoporosis is not a single disease but a family of disorders negatively affecting the human bone turnover and animals are despite all similarities in bone structure and metabolism obviously not humans. For this reason, every model struggles with specific pros and cons and can only be able to mimic certain aspects of the human disease. So the question remains, whether we can induce osteoporosis in animals comparable to the human situation?

In vitro analyses of different bone cell types are extremely helpful in answering important questions at the molecular biological level, in particular questions regarding intra- and intercellular signaling. Furthermore, these studies are able to
reduce the amount of animal experiments needed. However, these experiments can never address the highly relevant interactions of various organ systems, or structural and biomechanical issues in complex organisms. In addition, the American Food and Drug Administration (FDA) recommends ovariectomized animals as the preferred model for bone loss research [7] and due to the guidelines of the World Health Organization (WHO), drug effects must be demonstrated in appropriate animal models for osteoporosis [8]. But appropriateness in this context has different dimensions! Thus the specific animal model not only has to be appropriate in terms of imitating the human disease, but also when looking at costs and availability as well as ethical concerns. Reinwald and Burr defined concrete parameters that should be looked at when choosing a large animal model for osteoporosis, such as 1) appropriateness as a model of estrogen deficiency (i.e., significant bone loss induced by estrogen depletion), 2) specific biological and physiological characteristics (e.g., osteonal bone remodeling), 3) cost and availability, 4) housing/spatial requirements, 5) manageability during an experiment, 6) reproducible results, 7) minimal ethical/societal implications, and 8) predictive of skeletal effects of potential osteoporosis therapies in adult humans [9].

**Small animal models for osteoporosis**

Small animal models – namely rodents – are well established as models for osteoporosis. The ovariectomized mouse and rat are up-to-date standard animal models for postmenopausal bone loss [10–12]. In contrast to large animals, experiments with small animals are less costly and time consuming, requirements for housing and handling are of smaller dimensions, and ethical implications are in general lower in comparison to large animals. In addition, the possibility to specifically modify the genetic background of mice, made these animals extremely attractive for studying bone metabolism and disorders [13]. The genetic modification of single genes gives the great opportunity to identify the role of specific factors, membrane proteins, signaling pathways, or else. For example, our group could recently demonstrate the importance of the transmembrane receptor Kremen-2 (Krm2) in the regulation of bone formation in a knock-out mouse model (Fig. 1) [14]. Recently it succeeded to alter also the genetic background of rats, making them again more attractive as models for bone loss [15].

However, beside all these advantages of rodents, there are issues that can only be addressed in large animal models; such as metaphyseal fracture healing – the ‘hot-spot’ area of osteoporotic fractures [2] and advancement of orthopedic implants comparable to those used in humans [16,17]. In addition, repeated histomorphometric analyses, substantial blood and urine samples, as well as iliac crest biopsies can only be performed in large animals [18] (Fig. 2).

**Large animal models for osteoporosis**

Searching for an appropriate animal model for postmenopausal osteoporosis, it has to be considered that spontaneous menopause is only found in humans, Old World monkeys and great apes. Since most other mammalian species experience lifelong estrous cycles [9,19] bone-loss caused by estrogen deficiency cannot be observed naturally in these animals [18]. Furthermore, in all quadrupeds the static and biomechanical loads – especially of the extremities and spine – is different to those in humans [9,20].

In international literature different species are described as large animal models for bone loss, such as sheep, goats, dogs, pigs, and non-human primates. The latter obviously do show the most similarities to human bone structure and metabolism from all animal models available. However, ethical concerns and legal restrictions are highest in these animals. Disadvantageous is furthermore, that experiments with non-human primates are very cost intensive and only legalized in very few centers around the world [21–23]. This gives reason why these animals – although very close to human physiology – are not appropriate as standard models for osteoporosis.

Beside non-human primates pigs do have many characteristics of bone structure and metabolism in common with human. Additionally, their gastrointestinal system as well as the water and electrolyte homeostasis is close to human [24–26]. The similarities between both species are best documented through the fact that organs of pigs are used for Xeno-transplantations in humans [27]. Disadvantageous however is the fact, that adult domestic pigs weight up to 200 kg and especially male subjects tend to be aggressive. This critical combination makes it sometimes impossible to do further experiments or even to take blood samples without performing general anesthesia in these animals [25]. However, mini-pigs might be an attractive alternative to work with [28]. Recently genetic modifications in pigs via e.g. nucleus-transfer-technology were successfully performed, such as establishing inducible RANK-Ligand overexpression systems as models for inducible systemic bone loss [29,30].

Beagle dogs have also been characterized as models for human bone loss [31]. They do also show bone structure and metabolism comparable to humans with cortical and trabecular bone remodeled by bone multicellular units (BMUs) [32]. However, the data published about the effects of ovariectomy on bone structure and turnover are inconsistently and the effects vary significantly between different anatomical sites [33–36]. In addition, ethical issues, especially in the societies of the western hemisphere, are highly relevant using dog models, thus this model is also not appropriate as a standard model of bone loss.

Sheep in particular, have proven invaluable in orthopedic research [9,37,38] and should be therefore discussed in more detail on the following pages.

**The Ewe**

Female sheep (ewes) are well established as model animals in orthopedic research. Some of the advantages of sheep are: their docile compliant nature [18], their simple husbandry needs, low costs of acquisition and maintenance, and availability of aged results.
(>6 years) animals in large numbers [6,39]. In addition, ethical and societal implications are generally less sensitive compared to other large animal models [9].

The macro- and microarchitecture of sheep bone is comparable to human bone; trabecular and cortical bone with Haversian systems, as well as bone remodeling performed by bone multicellular units (BMUs) is found in both species [6,9,40] (Fig. 3). The cortical bone of young sheep is plexiform. Although older sheep (~1 year) show already bone remodeling with well-developed Haversian systems [6,18,39,41,42], remodeling of all primary osteonal bone is not observed until 7–9 years of age [9,37]. In addition, the relevance of biochemical bone turnover markers such as alkaline phosphatase, osteocalcin or crosslinks could also be demonstrated in sheep [40,43]. Beside the described similarities in bone structure and metabolism between human and sheep, there are some differences that have to be taken into account. Thus the bone mineral density (BMD) and bone mineral content (BMC) are significantly higher in sheep compared to humans (BMD lumbar spine (mg/cm³): human ~180 vs. sheep ~440, BMC (mg): human ~80 vs. sheep ~240) leading to an even more pronounced increase in mechanical stability (fracture stress (N/mm²): human ~1.2 vs. sheep ~13.2) [32]. These might explain why even ewes with marked bone-loss still show relatively high BMD and BMC values and osteoporotic fractures barely ever occur in these animals. Since BMD and bone turnover parameters change significantly in sheep throughout the year [40,41,44], appropriate control groups are essential and experiments should – whenever possible – span all four seasons to minimize these effects [18].

Sheep are predominantly polyestrous/seasonal short day breeders and therefore sensitivity of bone metabolism on estrogen deficiency varies with the season [45]. In addition, cycle characteristics vary significantly between human and sheep (e.g. cycle length (days): woman 28 vs. ewe 17; approximate estrogen peak (pg/ml): woman 300–600 vs. ewe 8–10) [9,46]. The less pronounced influence of estrogen on bone turnover in sheep might explain the minor effects on bone mass or structure after ovariectomy in these animals (see below).

A major disadvantage of herbivores/ruminants as animal models is the different gastrointestinal system. These animals are therefore obviously not suitable for studying effects of orally administered drugs [9,18].

Ewe models for osteoporosis

There are several sheep studies published focusing for example on fracture healing [47,48], orthopedic and dental implants [49,50], bone substitutes [51–55], as well as anti-osteoporotic drugs [56,57]. Whereas in the past predominantly aged, ovariectomized ewes or glucocorticoid sheep have been used as models, in the last decade sheep models for central bone regulation were additionally introduced [58–60].

Models for postmenopausal osteoporosis – ovariectomy

The ovariectomy (OVX) in sheep is a simple and safe surgical approach. Although BMD reduction is reported three and six months after OVX [61,62], long-term studies questioned the sustainability of the described bone loss. Several groups demonstrated that the BMD levels and bone turnover markers stabilized and returned to pre-OVX levels around six months after OVX [59,62–64]. The observed rebound effect is explained by histomorphometric analyses showing that the increase in bone resorption is compensated by a simultaneous increase in bone formation [59]. However, other groups reported significant changes of bone mass and micro-structural parameters, as well as biomechanical properties 12 and 24 months after OVX [65–69]. Whereas other studies failed to show significant changes 12 months after OVX [70]. Nevertheless, the relevance of hormonal influences on bone metabolism in sheep is supported by data from studies showing that estrogen and selective estrogen receptor modulators (SERM’s) are able to significantly increase bone mass in these animals [57,71].

Taken together, the influence of estrogen on bone metabolism in sheep seems to be comparable to those found in humans. However, significant changes in bone mass and/or structural parameters are due to distinct compensation mechanisms difficult to predict.

Models for steroid-induced osteoporosis

Glucocorticoid therapy in sheep leads to significant-to-dramatic bone loss, structural deterioration and biomechanical
Impairment comparable to the conditions found in steroid-treated humans [43,72]. Advantages of this approach are the ease and reliability of induction of pronounced cortical and trabecular bone loss [73,74]. Therefore, this model is the most widely used sheep model for systemic bone loss/osteoporosis. However, major disadvantages of this treatment regime are the need for continued glucocorticoid injections to achieve the bone loss and the compromised animal welfare with documented severe side effects, such as massive infections and hair-loss [43,73–75]. These side effects can be reduced by decreasing the number of glucocorticoid administrations (using equal total amounts) without reducing the impact on bone metabolism [76]. Nevertheless, ethical implications limit the value of this model as systemic side effects preclude studying skeletal physiology in bone loss situations. Furthermore, ethical implications in terms of severe side effects “cannot be overlooked” [77] in bone loss models based on polypragmatic treatment regimes, such as combination of OVX + glucocorticoid therapy + diet restriction + movement restriction [75,78], which is why these models are nowadays obsolete [38].

Models for centrally induced bone loss

Experiments in rodents gave us insights in central bone regulation and helped to identify Leptin as a potential candidate for regulation of this superordinate controlling system of bone metabolism [79–83]. With the intracerebroventricular (ICV) application of recombinant Leptin in ewe leading to significant decline in bone formation and bone mass, it could be demonstrated that this system is also important for bone regulation in large remodeling animals [59,84]. However, this model is not appropriate as a regular model for studying osteoporosis, due to the very high running costs (recombinant Leptin) and the very complex neurosurgical procedure and setting necessary for implementation [38].

Subsequently, our group implemented a ewe model for centrally induced bone loss by surgical disconnection of the hypothalamo-pituitary axis (HPD) [60]. This neurosurgical approach is sufficient to implement a profound bone loss that affects cortical as well as trabecular bone. Thereby the bone loss developed continuously over time and was sustained without any further treatment (Fig. 4), which is important in reducing running costs, as well as improving animal welfare. Histomorphometric analyses could identify a pronounced low turnover situation with simultaneously depressed osteoblast and osteoclast function as reason for the observed bone loss. However, surgical disconnection of the pituitary gland from the hypothalamus leads obviously to several systemic alterations as a result of blood level changes of different hormones (LH, FSH, T3, T4, IGF-1, cortisol, and leptin) [60]. These systemic changes need to be addressed when interpreting results generated in this model [38,85].

Another ewe model for central bone regulation described by Egermann et al. is based on melatonin deficiency caused by surgical pinealectomy [58]. Melatonin is not only secreted centrally by the pineal gland but also in bone marrow cells [86] and has a significant influence on osteoblast proliferation, differentiation and activity [87–89] as well as on bone mass and structure [90]. Significant reduction of bone mass is described in this model after 6 and 30 months post pinealectomy in comparison to control. Although the reported bone loss was limited, the decrease reached the level of significance and no other treatment was necessary beside the pinealectomy [58].

Conclusion

Osteoporosis is a chronic systemic bone disease of growing relevance due to the on-going demographic change. Since the underlying regulatory mechanisms of this critical illness are still not fully understood and treatment options are not satisfactorily resolved, there is still a great need for osteoporosis research. For this research animal models are still essential and also recommended from the American Food and Drug Administration (FDA) as well as the World Health Organization (WHO).

But can we induce osteoporosis in animals comparable to the human situation? Looking at osteoporosis as a disease causing a systemic bone loss – the answer is ‘Yes!’ However, this is very simplistic and not helpful in studying underlying regulatory mechanism of the disease itself. Furthermore osteoporosis is not one single disease but a family of disorders negatively affecting the human bone turnover and structure. That gives reason why there can never be just one or the perfect model! Every model struggles with specific pro and cons and can by its nature only be able to mimic certain aspects of the human disease. But this means vice versa, that even animal models representing only some aspects of the respective human condition/disease may be useful [91].

Ovariectomized rodents (mouse and rat) are up-to-date standard animal models for postmenopausal osteoporosis.
Furthermore, the possibility to specifically modify the genetic background of these animals gives the great opportunity to identify the role of specific gene products in bone metabolism and/or bone disease. However, some aspects can only be addressed in large animal models; such as metaphyseal fracture healing and advancement of orthopedic implants.

Among other large animal models sheep in particular have been proven invaluable for osteoporosis research. Beside the similarities in bone structure, metabolism and hormonal regulation, sheep have simple husbandry needs, a compliant nature, are available in large numbers, costs for acquisition and maintenance are in general low, and societal and ethical implications are low compared to other large animal models. However, study results always have to be interpreted against the background of strain, age, season, diet, skeletal site and hormone-cycle characteristics — therefore, appropriate control groups are crucial.

The ovariectomized ewe is an established model for post-menopausal osteoporosis due to the well-documented hormonal influence on bone metabolism in sheep. However, the unreliable rebound effect after OVX and the only minor impact on bone mass questioning this model suitable as a standard model for human osteoporosis. In contrast, Glucocorticoid treatment has a major impact on bone turnover in sheep and leads to conditions comparable to those found in steroid-treated humans. However, adverse side effects cause unacceptable discomfort and illness of the experimental animals and questioning this model — without substantial improvements of the animal welfare — as ethically acceptable. Last but not least, animal models of centrally induced bone loss are doubtless very complex systems. However, these models might be useful for studying central regulatory mechanisms of bone metabolism as well as testing new implants and/or bone substitutes and/or anti-osteoporotic drugs. The HPD model for example might be attractive for studying effects of anti-osteoporotic drugs in a pronounced low-turnover situation comparable to the situation found in patients suffering from senile osteoporosis [38].

In conclusion, we are able to influence the bone metabolism in animals causing a more or less pronounced systemic bone loss and structural deterioration comparable to the situation found in patients suffering from osteoporosis. However, there is not THE model for osteoporosis nor a perfect model, but a variety of models appropriate for answering specific questions. However, the appropriateness of an animal model for osteoporosis is not only defined in regard to the similarity to human physiology and the disease itself, but also in regard to acquisition, housing requirements, handling, costs, as well as ethical concerns and animal welfare. This implies that for specific questions many different aspects have to be taken into account — not only the impact on bone mass and structure caused by a therapeutic intervention.

Conflict of interest
The authors have no conflict of interest.

References


Fracture repair: general aspects and influence of osteoporosis and anti-osteoporosis treatment

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ABSTRACT

Bone differs from other tissues in its capacity to self-repair after a fracture. The low bone mass and structural deterioration of bone associated with osteoporosis increases the risk of fragility fracture compared with healthy individuals. The intention of this article is to review the complex process of fracture repair and essential requirements for a successful fracture healing response summarized as the “diamond concept” in terms of aging and osteoporosis. The current preclinical and clinical evidence for a beneficial or harmful influence of anti-osteoporosis medications such as bisphosphonates, parathyroid hormone (PTH), strontium ranelate and antibodies of Wnt-inhibiting signaling proteins on bone healing is presented and discussed. Literature suggests that there are no detrimental consequences of such therapeutics on fracture repair processes. Following a fragility fracture, it seems that early start of preventive anti-osteoporotic treatment right after surgery does not delay the union of the fracture, except perhaps in the case of very rigidly fixed fracture requiring direct bone healing. There is some promising experimental and clinical evidence for possible enhancement of the bone repair process via administration of systemic agents. Further well designed studies in humans are necessary to accumulate more evidence on the positive effects and to translate this knowledge into valid therapeutic applications.

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method of fixation chosen by the surgeon determines the type of bony union [5]. Bone healing occurs either by primary or secondary healing [6].

**Primary fracture healing or direct bony union**

Primary fracture healing or direct bony union occurs when there is no motion at the fracture site usually achieved after a surgical procedure: open anatomical reduction with a very rigid internal fixation [7]. A direct contact of compact bone is required and the fracture gap should be less than 200 μm so that cutting cones are formed at the end of the osteons closest the fracture site. This “contact healing” involves osteoclasts, which cross the fracture line and create small cavities. These cavities are filled by new bone generated by osteoblasts from the surrounding mesenchymal cells. Bony union and haversian remodeling occur simultaneously. This is a slow process, quite similar to intramembranous ossification during fetal skeletogenesis and to normal bone remodeling. The fracture heals directly without formation of a periosteal callus (Fig. 1). In the same mechanical and anatomical conditions, the process differs when the gap is wider but still less than 1 mm in any case. In this “gap healing” process, the gap is primary filled with lamellar bone remaining mechanically weak after 4 to 8 weeks and is followed by remodelling which starts as the “contact healing” cascade takes place.

**Secondary fracture healing or indirect bony union**

Secondary fracture healing or indirect bony union is the most common process through which bony union occurs after a fracture. If the anatomical reduction and the mechanical stability of the fracture are fundamental prerequisite to get union, the rigidity of the fixation can be less rigid as described above. In such cases, with some elasticity remaining at the fracture site, the biological response under loading is the formation of an external callus bridging the fracture gap. The fracture is considered healed when bone continuity is visible on x-rays. Indirect bone healing is characteristic in non-operative fracture treatment and in elastic fixation preserving some micro-motion at the fracture level such as intramedullary nailing, external fixation or plate fixation in complex and comminuted fractures. The process recapitulates the steps of the endochondral ossification during the fetal period [8].

The histological morphology of bone after fracture was first described in 1930 by Ham. Later, McKibbin has emphasized the cellular mechanism [9]. The better understanding of bone biology over the last decades has increased the knowledge of the molecular control of the cellular events [10].

The healing process involves a combination of intramembranous ossification and endochondral ossification similar to bone formation during osteogenesis.

The fracture repair follows a characteristic course which can be divided into three partially overlapping phases: inflammatory, repair and remodelling [11]. The first two phases last 10 to 18 weeks and correspond to the restoration of the bone continuity and the mechanical properties to allow a full weight bearing. The last phase takes months to years and can be considered a gradual adaptation of the restored bone to the usual strains of the life.

**Hematoma and inflammatory phase**

Hematoma and inflammatory phase are the immediate reactions to the fracture: bleeding occurs from the bone and the surrounding soft tissues; the microvascular disruption leads to hypoxia and bone necrosis. The hematoma coagulates around bone extremities and within the medulla forming a template for callus formation. The fracture hematoma houses blood derived inflammatory cells which release cytokines and initiate the inflammatory response: increased blood flow, increased vessel permeability, increased cell migration [12]. Osteoclasts are activated to resorb bone debris and vascular proliferation provides stem cells which differentiate into cells with osteogenic potential based upon mechanical environment and signalling molecules. This inflammatory response peaks within 24 hours and is complete after 7 days. A tissue called callus forms at the fracture site and stiffens as it calcifies.

**Repair phase**

Its nature is dependent on mechanical and anatomical conditions in the fracture healing zone (primary or secondary healing). In the secondary healing process, the fracture repair has been classically divided into the formation of soft callus which subsequently calcifies to form the hard callus. During the soft callus formation (3–4 weeks) the clot is invaded by a fibrin-rich granulation tissue. Within this tissue an endochondral formation develops between the bone extremities and external to the periosteum. This chondroid cartilaginous matrix rich in proteoglycans and type 2 collagen is replaced by an osteoid matrix rich in type 1 collagen. The ossified cartilage is replaced progressively by a woven bone. The soft callus enveloping the bone extremities becomes more solid and mechanically rigid. The hard callus formation (3–4 months) is characterized by an intramembranous ossification occurring in the subperiosteal area adjacent to the distal and proximal ends of the fracture forming the peripheral hard callus (Fig. 2). The inner layer of the periosteum contains osteoblasts which synthesize a matrix rich in type 1 collagen and directly generates calcified tissue [13]. This final central bridging by woven bone provides the fracture with a semi-rigid structure allowing weight bearing and restoring function of the limb. At this stage the woven bone is identical to the secondary spongiosa of the growth plate and the fracture is considered healed.

**Remodeling phase**

Once the fracture has been bridged by the callus, the process of fracture repair slowly replacing the new woven bone with lamellar bone continues. The remodelling results in a balanced resorption of the hard callus by osteoclasts and lamellar bone deposition by the osteoblasts. This last phase is initiated as early as the first month and it takes years to achieve the reconstruction of the original bone structure.
of the trabeculae and a decrease in connectivity [21]. Clinical callus were also disrupted, with decreased strength, and a reduction around 20% in BMD. Mechanical properties delay in ossification, a decrease of 20% to 40% in callus area, Despite some contradictory results, more studies support a rodent animal model with a tibia or femur osteotomy [20].

Despite significant effect in several clinical studies, there is so far no high level of evidence that osteoporosis per se increases the incidence of fracture non-union [2,23]. Cohorts of patients are heterogeneous, randomized studies comparing osteoporotic patients versus non osteoporotic are missing.

Osteoporosis is closely linked with aging. Fracture healing in elderly is compromised by the decline of capacity of bone formation [17]. The loss of osteoblasts in the aging skeleton has been attributed to a decrease in the number of mesenchymal stem cells (MSCs) and their ability to differentiate in progenitors towards the osteoblastic lineage [24]. Due to the augmentation of the life expectancy the absolute number of fragility fracture and its corollary, the absolute number of delayed or non-union increase and the consequences are an augmentation of the mortality and morbidity in this population. The main determinants for deficient fracture healing can be divided in biological and surgical factors [22] (Fig. 3).

The treatment of fragility fracture in elderly remains challenging for the orthopaedic surgeon. The poor quality of bone and the frequent fracture comminution make fixation of osteoporotic fracture difficult despite the development of new fixation devices like locked plating or locked intra medullary nailing, both having revolutionized the fracture fixation in weak bone [25]. Augmentation with cement or bone substitutes may fill the bone void or enhance the strength of the fixation. As in hip fracture, where the indications of joint replacement have been well described for a long time, some complex epiphyseal fractures (shoulder, elbow, knee), may benefit from primary prosthetic replacement. This option of replacement instead of fixation in comminuted articular fracture of the shoulder, the knee or the elbow has faster and better functional results in very elderly people compared with a mechanically poor fracture fixation [26].

Influence of anti-osteoporosis medications

The anti-osteoporosis drugs have been shown either to reduce bone resorption or stimulate bone formation in order to prevent fractures and to increase bone strength. The different classes of drugs, anti-resorptive bone forming or dual-effect agents have been investigated in preclinical and clinical studies to evaluate how they could influence the early stages of fracture healing. So far there was no evidence that any anti-osteoporosis treatment has negative effect on initial union of fractures in animal model [27–29]. However, the investigations were conducted in a setting of an indirect healing process. Recently in a rodent model of rigid compression plate fixation of a tibial osteotomy, an inhibitory effect of bisphosphonates (BiPhs) has been shown on primary healing [30].

The clinical evidence of the current and new osteoporosis treatment on bone healing are reviewed below.

Anti-resorptive agents

Bisphosphonates are the most widely used medications to treat osteoporosis. Various studies have demonstrated no increased risk of non-union or of deleterious effect on fracture healing compared with a control group, independent of the post fracture timing of administration of zoledronic acid or risedronate in inter-trochanteric hip fracture [31] or risedronate in distal radius fractures. The same results were observed with denosumab, a receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor, in the post-hoc analysis of a phase 2 clinical trial [32].

Bone forming agents

The impact of parathyroid hormone (PTH) peptides on bone repair has strong evidence in preclinical studies and there is a
A clinical trial of distal radius fracture has not demonstrated any significant radiological or clinical effect of the ronacaleret, a CaSr antagonist on fracture healing [42].

**Conclusion**

The number of osteoporotic fracture is increasing, especially among the elderly population. Fracture treatment in elderly osteoporotic patients remains challenging. Fracture healing is often compromised both by a high rate of fixation failure due to weak bone and the biological consequences of aging and comorbidities on the bone repair process. So far impaired healing is treated by mechanical improvement in bone fixation and local biological stimulation by autogenous bone graft or more recently “ostebiologics”. With the growing number of anti-osteooporotic drugs to prevent fracture and increase bone quality, it was a priority to investigate their impact on the fracture healing process.

The evidence for the effects of anti-osteoporotic drugs on fracture healing is rather positive. The concerns for potential detrimental consequences of such therapeutics on fracture repair process seem to be overwhelmed by preclinical and clinical data. Following a fragility fracture there is no reason to delay a preventive anti-osteoporotic treatment till the union of the fracture, except perhaps in the case of very rigidly fixed fracture requiring direct bone union. There is some promising experimental and clinical evidence for possible enhancement of the bone repair process via a systemic agent. Further well designed studies in humans are necessary to accumulate more evidence on the positive effects and to translate this knowledge into valid therapeutic applications.

**Conflict of Interest**

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**References**


Main differences in osteoporotic fracture models: which should I use?

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ABSTRACT
Osteoporosis is a global public health problem currently affecting more than 200 million people worldwide. Major research efforts are being made to improve the outcomes for patients with osteoporosis. However, the treatment of fractures associated with osteoporosis remains unsatisfactory. Animal models continue to be an important tool for establishing strategies to treat osteoporotic fractures, and various methods of inducing osteoporosis have been used. Investigators must select a model that best reflects the clinical problem being studied, and the underlying pathophysiology of the osteoporosis in the target patient group. In particular a model for Type I post-menopausal osteoporosis should mimic a fall in oestrogen and rise in osteoclast activity observed with this condition, whereas a model for type II ‘senile’ osteoporosis should mimic the fall in osteoblast activity. Unfortunately, there is no single all-encompassing model that precisely imitates the underlying osteoporosis or the fracture patterns seen in humans. As such the choice of species and model must be individualised to the scientific question being addressed. This article summarises general considerations when choosing an osteoporotic fracture model and outlines existing models of osteoporosis. The most appropriate model in a range of osteoporotic fracture research scenarios are subsequently considered.

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Introduction

Current demographic trends, with an increased number of individuals surviving past 65 years, have resulted in an increased number of osteoporotic fractures. Worldwide the number of individuals at high risk of osteoporotic fracture is expected to double by 2040 [1]. Osteoporosis is characterized by loss of bone mass and deterioration of bony microarchitecture, resulting in increased bone fragility and susceptibility to fracture. While a drop in oestrogen following the menopause, increasing age, prolonged immobilization, chronic disease and use of certain medications can ultimately result in osteoporosis, the pathophysiology underlying the imbalance between the resorption and formation of bone in each case is still not fully understood. The treatment of fractures associated with osteoporosis remains unsatisfactory. Conventional implants have a higher complication rate in osteoporotic bone, with fixation failure occurring as a consequence of the weak bone structure [2,3]. Major research efforts have been made to improve the outcomes for patients with osteoporosis. Research has largely focused on gaining a better understanding of bone behaviour in osteoporosis, examining the response of osteoporotic bone to potential therapies, examining osteoporotic fracture repair, examining the role of medical therapies in osteoporotic fracture repair, and understanding the mechanical behaviour of osteoporotic bone. Various animal models are widely used to investigate the pathogenesis of osteoporosis and for the clinical testing of anti-resorptive drugs. However, animal models designed to investigate mechanical properties of osteoporotic bone and the surgical fixation of these fractures must fulfill distinct requirements. As such the choice of osteoporotic fracture model should consider a model best suited to the research question under investigation (e.g. biology, mechanics or fracture fixation). In addition, the animal model should reflect the underlying pathophysiology of osteoporosis in the target patient group. There is currently no all-encompassing model and the choice of species and model used must be individualised to the scientific question being addressed. This review we highlight general considerations when selecting an animal model of osteoporotic fractures and consider how these may be most appropriately used in scenarios of osteoporotic fracture research.

General considerations when choosing an osteoporotic model

There are a number of factors that need to be taken into consideration when choosing an osteoporotic fracture model [4].

Pathophysiology underlying different types of osteoporosis

It is imperative to appreciate the differences in pathophysiology underlying the different causes of osteoporosis – an experimental model that best reflects the mechanisms of...
Reduced bone mass in the target human population should be used.

Bone remodelling is a physiological process in which old or damaged bone is removed by osteoclasts (i.e. bone resorbing cells), then replaced by new bone formed by osteoblasts (i.e. bone forming cells). In a healthy individual the rate of bone resorption is closely linked to the rate of bone formation. Systemic hormones and local paracrine factors as well as gravity, physical activity, and weight bearing regulate the recruitment, replication, and function of the osteoblasts and the osteoclasts.

Osteoporosis is a disorder of bone remodelling characterized by low bone mass and structural deterioration resulting in bone fragility and an increased vulnerability to fractures. Osteoporosis represents a group of distinct pathological conditions, rather than a single entity, and is traditionally classified into primary and secondary types [6] (Fig. 1). Primary osteoporosis is historically further divided into two subtypes: type I osteoporosis and type II osteoporosis [7]. Type I osteoporosis (also referred to as postmenopausal osteoporosis) is common in postmenopausal women and is caused primarily by the resulting oestrogen deficiency, whereas type II osteoporosis (also known as age-related osteoporosis or senile osteoporosis) is associated primarily with aging. In contrast, secondary osteoporosis refers to bone disorders that are secondary complications of various other medical conditions, consequences of changes in physical activity, or adverse results of therapeutic interventions for certain disorders [6]. While this classification has traditionally been popular, the genesis of osteoporosis is much more complex and as yet not fully understood. As such, it is likely that there are numerous genetic variations and mutations, and discrete conditions within conventionally termed primary or idiopathic osteoporosis that are still to be recognised.

While the underlying regulatory mechanisms are not fully understood, type I primary osteoporosis is thought to be caused primarily by the decline in oestrogen levels associated with menopause [8]. Both bone resorption and bone formation are increased in postmenopausal osteoporosis; however, the extent of increased bone resorption exceeds that of augmented bone formation, which causes an imbalance between bone resorption and bone formation in favour of bone resorption [9–11]; i.e. there is a marked increase in osteoclast activity.

Type II primary osteoporosis, is typically seen in men and women after the age of 60 to 70 [12,13]. In this form of osteoporosis, a progressive decline in the supply of osteoblasts and a decrease in their activity occurs without an increase in osteoclast activity [14,15]. The decreased bone formation caused by changes in reactive oxygen species (ROS), insulin-like growth factor 1 (IGF-1), and parathyroid hormone (PTH) levels associated with aging plays a predominant role in the pathogenesis of age-related osteoporosis [16,17]. Thus, the pathogenesis of osteoporosis in elderly women may consist of two components: both mechanisms of primary osteoporosis – i.e. both type 1 and type 2.

Secondary osteoporosis is the bone loss secondary to chronic diseases such as Cushing’s Syndrome and patients on long-term steroids. Glucocorticoids are potent immunomodulatory drugs that are commonly used to treat a variety of inflammatory conditions and autoimmune disorders. Bone loss occurs within several months of initiating glucocorticoid treatment, and prolonged therapy leads to a significant decrease in bone mass and an increased risk of fracture [18]. Although physiological levels of glucocorticoids are required for normal osteoblast differentiation, excess glucocorticoids exert an inhibitory effect on osteoblast differentiation.

One of the major functions of bone remodelling is to adapt bone material and structural properties to the mechanical demands that are placed on the skeleton, including mechanical loading and weight bearing. Prolonged bed rest or immobilization (such as casting of a limb) reflects a decrease in mechanical requirements, which results in altered bone remodelling and is termed immobilization-induced (or disuse) osteoporosis.

**Relevance to human physiology**

As the ultimate goal is to use an animal model that best replicates the bone and endocrine changes occurring in humans, an important consideration is the “physiological proximity” and biology of bone metabolism, which can vary from species to species. From a physiological point of view, non-human primates and larger animals such as sheep offer considerable advantages over small mammals. Their longer age spans, extended adult skeletal phases, and hormonal patterns that can mimic oestrogen and progesterone cycling exhibited by human females make them excellent models for studying the human skeleton [19,20]. Such larger models also allow for the effects of treatments to be followed in a longitudinal fashion, as it is more feasible in larger animals to obtain multiple bone biopsies for histomorphometric evaluation [21]. There has been a long standing debate as to the appropriateness of rodent models, mainly given their lack of true skeletal maturity (lack of epiphyseal growth plate closure, lack of Haversian (osteonal) remodelling and a lack of a true menopause despite the fact that irregular oestrogen cycles are observed.
with age [22]). Despite this, recent studies have overwhelmingly established the relevance of both rat and mouse models for studying postmenopausal as well as age-related, drug induced and pathology-related bone loss.

Biomechanical considerations

The selection of an animal model for investigation of fracture fixation in osteoporotic bone has so far been based on the comparable size of long bones in animals and humans. In this regard sheep have been preferred to smaller animal models such as rats or mice. Despite the development of advanced implants and sophisticated testing devices specifically designed for use in small animals, significant challenges in their use remain. The surgical procedures are technically challenging and minor deviations in the testing setup may significantly affect the test results [23].

Species availability, cost and time frame considerations

While relevance to human physiology and mechanics should be used to select an appropriate animal model, the “best” model may not be feasible or practical. For example, nonprimate species pose significant challenges in terms of cost and space availability. While the FDA require that large mammalian models are used prior to the initiation of clinical trials for drug candidates, the cost involved, potential ethical concerns and the time required to conduct large animal studies lead to most pre-clinical evaluation and mechanistic studies being conducted in rodent models. The expense for purchase, breeding, and holding of rodents is relatively low when compared to larger animals while the time course to fracture healing is reduced [24]. The implementation of larger groups in the experimental study design is therefore more feasible. However, long-term studies that require several biopsies, or large blood samples, are rarely possible. Mice are highly adaptable to experimental manipulation and a broad spectrum of antibodies and tools for molecular characterization are readily available.

Ethical and societal implication

The general public is accustomed to the role of rodents in research, and their use is associated with fewer ethical and societal implications than larger animals.

Potential contribution of transgenic mice

The accessibility of mice to genetic manipulation has made them the most commonly used laboratory animal and their use is now often favoured over rats in many applications for musculoskeletal research. The sequencing and analysis of the mouse genome has enabled many genes to be targeted and studied using this technology [25,26]. Transgenic technology can be used to silence existing genes (knock-out and knock-down models), introduce new genes or increase gene expression to levels above normal (knock-in models), and to modify gene expression in specific tissues and/or at specific times (conditional transgenic models) [27]. In addition to modifying native gene expression, transgenic technology can be used to insert fluorescent proteins into specific target cells. In particular, fluorescent reporters of Cre recombinase activity are important for defining the spatial and temporal extent of Cre-mediated recombination. As such, the descendants of stem and progenitor cells can be traced by crossing a Cre mouse with a reporter mouse strain permanently expressing a reporter gene after Cre-mediated recombination. These systems could potentially be used to dissect molecular mechanisms of both the development of osteoporosis and the healing of fractures occurring in osteoporotic bone that would not be possible using wild type or larger animals.

Genetic uniformity

The genetic uniformity seen with inbred strains of mice and rats reduces individual differences so fewer animals can be used (or the power of experiments increased) compared to the number that would be needed if the experiments were conducted with an outbred strain.

Animal models of osteoporosis

Various methods of inducing osteoporosis have been used and described. Most commonly these involve surgical procedures such as ovariectomy or other endocrine surgeries such as orchidectomy, hypovasectomy, parahydroectomy. In other studies, immobilisation, modifications of the diet and drugs such as steroids have been used. Aged animals have been used to mimic aspects of senile osteoporosis and various genetic modifications have been used including those to mimic aging. The methods of inducing osteoporosis in animals and the type of osteoporosis that they best reflect are listed in Table 1.

Ovariectomy

Ovariectomy is a relatively safe and simple method of inducing osteoporosis in animals. The ovariectomised rat is one of the most commonly used osteoporosis models although their bone physiology must be carefully considered in planning experiments. The transition from modelling to remodelling occurs in cancellous bone in the lumbar vertebrae from the age of 3 months, but in the proximal tibial metaphysis this does not occur until 6–9 months. Remodelling first appears in cortical bone approximately 3 months later than in the adjacent cancellous bone. Studies using ovariectomised rats indicate that osteoporosis influences the late period of fracture healing in rats. The co-existence of oestrogen deficiency and low calcium does not have a marked influence on early healing, whereas there is a profound negative effect in the late period [28]. Rats respond well to ovariectomy [29]. Very little reduction in bone mass occurs following ovariectomy in cats and dogs, as illustrated by the rarity of spontaneous fractures despite huge numbers of ovariectomised pets. The guinea pig is a poor model for post-menopausal osteoporosis as there is no response to ovariectomy or to steroids. The ovariectomised sheep and mini-pig have also been used as large animal models of type 1 osteoporosis. Although oestrogen deficiency doubtless influences bone metabolism in sheep, the variable effect of ovariectomy on bone mass and structure in the ewe must be considered when using this as a model of type 1 osteoporosis.

Aged animals

Aged animals have been used to model primary type 2 osteoporosis. Rats aged over 1 year and sheep aged over 9 years exhibit senile osteoporosis [30]. However, the costs associated with the housing of animals for such extended periods are significant and the variability of the ageing process between individual animals can be marked. Age related bone loss has been well documented in mice. Laboratory mice usually live for 2–3 years with bone mass peaking at 4–8 months of age, followed by an age-related decline. The popular laboratory mouse strain, C57BL/L and BALB/c, develops a senile osteoporosis-
like bone phenotype with decreased bone mass and quality [31–34]. Meyer et al., observed that young irradiated rats that are then transplanted with bone marrow from aged individuals demonstrated delayed healing [35]. This may provide a potential future model for primary osteoporosis type 2. Conversely, spontaneous vertebral fractures have been observed in older mini-pigs. However, as mentioned previously, the use of aged animals is particularly problematic in large animals due to costs relating to maintenance and housing of animals. The menopause generally does not occur in animals with the exception of non-human primates.

**Accelerated ageing strains**

Senescence accelerated mouse (SAM) lines exhibit an accelerated ageing phenotype with shortened lifespan with a marked ageing phenotype apparent by 6–8 months [36]. The Senescence accelerated mouse prone-6 (SAMP6) mouse model has many features that reflect senile osteoporosis in humans, including a low adult bone mineral density, osteoblast insufficiency, and spontaneous fractures in later life [37–39]. The predominant phenotype in these mice is of impaired bone formation due to compromised osteogenesis in the bone marrow [18,19] that may be related to suppression of Wnt signalling pathways [21]. Although a number of phenotypic characteristics are thought to be consistent between ageing mice and humans, they have complicated genetic backgrounds and their value as a model of human-osteoporosis remains controversial [40]. Because SAMP6 mice already have decreased bone strength at maturity, and because a part of the bone fragility originates during growth, the term “senile osteoporosis” must be used with caution. Mice, including SAMP6 strains, have been used in a number of studies to evaluate outcomes following fracture [41–45].

**Surgical disconnection of the hypothalamus and pituitary**

Oheim et al. performed skeletal characterization in sheep following hypothalamic-pituitary disconnection (HPD). They found that this model resulted in a low turnover form of administration. Their data strongly support the hypothesis that this model resulted in a low turnover form of administration. Conversely, spontaneous vertebral fractures have been observed in older mini-pigs. However, as mentioned previously, the use of aged animals is particularly problematic in large animals due to costs relating to maintenance and housing of animals. The menopause generally does not occur in animals with the exception of non-human primates. The vast majority of current fracture models are created in the shaft of long bones, because it is easier to create a reproducible fracture in the midshaft and to stabilise an osteotomy at this.

**Secondary osteoporosis models**

Wherever possible, the agent causing the disease in man should be mimicked in animal models of secondary osteoporosis. For instance, the most common form of secondary osteoporosis can be mimicked in animals with prolonged steroid administration.

### Scenarios of osteoporotic fracture research: which animal model should I use?

There are a number of scenarios for which animal models of osteoporotic fractures can be insightful, reflecting different aspects of the clinical problem. Osteoporotic models are chosen to investigate different aspects of the clinical problem.

**Scenario 1: Intact osteoporotic skeleton**

**Scenario 1a: Examining bone pathophysiology in osteoporosis**

A number of studies are aimed at establishing the underlying mechanisms that result in the loss of bone mass, such as the cells involved in the different types of osteoporosis and the local and humoral factors that control them. Several of the small animals models are useful for testing hypotheses in this scenario. In particular the ovariectomised rat and the aged rat are useful for type 1 primary osteoporosis and type 2 primary osteoporosis respectively as there is a wide range of well-validated and reliable tools including antibodies available for the rat. Similarly, assays and tools are readily available for mice and certain transgenic mice have added advantages, such as those that identify the origin of cells within the bone. For certain research questions concerning type 2 primary osteoporosis the SAMP6 mouse is particularly useful.

Many of the studies in this scenario ultimately seek to establish therapeutic strategies for preventing osteoporotic fractures, such as novel compounds for preventing, treating and even reversing osteoporosis. These are considered in scenario 1b.

**Scenario 1b: Examining the response of osteoporotic bone to therapies**

Currently the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) require that new anti-osteoporotic drugs are tested in two species and recommend the adult ovariectomised rat and a large animal which should have cortical bone remodelling such as non-human primates, sheep, or pigs. As discussed previously, small animals, such as the mouse and rat are advantageous due to wide availability of well-validated and reliable tools including antibodies and transgenic mice. The preferred large animal model varies geographically; in the USA there is societal acceptance of dog models, whereas in Europe, sheep and pigs are more widely accepted; and the species may be selected on the expertise of the animal research unit. For type 1 Primary osteoporosis, the animal would undergo ovariectomy and for type 2 primary osteoporosis, sheep undergoing hypothalamic-pituitary disconnection (HPD) would be used.

**Scenario 2: Fracture repair in osteoporotic skeleton**

**Scenario 2a: Examining osteoporotic fracture repair**

The vast majority of current fracture models are created in the shaft of long bones, because it is easier to create a reproducible fracture in the midshaft and to stabilise an osteotomy at this.
site. However the majority of osteoprotic fractures occur in the metaphysis [47,48]. With this in mind, Murray [49] used an intramedullary pin inserted percutaneously to stabilise a closed metaphyseal fracture and Stuermer et al. developed an osteotomy in the rat tibia proximal metaphysis and stabilised this with a small "T" plate [50]. Subsequently Thormann et al. [47] and Alt et al. [51] described healing in the metaphysis of ovariectomised rats stabilized with T plates. It is important that studies investigating osteoporotic fracture repair consider the differences in the fracture repair process in metaphyseal vs diaphyseal bone as well as the difference that occur in osteoporotic bone. The different diaphyseal fracture healing scenarios have been reviewed by Mills and Simpson [52] and small and large animal models suggested for each of these. To investigate osteoporotic repair in these scenarios the small and large animal species would be selected as outlined in scenario 1.

Scenario 2b: Examining osteoporotic fracture repair with therapies

As in scenario 1b, there is a requirement that novel agents are evaluated in a small and large animal model. It is also important to consider both primary and secondary types of fracture repair as many of the osteoporosis drugs act against the osteoclast and these agents will thus have profoundly different effects on primary and secondary fracture repair mechanisms. Taking this into account, the model is then chosen as outlined in scenario 2a.

Scenario 3: Mechanical features of the osteoporotic skeleton

In the majority of animal models the ratio of the bones (the ratio of the length and the width) is less than it is in humans. Currently, calculations for bending strength use Euler buckling theory. However this relies on the assumption that the length is far greater than the width. Ideally this should be greater than 50, but in practice the length should be at least 20 times the width. In species used in orthopaedic research such as sheep, rats and mice the ratio is commonly below 10. This problem is further exacerbated in knockout animals and needs to be taken into account when calculating mechanical parameters in fracture models.

Small animals are inappropriate to serve as models for human implant testing, because the skeletal size, anatomy and morphology is not comparable with humans [53]. Larger animals would be more appropriate for this purpose. The small size of rodents makes surgery technically challenging. Therefore, large long bones are most suitable for studies on bone repair, fracture stabilization or biomechanical testing so far not applicable in these small, irregularly shaped bones. In most rodent models, midshaft fractures are used to analyze bone repair. Diaphyseal fracture models, which are most established in mice, are appropriate for studies on cortical bone healing. However, only metaphyseal fracture models are suitable to investigate the impact of osteoporosis on cancellous bone healing [53].

### Algorithm for selecting an appropriate model

We have developed an algorithm for selecting the most appropriate animal model in each scenario in our practice (Table 2). For primary osteoporosis type 1 (or post-menopausal osteoporosis) the ovariectomy with or without a low calcium diet can be used in both rats and sheep to induce a representative model. For primary osteoporosis type 2 (or senile osteoporosis) the aged rat is a reasonable small animal model while sheep that have undergone a hypothalamic-pituitary disconnection is a reasonable large animal model. SAMP6 or transgenic mouse lines are helpful for investigating bone behaviour and osteoporotic fracture repair with secondary bone healing. For primary bone healing, plating in association with a small amount of periosteal and endosteal stripping can be used to investigate the role of agents such as bisphosphonates that inhibit osteoclasts. For secondary osteoporosis, we suggest that the primary cause be mimicked.

### Conclusions

Animal models continue to be an important tool for establishing strategies to treat osteoporotic fractures. There is no single all-encompassing model that precisely imitates the underlying osteoporosis or the fracture patterns seen in humans. As such the choice of species and model must be individualised to the scientific question being addressed.

### Conflict of interest

The authors declare no conflict of interest.

### References

Bone mineral density aspects in the femoral neck of hip fracture patients

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ABSTRACT

Elderly people, due to neurological conditions and muscular atrophy, present a greater propensity to falls and thus are very susceptible to hip fractures. Other variables, such as osteoporosis, may also be related to the etiopathogenesis of hip fractures, although osteoporosis is in fact a concurrent disease, and merely a coadjuvant cause. Nonetheless, osteoporosis can make fracture patterns more severe and interfere with osteosynthesis. Osteoporosis is the radiological image of osteopenia, a pathological concept meaning a smaller quantity of bone per unit of volume. The radiological expression of osteopenia is therefore that of bone tissue with a lower radiological density than normal. In the context of hip fractures, bone mineral density and bone architecture of the femoral neck together with protein expression profiles and cross-links of this anatomical area are of special interest which is reviewed in the current paper. Spatial variations in bone mineral density in the femoral neck were found in the literature with increased porosity from the periosteal to the endosteal region and also from the distal to the proximal part of the femoral neck. Furthermore, increased crystal size, increased cortical porosity, reduced osteocyte lacunar density and an increased Ca/P ratio associated with higher concentrations of Ca and P were described in hip fracture patients compared to control patients. Osteocalcin/collagen type I expression ratio and enzymatic cross-link content in high-density bone was found to be significantly lower in hip fractures compared to controls. In conclusion, further research in bone mineral density and associated parameters are of interest to deepen the understanding of osteoporotic hip fractures.

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Introduction

A hip fracture immobilizes, restricts autonomy, shortens life expectancy and results in a high cost for any health care system [1–5]. Elderly people suffer more frequently from hip fractures because they have a higher propensity to falls, as a result of neurological conditions and muscular atrophy. Other variables are also related to the etiopathogenesis of hip fractures, but in recent decades the debate in this field has mainly focused on osteoporosis, to the extent that hip fractures are included within a group of “osteoporotic fractures”, despite the fact that osteoporosis is a concurrent disease and only a coadjuvant cause.

Anyone may suffer a hip fracture, whether or not they have osteoporosis, but, apart from pathological fractures, a traumatism is usually a necessary cause for a hip fracture to occur [6]. However, osteoporosis makes fracture patterns more severe and also interferes with osteosynthesis. Therefore, effective treatment of osteoporosis is a major issue, although the issues of pathogenesis, the outcome of treatment – particularly as concerns the prevention of fractures – and how health economic budgets must be distributed for areas such as fall avoidance, drug administration, hip fracture management and aftercare are all under discussion.

Osteoporosis is the radiological image of osteopenia, which is the understanding of less bone mass resulting in less amount of bone per unit of volume, together with bone architecture deterioration. This apparently very clear explanation of the radiology-pathology correlation may become somewhat confusing when treatment aimed at mineralizing the remaining bone structure – cortex and cancellous – is applied. Mineralization results in a radiologically denser bone but one that presents the same bone mass density (amount of mineralized osteoid substance per volume) or, in other words, a structure that is equally weak, but stiffer. Bones must be stiff enough so that they do not bend when loaded, but not so stiff as to lack the necessary flexibility to absorb energy by elastic and plastic deformation, thus decreasing the energy that can provoke tissue damage and possible bone fracture. Failure may occur if bones become deformed too much or too little, and exceed their peak stress limits [7].

The biomechanical properties of bone present many variables in relation to hip fractures. Bone strength depends on a large
number of components including, but not limited to, bone microarchitecture, geometry, cortical and trabecular porosity, and tissue mineralization density [8,9]. Also relevant are the rate of bone turnover, bone size, trabecular connectivity, molecular crosslinking, tissue maturation, microdamage burden, osteocyte density, gender, race and other factors. These properties are sometimes collectively referred to as bone quality [10]. Determining the proportional contributions made by each of these aspects is a challenge yet to be addressed [7].

In order to achieve solutions to biomechanical problems, the material and structural properties of bone that determine its strength must be quantified. Many pharmacological treatments have been proposed to reduce osteoclastic bone resorption and to increase osteoblastic bone formation and thus prevent the progression of fragility, but the main problem remains bone structure and its relation with biomechanical behaviour. A stiffer, less flexible bone is not one that is stronger and less susceptible to suffer a fracture. We have learned from engineers and architects working in earthquake-prone areas, structures must be made more flexible [11,12] in order to resist shocks, e.g. with the help of “mass dampers” [13,14]. Elasticity is known to be a property of solid bodies, one that preserves the construct from breakage but without dramatically enlarging the body mass. On the other hand, the worst combination appears to be that of a very stiff structure – such as would result from overmineralization – together with decreased body mass.

**Mineralization and hip fractures**

Bone quality is a very important concept with respect to hip fractures. It comprehends both bone structure and bone composition, which, in turns, includes cells, proteins and mineralization.

**Bone density, microarchitecture and mineral composition in hip fracture patients**

In a study using a synchrotron radiation microcomputed tomography system coupled with a multiscale biomechanical model to determine the 3-D anatomical dependence of tissue mineral density and of the elastic constants from bone specimens taken from the lower part of the femoral neck of patients undergoing joint replacement, it was found that porosity increased in the radial direction, from the periosteum inwards, but did not vary markedly along the bone axis. Tissue mineral density was significantly higher in the periosteal region than in other bone locations and decreased from the periosteal to the endosteal region, this decrease being faster in the porous part of the samples than in the dense cortical bone. This decrease also took place from the distal to the proximal part of the femur neck. Mineral density variations in the radial direction induce weak changes in bone properties compared to constant tissue mineral density; similarly, tissue variations in the axial direction are responsible for significant variations in the elastic constants. According to the authors of this study, spatial variations in tissue mineral density should be taken into account to properly describe the spatial heterogeneity of elastic coefficients of bone tissue at the organ scale [15,16]. These parameters might be of great importance in the genesis of hip fractures.

Other research into the nano-structure, composition and micro-architecture of the superolateral femoral neck in elderly hip fracture patients versus healthy controls has shown that mineral crystals at external cortical bone surfaces of the fracture group are larger, and also have a higher mineral content and a more homogeneous mineralization profile. However, the hip fracture cases presented cortical porosity values that were almost 35% higher but presented a significantly lower osteocyte lacunar number density compared to controls [17]. Together with increased crystal size, the shift toward higher mineralization, increased cortical porosity and reduced osteocyte lacunar density indicate that the cortical bone of the superolateral femoral neck bears distinct signs of fragility at various levels of its structural organization [18].

Higher mineral contents and greater porosity is the worst combination for bone strength in relation to fracture risk. This is borne out by previous studies that have shown hypermineralized osteocyte lacunae, relative to the total number, to be greater in patients with osteoporosis and osteoarthritis than in femur bone obtained at autopsy [19]. Osteoporosis is characterized by increased hypermineralized osteocyte lacunar number density whereas osteoarthritis presents decreased osteocyte lacunar number density and total osteocyte lacunar number density. The calcium-phosphorus ratio does not appear to differ between hypermineralized osteocyte lacunae and bone matrix in osteoporosis and osteoarthritis groups. Although the role of hypermineralized osteocyte lacunae in bone remodelling and bone biomechanical properties requires further research, these findings are very interesting in relating hypermineralization with neck fracture susceptibility [19], and extend our understanding of the bone stiffness-flexibility relation.

An earlier study compared the degree of mineralization of bone tissue in femoral neck cortex specimens between women with hip fractures and a control group and reported that bone fragility may be related to a greater degree of mineralization of bone tissue heterogeneity in osteons and interstitial tissue [18]. Another study, of the degree of bone mineralization, using quantitative microradiography calibrated with an aluminium step wedge in the femoral neck cortex of patients with hip fractures, compared to a control group, found that the degree of bone tissue mineralization was significantly lower in the osteons than in the interstitial tissue in both groups, whereas osteons and interstitial tissue were significantly greater in the hip fracture patients than in the controls. These data further support the view that bone fragility may be related to a higher degree of tissue mineralization [20].

We studied bone density and mineral composition in hip fracture versus osteoarthritic non-fracture patients undergoing total hip replacement, but the specimens were retrieved from the base of the femoral neck, because in osteoarthritis the femoral head becomes sclerotic, and this might provoke the introduction of bias when comparing hip fracture patients with osteoporosis to osteoarthritic patients. Studies for bone density and mineralization were performed by pinpoint electron beam at 40000× magnification (Fig. 1). The peak-to background ratio (P/B) method was used to measure the concentrations of calcium and phosphorus in each group of patients. Microcrystalline salt standards were used to quantify Ca and P as described in previous publications [21]. All results were calculated as weight fraction percentage of Ca and P [22]. Differences were statistically significant for Ca, P and the Ca/P molar ratio. These results reveal that cancellous bone obtained from patients with hip osteoarthritis is stoichiometrically similar to normal bone, which is characterized by a Ca/P molar ratio corresponding to hydroxyapatite (1.67). Therefore, this bone can be considered normal from the microanalytical standpoint. However, the cancellous bone in our hip fracture patients had an increased Ca/P ratio, associated with higher Ca and P concentrations (Fig. 2). The finding that hip fracture patients have an increased Ca/P ratio associated with altered Ca and P concentrations, whereas cancellous bone obtained from osteoarthritic patients can be considered normal from the microanalytical point of view, refuted the idea of increasing calcium intake or administering...
drugs to “improve mineralization” in osteoporotic patients for hip fracture prevention (unpublished data). Some authors recommend determining calcium and phosphorus fractions in bone mineral density measurements, in order to enhance fracture risk assessment and achieve more targeted therapies [23].

In the same line, but in diabetic patients, a study was made of proximal femur specimens obtained during total hip replacement, from patients older than 65 years with and without type 2 diabetes; a scanning electron microscope was used for the quantitative backscattered electron imaging analysis of trabecular bone samples from the femoral neck. This analysis revealed an increased mean calcium concentration in bone and a lower level of mineralization heterogeneity in adults with type 2 diabetes than in patients with no diabetes. The authors interpreted this to mean that in adults with type 2 diabetes the biomechanical properties of bone are affected, increasing the risk of hip fracture [24].

Protein expression profiles, osteogenic potential and protein cross-links in hips fracture patients

Proteins are essential to bone strength, not only because they shape the microstructure, but also because they determine the mineral phase in bone tissue. Osteocalcin is the most abundant non-collagen protein in bone matrix, and is crucial for mineralization. In a study of patients undergoing joint replacement, presenting either hip fracture or osteoarthritis, the bone osteocalcin/collagen type 1 ratio was significantly lower in the hip fracture patients than in the osteoarthritis patients, adjusted for age, gender and body mass index. This ratio was associated with the hip fracture event, independently of the group assigned and of the clinical characteristics [25]. In the hip fracture patients, multivariate analysis showed that the low osteocalcin/collagen type 1 expression ratio was significantly associated with impaired trabecular strength and stiffness. However, no differences were found regarding total osteocalcin, apolipoprotein E and Vitamin K, nor between undercarboxylated osteocalcin and bone mechanics. In this study, the multivariate analysis revealed that serum total osteocalcin in osteoarthritis patients was negatively associated with strength and stiffness.

The authors concluded that a low bone osteocalcin/collagen type 1 expression ratio is an independent predictor of worse trabecular mechanical behaviour and of the hip fracture event, and that in hip fracture patients the imbalance of bone osteocalcin/collagen type 1 expression ratio reflects disturbances in osteoblast activity leading to bone fragility [25].

In another study comparing osteogenic potential and the responsiveness to leptin of mesenchymal stem cells from bone marrow, between postmenopausal women with osteoarthritis or osteoporosis, it was found that, under the same osteogenic supplements condition, the mRNA expression of osteogenesis-specific genes, both osteocalcin and alkaline phosphatase were higher in the osteoarthritis group. Comparison of bone matrix mineralization produced results comparable to those for mRNA expression. The level of bone-specific alkaline phosphatase was higher in cells from donors with osteoarthritis, whereas osteoprotegerin, the protein released by osteoblasts preventing RANK and RANKL from coming into contact, was higher in the osteoporosis group. This difference in bone-specific alkaline phosphatase expression ceased to be statistically significant after the administration of leptin. Although leptin upregulated the expression of osteoprotegerin, a significant difference was still apparent between osteoarthritis and osteoporosis. Differences were observed in osteogenic potential and responsiveness to leptin of mesenchymal stem cells, between postmenopausal women with osteoarthritis or osteoporosis; this finding seems to be related to the different distribution of bone mass among populations with osteoarthritis or osteoporosis [26].

Protein cross-links are also of fundamental importance in bone structure and mineralization in relation to hip fracture patterns. Enzymatic and glycation-induced nonenzymatic cross-links play important roles in the expression of bone strength. Altered collagen crosslinking, in both low and highly mineralized bone, impairs bone quality in osteoporotic patients. In a study on intracapsular hip fracture in patients with a mean age of 78 years, compared to 25 age-matched post-mortem controls, the authors found that in the fracture cases, enzymatic cross-link content was reduced in high density bone, while that of pentosidine was increased in both low and high density bone; higher levels of plasma homocysteine and lower levels of pyridoxal were observed, compared with the non-fracture controls. In the latter
group, there was no difference in total enzymatic cross-links between low and high density bone, but the pentosidine content was significantly higher in the high density bone. According to the authors, these results suggest that the combination of reduced mineralization, enzymatic cross-links and the excessive formation of pentosidine may play an important role in explaining poor bone quality in osteoporosis [27].

**Outlook**

New lines of research relating proteins and mineralization for a more precise explanation of the pathogenesis of osteoporosis-related fractures are currently in progress, but study designs need to be aimed at enhancing basic science as well as achieving good epidemiological analyses. In the Health Aging and Body Composition study, in which 3075 well-functioning black and white persons aged 70–79 years were enrolled, the authors studied the association of fetuin-A, a hepatic secretory protein that promotes bone mineralization in vitro, with bone mineral density. They concluded that higher fetuin-A levels are independently associated with higher bone mineral density in older women but not in older men, and concluded that future studies should be made to evaluate whether fetuin-A may refine fracture risk assessment in older women [28].

**Conclusion**

Research in bone mineral density and associated parameters are of interest to deepen the understanding of osteoporotic hip fractures. Spatial variations in bone mineral density in the femoral neck were described with increased porosity from the periosteal to the endosteal region and also from the distal to the proximal part of the femoral neck. Furthermore, increased crystal size, increased cortical porosity, reduced osteocyte lacunar density and an increased Ca/P ratio associated with higher concentrations of Ca and P were described in hip fracture patients compared to control patients. Osteocalcin/collagen type I expression ratio and enzymatic cross-link content in high density bone was found to be significantly lower in hip fractures compared to controls.

**Conflict of interest**

All authors declare they have no conflict of interest. All ethical issues have been duly complimented according to Spanish, European and international laws.

**References**

Hip fracture programs: are they effective?

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KEYWORDS

fragility fracture program
geriatric fracture center
hip fracture
systems of care
hip fracture outcomes

ABSTRACT

This manuscript will evaluate the published evidence on efficacy of organized hip fracture programs to determine if they improve patient outcomes. A detailed literature search was conducted to find manuscripts published in the past 20 years about organized hip fracture care programs. Seventeen programs with published results were identified from this detailed search and these were evaluated and synthesized in the following manuscript. Organized hip fracture programs offer significant benefits to patients, care providers and health systems. The more complex program designs have a more profound effect on improvement in outcomes for hip fracture patients. Most programs have reported reduced length of stay, reduced in-hospital mortality rates, and reduced complications. Some programs have reported reduced costs and reduced readmission rates after implementing an organized hip fracture program.

Introduction

Hip fracture is a common injury in older adults that is both greatly feared and harmful. Although the incidence of hip fracture has slightly decreased, the prevalence is increasing with the aging of the population. The outcomes of hip fracture care have improved little over the past 30 years despite improvements in anticoagulation, anesthetic management, hip fracture implants, and pharmacologic advances [1]. This situation has led many physicians and surgeons caring for hip fracture patients to look for other methods to improve patient outcomes. Altering the model of care has been one such improvement attempted in many centers. Over the past 20 years, several such models have been implemented and studied. Giusti and colleagues have already published a thoughtful and detailed analysis of these models and their attributes [2]. This manuscript will evaluate the published evidence on efficacy of organized hip fracture programs to improve patient outcomes.

Materials and methods

The author searched PubMed (1999–present) databases of the National Library of Medicine and Google Scholar (1999–present) for appropriate articles addressing the impact of hip fracture programs. The key words, which were searched, were the terms “geriatric fracture programs”, “hip fracture programs”, and “geriatric fracture centers”. Searching of the reference lists of potentially relevant original papers was also performed. Inclusion criteria: papers written in English in peer-reviewed journals. Exclusion criteria: articles using language other than English, letters and expert opinion articles. Articles meeting these criteria were retrieved and each of the papers was examined.

Results

From the above search strategy, 1403 results were obtained. Most of the articles were excluded by title or review of the abstract as being irrelevant to this search. This left 28 manuscripts that addressed the question of efficacy of organized hip fracture programs. When several manuscripts addressed the same model at the same institution, they were condensed into one. This left 17 programs with a reasonable quality of outcome reporting to examine in this manuscript. Programs reporting results were located in Europe, the US and Asia. One of the problems with comparing results of these programs is a lack of consistency of outcome measures selected by the authors to report on. Liem and colleagues have recently reported on a suggested set of outcome measures to study hip fracture programs [3,4]. Unfortunately, these suggested outcome measures have not yet been widely applied in published studies. Therefore, common outcome measures will be examined in this manuscript, including length of hospital stay, short-term mortality, in-hospital complications, and time to surgery.

Discussion

Elements of highly organized hip fracture programs include standardized order sets [5], use of a clinical care pathway [5], co-management with a medical physician and orthopaedic surgeon [5], early surgery [5,6], use of lean business principles to optimize patient care [7], early mobility with weight bearing permitted [6], and early discharge planning [5]. Additionally, incorporation...
of “best practices” into each aspect of care is typically done in the most complex and organized hip fracture care programs [2]. Although each individual intervention mentioned above may improve outcomes, a combination of all of these methods in one program yields even better outcomes.

Table 1 shows the results of several different types of organized care programs implemented in different centers around the world. The Geriatric Fracture Center (GFC) model consistently has been shown to provide the best outcomes to date [2,5,8,9]. The Orthogeriatric model of care has also been shown to be better than usual care [2,10], but does not have as profound an impact on care as the GFC model. The multidisciplinary fracture service MDFS model usually improves care quality as well [11,12], but does not have as profound an impact on outcomes as the GFC model [2]. Once an organized hip fracture program is implemented, it tends to raise the standard of care, making it unethical to perform a RCT study of usual care versus the organized model. This has made it difficult to perform a head to head assessment of one method versus the other. Friedman and colleagues compared the GFC model against the organized model. This has made it difficult to study their outcomes of care [9,11,12,14–23].

Neuman and colleagues performed a meta-analysis of care pathway use for hip fractures and found improvement in outcomes when a care pathway alone was used [24], Grigoryan and colleagues performed a meta-analysis of three types of hip fracture care including: routine geriatric consultation, geriatric ward with orthopaedic consultation, and shared care [25]. They found an association between Orthogeriatric pathway use and a reduced risk of four common complications (deep vein thrombosis, surgical site infection, urinary tract infection and pressure ulcer) during the hospitalization after hip fracture. They recommended that additional studies be conducted to analyze these programs [25]. In particular, hospital length of stay seems to improve with implementation of the GFC care model. However, the particular region’s health system and resources seem to have a major effect on length of stay. Several programs have reported reduced costs of care based on implementation of the GFC care model [2,17,26]. Giusti and colleagues have reported that the more complex and comprehensive programs achieved better outcomes when compared with the simpler interventions [2].

Conclusions

Organized hip fracture programs offer significant benefits to patients, care providers and health systems. The more complex program designs have a more profound effect on improvement in outcomes for hip fracture patients. Most programs have reported reduced length of stay, reduced in-hospital mortality rates, and reduced complications. Some programs have reported reduced costs and reduced readmission rates after implementing an organized hip fracture program.

Conflict of interest

Grant support: PCORI, AOTrauma. Consultant: Surgical Excellence.

References


Table 1

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Location</th>
<th>Model Type</th>
<th>Time to surgery as reported</th>
<th>In hospital complication rates</th>
<th>Inpatient mortality (%)</th>
<th>Length of stay (days)</th>
<th>Readmit rates at 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khan [27]</td>
<td>2002</td>
<td>Surrey</td>
<td>OGU</td>
<td>NR</td>
<td>NR</td>
<td>11.1%</td>
<td>26.88</td>
<td>NR</td>
</tr>
<tr>
<td>Kashaghi [19]</td>
<td>2005</td>
<td>Baltimore</td>
<td>MDFS</td>
<td>63% &lt;24 h</td>
<td>36% – down</td>
<td>NR</td>
<td>5.7</td>
<td>NR</td>
</tr>
<tr>
<td>Friedman [13]</td>
<td>2009</td>
<td>Rochester</td>
<td>GFC</td>
<td>24.1 h</td>
<td>30%</td>
<td>1.5%</td>
<td>4.6</td>
<td>9.7%</td>
</tr>
<tr>
<td>Kammerlander [18]</td>
<td>2011</td>
<td>Innbruck</td>
<td>GFC</td>
<td>70.5% &lt;24 h</td>
<td>20.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adunsky [14]</td>
<td>2011</td>
<td>Sheba</td>
<td>OGU</td>
<td>3.6 d</td>
<td>4.1% “major complications”</td>
<td>3.2%</td>
<td>29.9</td>
<td>NR</td>
</tr>
<tr>
<td>Leung [20]</td>
<td>2011</td>
<td>Hong Kong</td>
<td>GFC</td>
<td>45 hrs</td>
<td>55%</td>
<td>1.1</td>
<td>9.3</td>
<td>9.3%</td>
</tr>
<tr>
<td>Dy [11]</td>
<td>2012</td>
<td>New York</td>
<td>MDFS</td>
<td>1.91</td>
<td>34.6%</td>
<td>0.6%</td>
<td>8.9</td>
<td>14.2% at 90 days</td>
</tr>
<tr>
<td>Gregersen [12]</td>
<td>2012</td>
<td>Aarhus</td>
<td>MDFS</td>
<td>NR</td>
<td>NR</td>
<td>6%</td>
<td>13</td>
<td>NR</td>
</tr>
<tr>
<td>Gonzalez-Montalvo [23]</td>
<td>2012</td>
<td>Madrid</td>
<td>OGU</td>
<td>5 days</td>
<td>NR</td>
<td>NR</td>
<td>12</td>
<td>NR</td>
</tr>
<tr>
<td>Lau [9]</td>
<td>2013</td>
<td>Hong Kong</td>
<td>GFC</td>
<td>1.5 days</td>
<td>NR, less infections</td>
<td>1.25%</td>
<td>6.4</td>
<td>NR</td>
</tr>
<tr>
<td>Folbert [21]</td>
<td>2013</td>
<td>Amelo</td>
<td>GFC</td>
<td>95% &lt;48 h</td>
<td>39% up slightly</td>
<td>5%</td>
<td>11</td>
<td>Reduced</td>
</tr>
<tr>
<td>Bhattacharya [28]</td>
<td>2013</td>
<td>Glasgow</td>
<td>OGU</td>
<td>&lt;48 h</td>
<td>“better care”</td>
<td>8.4%</td>
<td>19.5</td>
<td>NR</td>
</tr>
<tr>
<td>Della Rocca [17]</td>
<td>2013</td>
<td>Columbia</td>
<td>GFC</td>
<td>29.9 h</td>
<td>&gt;ICU stays</td>
<td>4.3%</td>
<td>7.1</td>
<td>14%</td>
</tr>
<tr>
<td>Collinge [16]</td>
<td>2013</td>
<td>Ft. Worth</td>
<td>GFC</td>
<td>39 h</td>
<td>NR</td>
<td>3%</td>
<td>6.5</td>
<td>NR</td>
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<td>Doshi [29]</td>
<td>2013</td>
<td>Singapore</td>
<td>GFC</td>
<td>36.7 h</td>
<td>5.1%</td>
<td>2.3%</td>
<td>10</td>
<td>NR</td>
</tr>
<tr>
<td>Biber [15]</td>
<td>2013</td>
<td>Nurnberg</td>
<td>GFC</td>
<td>2.1 days</td>
<td>9.6% – up slightly</td>
<td>13.9%</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>Flikmeert [22]</td>
<td>2014</td>
<td>Groningen</td>
<td>MDFS</td>
<td>92% &lt;24 h</td>
<td>NR</td>
<td>2%</td>
<td>7</td>
<td>NR</td>
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</tbody>
</table>

GFC-Geriatric Fracture Center model of care. OGU -Orthogeriatric unit model of care. MDFS-Multidisciplinary Fracture Service model of care. NR- not reported.
Atypical fractures, a biased perspective

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KEYWORDS

atypical fractures
bisphosphonates
proximal femoral fractures
osteoporosis

ABSTRACT

When stress fractures started to show up in the femurs of elderly ladies, it was soon evident that bisphosphonate use lay behind, and the absolute risk increase due to bisphosphonate use was reasonably well estimated already in 2008. Thereafter followed a period of confusion: the term atypical fracture was introduced, with a definition so vague that the true stress fractures tended to disappear in a cloud of ambiguity. This cast doubt on the association with bisphosphonates. The association was then re-established by large epidemiological studies based on radiographic adjudication. Atypical fractures are largely caused by bisphosphonates. With a correct indication, bisphosphonates prevent many more fractures than they cause, at least during the first years of use. With an incorrect indication they are likely to cause more harm than good.

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statistical strength of the findings, such studies simply had to be large, all experts said. After more than a year, finally, the results were clear, showing the same inverse relationship for a large cohort. In a registry-based study of 1.5 million women, the findings were quite similar to the smaller study, but with some additions. Now, in New England Journal of Medicine, an interested audience was waiting for more. However, the multiple revisions requested took over a year, and during this year important things happened.

The osteoporosis research community, mainly epidemiologists and endocrinologists, had become interested, and a number of papers came out, disputing or playing down the connection between stress fractures and bisphosphonates. The methods seemed similar to our New England paper, but there were some obvious methodological flaws. For example, a published radiograph said to illustrate an “atypical” fracture showed a fracture with cracks in the major trochanter, and no mention was made of other skeletal abnormalities. The American Society for Bone and Mineral Research (ASBMR) formed a large “task force” to make a better definition and clarify the matter. Their definition was still somewhat vague (short oblique fractures), multiple risk factors and drugs were mentioned, and the connection to bisphosphonates was merely described as possible [7]. We were forced to accept and use the term atypical fracture in our New England paper [8].

A year later, another large registry-based, industry-sponsored paper came out, claiming that the relation between bisphosphonates and atypical fractures was weak or absent [9]. The methods seemed similar to our New England paper, but there were some obvious methodological flaws. For example, a published radiograph said to illustrate an “atypical” fracture showed a fracture with cracks in the major trochanter, and no mention was made of other skeletal abnormalities. The American Society for Bone and Mineral Research (ASBMR) formed a large “task force” to make a better definition and clarify the matter. Their definition was still somewhat vague (short oblique fractures), multiple risk factors and drugs were mentioned, and the connection to bisphosphonates was merely described as possible [7]. We were forced to accept and use the term atypical fracture in our New England paper [8].

The generally accepted theory of how bisphosphonate cause atypical fractures involves that the skeleton in general becomes more brittle because reduced remodeling gives the bone more time to accumulate microcracks before it is replaced. This view is based on the idea that bone remodeling is too slow and is never completely replaced. However, it is now recognized that areas of microdamage attract bone resorption via RANKL expression, so that they are selectively replaced with new bone. We noted that the risk of atypical fracture seems to be related to current use, rather than drug accumulation in the bone. It increases steadily during bisphosphonate use, but decreases rapidly after bisphosphonate cessation. Areas of microdamage, subjected to targeted remodeling, may appear deeply in the bone, where bisphosphonates have not reached. Thus, only during current use, will bisphosphonates be present in the circulation and have a chance to reach these areas. After cessation, there will be no obstacle for targeted remodeling deep inside the bone. The rapid decrease in risk after cessation, suggests bisphosphonates cause atypical fractures mainly by reducing targeted remodeling [18].

Risk versus benefit

In general, bisphosphonates prevent many more fractures than they cause. However, the benefit of prolonging bisphosphonate use more than a few years is unclear, as the drug remains in the bone many years after cessation. The risk of atypical fracture decreases rapidly after cessation. This means that the risk of any type of fracture might be at its lowest after cessation following a few years of use.

If a drug is taken without a proper indication it has no benefit, but the side effects still remain. The benefit of antiresorptive treatment has been shown mainly in healthy women, under the age of 80 years, with a clear diagnosis of osteoporosis. Despite attempts, there is no good support for bisphosphonate use in the oldest old, or in women without osteoporosis [19]. Therefore, the label use of bisphosphonates might cause more harm than benefit.

The parallel to the lung cancer debate

So, the role of the bisphosphonates that had seemed evident from start, and was supported by a national registry-based analysis, had become a highly questionable matter of debate. We have lately published an extension of our previous study, now based on 2.9 million individuals, again showing a relative risk of over 100 after 4 years of bisphosphonate use [14,15]. The risk increases steadily with duration of use, and different bisphosphonates incur different risks. There is no demonstrable connection with any other drug. There is a biologically and mechanistically plausible explanation. The ASBMR task force issued a new statement 2014, with a refined definition of atypical fracture, mainly based on our demonstration that they are a distinct subgroup ofshaft fractures, based on fracture angle [16]. The report discusses the association with bisphosphonates comprehensively, but they do not write plainly that bisphosphonates cause these fractures.

It is evident that mechanical stress plays a role for the risk of atypical fractures, and some elderly women with atypical fractures have not been exposed to bisphosphonates. Most patients on bisphosphonates do not get atypical fractures. Still, the association between bisphosphonates and atypical fractures is stronger than the association between smoking and lung cancer, and the arguments for a causative role are also stronger [17]. Most smokers don’t get lung cancer, and non-smokers sometimes get it. Still, smoking as a cause of lung cancer is not disputed.

Bisphosphonates may block the healing of microcracks rather than cause brittleness

The generally accepted theory of how bisphosphonate cause atypical fractures involves that the skeleton in general becomes more brittle because reduced remodeling gives the bone more time to accumulate microcracks before it is replaced. This view is based on the idea that bone remodeling is too slow and is never completely replaced. However, it is now recognized that areas of microdamage attract bone resorption via RANKL expression, so that they are selectively replaced with new bone. We noted that the risk of atypical fracture seems to be related to current use, rather than drug accumulation in the bone. It increases steadily during bisphosphonate use, but decreases rapidly after bisphosphonate cessation. Areas of microdamage, subjected to targeted remodeling, may appear deeply in the bone, where bisphosphonates have not reached. Thus, only during current use, will bisphosphonates be present in the circulation and have a chance to reach these areas. After cessation, there will be no obstacle for targeted remodeling deep inside the bone. The rapid decrease in risk after cessation, suggests bisphosphonates cause atypical fractures mainly by reducing targeted remodeling [18].

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The sociology of science

I have a perception that the debate over the causative role of bisphosphonates was harsh and emotional. At a superficial look,
it may seem that some of the involved persons were trying to protect the drug industry that supported their research. I don’t think this was the case, and I don’t want to accuse anybody of that. However, I have seen in several fields how researchers become emotionally attached to their ideas, especially the good ones, and the bisphosphonate development is an extraordinary success story. It is not hard to understand that osteoporosis researchers reacted by rejecting arguments from an outsider who started to blame drugs that they have devoted their lives to. Moreover, they feared that our findings could cause an overreaction in the general public, so that bisphosphonates would not be used even by those that would have a clear benefit from it. Still, we have to face the facts.

Conflict of interest

Per Aspenberg has shares in Addbio AB and has received institutional research support from Eli Lilly and Amgen.

References

Anti-sclerostin - is there an indication?

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**Keywords**
sclerostin
sclerostin antibody
osteoporosis
fracture healing
anabolic
romosozumab

**Abstract**
Several decades ago, a clinical condition that included severe bone overgrowth was described in a few patients in South Africa. The autosomal-recessive disease that later was named sclerosteosis was found to be caused by a mutation in the SOTS gene causing a lack of the protein sclerostin. This protein is produced by osteocytes and exerts its effect as an inhibitor of bone formation by blocking the Wnt signaling pathway. By the use of a monoclonal antibody that can block sclerostin a novel therapeutic pathway for rebuilding bone has been described. Preclinical studies have shown increased bone mass following subcutaneously administered anti-sclerostin antibody in animals with induced postmenopausal osteoporosis as well as in intact male rats and non-human primates. In a phase II study the efficacy and safety of an anti-sclerostin antibody, romosozumab, has been evaluated in 419 postmenopausal women for 12 months. 70, 140 or 210 mg was given subcutaneously monthly or every three months and compared to 70 mg of oral alendronate given once a week or 20 μg of teriparatide subcutaneously once daily. All dose levels of romosozumab were associated with significant increase in BMD with the most pronounced gain in the group receiving 210 mg where lumbar spine BMD increased with 11.3% from baseline. The BMD for the placebo group decreased by 0.1% while the alendronate group increased 4.1% and the teriparatide increased 7.1%. Biochemical markers revealed a transitory increase in the bone formation marker P1NP while no change in the bone resorption marker β-CTX. In comparison, teriparatide resulted in an increase for both P1NP and β-CTX for the complete study period. Even though the rapid gain in BMD is promising when considering a treatment option for osteoporosis and other conditions with bone loss, there are so far no published studies on whether anti-sclerostin can reduce the number of fractures. Wnt signaling might also play an important role in fracture healing with substances that causes an upregulation of the Wnt pathway producing enhancement of the fracture healing process. Healing of experimental fractures in various animal models have shown improvement following subcutaneously administered anti-sclerostin antibody. While there are no published reports on the potential effect of systemically administered anti-sclerostin antibodies on fracture healing in humans.

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**Introduction**

In 1958 Truswell [1] in a case report described a condition characterized by generalized skeletal overgrowth that was mostly pronounced in the skull and mandible but that also affected the extremities. In the two cases described by Truswell syndactyly was also present leading to the description osteopetrosis with syndactyly. In 1967 Hausen [2] introduced the term sclerosteosis based on findings that made this condition distinct from osteopetrosis.

The condition is very rare with less than 100 described individuals being affected. The vast majority of the identified persons are present in the Afrikaner population of South Africa although the condition has also been described in the US, Brazil, the Netherlands as well as isolated cases from other countries.

The inheritance of the disease is through an autosomal recessive mode [3].

The natural history of the condition reveals that both genders can be affected, with no differences in clinical findings and outcome between women and men. The more severe clinical symptoms are mainly secondary to the thickening of the skull. Increased intracranial pressure that can lead to sudden death, usually in early adulthood, is a frequent complication due to the reduced intracranial volume caused by the progressive bone formation. Due to the extensive amount of bone being formed cranial nerves can be entrapped. The most frequent manifestation are due to entrapment of the seventh and/or eighth cranial nerve causing hearing loss. Although hearing loss can also be an effect of extensive bone formation leading to reduced movement of the middle ear ossicles [4].

The skeleton in patients suffering from sclerosteosis is very resistant to trauma with no known fracture among
affected persons, i.e. the excessive bone formation observed on radiographs represent bone that is not only dense but also very strong and resistant to fracture. The condition is not associated with any known abnormalities in the endocrine system or any involvement of the cardiovascular system.

Sclerostin and anti-sclerostin antibodies

The disease sclerosteosis has been linked to mutations in the SOST gene that lead to inactivation of the glycoprotein Sclerostin [3,5]. Sclerostin is expressed and secreted by osteocytes. It has an antagonistic effect on Wnt signaling in bone forming osteoblasts whereby it acts as a negative regulator of bone formation. It has been suggested that osteocytes exerts a regulation of the local bone formation through sclerostin [6]. The exact molecular mechanism of action is still not known. Absence of sclerostin leads to over activity of Wnt signaling in bone tissue with increased bone formation as seen in patients with sclerosteosis, i.e. sclerostin inhibits osteoblast mediated bone formation. When the clinical features of the disease sclerosteosis had been described, the protein sclerostin identified and the mutation in the SOST gene that encodes sclerostin had been found, the accumulated knowledge opened up for a potential new therapeutic pathway promoting bone formation by the use of anti-sclerostin antibodies [7].

Preclinical anti-sclerostin studies

In a study using mice where through gene targeting the SOST gene was inactivated it was shown that the SOST knockout mice had a phenotype with high bone mass throughout the skeleton and increased bone strength [8]. The increase in bone volume was significant in both cortical and trabecular bone when assessed with DXA and micro-CT. Histomorphometry revealed a significant increase in osteoblast surface and no significant change in osteoclast surface in knockout mice when compared with wildtype mice. The results for male and female knockout mice were similar. The study confirmed that sclerostin is a key negative regulator that acts on both trabecular and cortical bone.

By blocking sclerostin with an antibody an increase in bone mass have been reported when using various preclinical models. In a study by Li and others [9] ovariectomized rats were left untreated for one year to induce estrogen deficiency bone loss as a model for postmenopausal osteoporosis. A monoclonal anti-sclerostin antibody was then administered subcutaneously twice every week for 5 weeks. The bone anabolic effect was very pronounced with marked net bone formation including both trabecular and cortical bone. Lumbar vertebra and femur-tibia were used for assessment of bone formation as well as for mechanical testing. In vivo DXA revealed that at 5 weeks, BMD in the group treated with the monoclonal antibody was increased by 26% in the lumbar vertebra and by 17% at femur-tibia relative to baseline at the time of the first injection. By the use of micro-CT the distal femur were analyzed. Five weeks of treatment with the monoclonal antibody completely restored trabecular bone mineral density back to control levels through a 57% increase in trabecular thickness and an increase in trabecular number. Mechanical testing using compression of the vertebra and four-point bending of the femur midshaft revealed a significant increase in strength. Vertebrae from animals treated with the monoclonal antibody were stronger in compression than vertebrae from both ovariectomized animals not treated with the monoclonal antibody as well as non ovariectomized animals. The difference was most pronounced in energy to failure and load at failure while slightly less pronounced in stiffness. In the femur the load at failure and energy to failure increased by 47% and 90%, respectively, when compared with ovariectomized controls that had not been treated with the monoclonal sclerostin blocking antibody, while there was no significant difference in stiffness. Femora from animals treated with the monoclonal antibody were even stronger than femora from non ovariectomized animals. Histomorphometry revealed that following five weeks of treatment with the monoclonal antibody blocking sclerostin, the cortical bone area and the cortical thickness had been restored back to sham control levels. Animals treated with the sclerostin blocking antibody had markedly increased bone formation on both the periosteal and endocortical surfaces of the femoral midshaft compared with ovariectomized controls.

In a study with a similar set-up for assessment as in the previous study, the effect on the skeleton of two different dosages, 5 and 25 mg/kg, of the sclerostin blocking antibody were assessed in gonad intact male aged rats [10]. The antibodies were administered through subcutaneous injections twice every week for five weeks. In vivo DXA of the lumbar vertebra as well as of long bones showed a marked increase in bone mineral density in both dose groups when compared with non-treated controls already at 3 weeks following the first injection. There was no significant difference in BMD between the two different dosage levels. Micro-CT ex vivo revealed a dose-dependently greater trabecular bone volume and trabecular thickness than in controls within the lumbar vertebra. In the distal femur both doses of the monoclonal anti-sclerostin antibody caused an increase in trabecular volume, trabecular thickness as well as trabecular number. In the midshaft of the femur cortical thickness was significantly greater in both treated groups compared with non-treated controls, while there was no difference between dose groups. Histomorphometry revealed a marked increase in bone formation with both doses of the antibody. Mineralizing surface, mineral apposition rate and surface-based bone formation were all significantly greater in animals treated with the anti sclerostin antibody compared with controls. The compressive strength of lumbar vertebra showed a significant dose dependent increase when compared with non-treated controls. The increase in load to failure in the group treated with the higher dose was 99%, while the corresponding increase in the lower dose group was about 34% when compared with the strength of vertebra from non-treated controls. The femoral strength in the shaft as well as in the femoral neck showed a significant increase in both treated groups when compared with controls, with no significant difference between the two dose groups except for a significantly higher energy to failure in the femoral neck for the group treated with a higher dose compared with the low dose group.

In a study on non-human primates [11] a humanized sclerostin monoclonal antibody was administered to female gonad intact monkeys. Two once-monthly subcutaneous injections of the antibody were administered at three dose levels (3, 10 and 30 mg/kg) with study termination at two months. There were 2–3 animals in each treatment group and 4 controls receiving vehicle alone.

Measurement of biochemical markers in the serum for bone turnover showed an increase in markers for bone formation, described by P1NP and osteocalcin, while there was no change for the serum marker CTX that was used for assessment of bone resorption. P1NP peaked at two weeks after the first injection and at one week after the second injection with significant increases in all three dose groups. The peak for osteocalcin occurred about one week later than the P1NP peak. Four weeks after the injection of the antibody the serum levels of the markers for bone formation had returned to base levels.

Static and dynamic histomorphometry showed a dose-dependent increase in trabecular bone volume and trabecular thickness when compared with vehicle treated animals. These
changes were associated with dose-dependent increase in mineral apposition rate and mineralizing surface in the trabecular bone in both the lumbar vertebra and the proximal tibia in animals treated with the antibody. In the cortical bone at the midshaft femur a significant increase in bone formation was observed. Quantitative analysis showed a dose-dependent increase in mineralizing surface, mineral apposition rate and bone-formation rate at both the periosteal and endosteal surfaces.

Bone strength was assessed through compressive loading of lumbar vertebra and by three-point bending of the femoral shaft. Due to the limited number of animals in each group the study was not primarily powered for assessment of bone strength. Despite the limited number of observations available the vertebra from the three animals treated with the highest dose of the antibody, i.e. 30 mg/kg, were significantly stronger with load to failure being 97% higher and energy to failure 183% higher than the four vehicle treated controls. While the femoral shaft from animals treated with the highest dose showed a non-significant increase in load at failure, stiffness as well as at energy to failure when compared with the vehicle treated controls.

Taken together, the preclinical studies in rodents as well as in non-human primates have shown a marked bone building effect by utilizing the effect of an antibody that neutralizes the effect of sclerostin opens up for a therapeutic approach whenever bone formation is aimed for. Two such conditions are osteoporosis and fracture healing.

**Anti-sclerostin and osteoporosis**

Current established pharmacological treatment of osteoporosis is almost exclusively based on anti-resorptive compounds while there is only one anabolic drug approved, i.e. a drug that stimulate bone formation. Among the anti-resorptive compounds, bisphosphonates constitutes by far the largest class. Some of the bisphosphonates represent fairly inexpensive treatment options for osteoporosis which is one reason for the dominance as first line drug versus various forms of osteoporosis. Bisphosphonates have a high affinity for bone and work by blocking the osteoclasts. Based on type of bisphosphonates administration can be either oral or intravenous. There is a wide variation in dosing regimen ranging from daily oral intake to one intravenous injection once a year. Compliance due to side effects is a potential issue, especially when administered orally. Other classes of anti-resorptive drugs include raloxifene, strontium ranelate and denosumab. Among the anti-resorptive agents denosumab represent a novel option whereby a monoclonal antibody antagonise RANKL, and thereby its prominent role in osteoclastogenesis. Due to the high affinity for RANKL the dosing interval for denosumab can be six months. Even though both bisphosphonates and denosumab are anti-resorptive treatment options there are several characteristics that separate them. Denosumab targets RANKL and is not incorporated into the bone mineral. This means that denosumab has got a better reversibility. The gastro-intestinal side effects known to accompany many of the bisphosphonates do not occur when using denosumab and the biannual subcutaneous administration will act in favor of improved adherence.

Even though anti-resorptive drugs are effective by preventing further bone loss, a faster way to gain bone mass could be through anabolic substances. This option might be especially attractive in situations with severe osteoporosis or extensive bone loss. However, bone anabolic drugs are at present limited to parathyroid hormone, PTH, which is available in two forms. Either the full length PTH 1–84 or the N-terminal fragment, teriparatide, or PTH 1–34.

The use of anti-sclerostin antibody as an anabolic substance is a potential therapeutic approach for severe osteoporosis as well as for conditions associated with severe bone loss as based on preclinical studies mentioned above. In a first-in-human randomized and double blinded placebo controlled study a single dose of a sclerostin monoclonal antibody was administered to healthy men and postmenopausal women [12]. Dose related increases in the bone formation markers procollagen type 1 N-propeptide (P1NP), bone-specific alkaline phosphatase (BAP) and osteocalcin were observed along with a dose related decrease in the bone resorption marker serum C-telopeptide (CTX). A biochemical pattern that indicate a large anabolic window. These findings point to a possible situation where at the same time bone formation will be increased and bone resorption will be decreased. That is a different pattern of bone turnover when compared with for instance PTH. Measurement of bone mineral density, BMD, using dual energy X-ray absorptiometry (DXA) revealed a significant increase of 5.3% in the lumbar spine and 2.8% in the hip at the final examination at 85 days after receiving the substance.

In a phase II multicenter, randomized, placebo-controlled study [13] the efficacy and safety of an anti-sclerostin antibody, romosozumab, was evaluated in 419 postmenopausal women with low bone density. The substance was administered subcutaneously monthly or every three months in one of three dosing levels, 70 mg, 140 mg or 210 mg. A comparison was made with 70 mg of oral alendronate given once a week or 20 μg of teriparatide administered subcutaneously once daily. The primary endpoint was change from baseline in bone mineral density at the lumbar spine at 12 months. The results showed that all three dose levels of the romosozumab were associated with significant increase in BMD at the lumbar spine. The most pronounced gain was observed in the group receiving a monthly dose of 210 mg where the lumbar spine BMD increased with 11.3% from baseline. The corresponding change for the placebo group was a decrease by 0.1% while the alendronate group showed an increase by 4.1% and the teriparatide treated patients had an increase in lumbar BMD by 7.1% at 12 months. A similar increase of BMD for the various groups was also seen in the total hip and the femoral neck with patients treated with 210 mg of romosozumab gaining significantly most among the various groups studied. In contrast to the findings in the lumbar spine there was no difference in BMD gain in the hip between the groups treated with alendronate or teriparatide. Biochemical markers revealed a transitory increase in the bone formation marker P1NP while no change in the bone resorption marker β-CTX. In comparison, treatment with teriparatide resulted in an increase for both P1NP and β-CTX for the complete study period while the alendronate treated patients had lowered levels of both markers during the entire study period. The authors concluded that in women with low bone mass, romosozumab was associated with increased bone mineral density and bone formation and with decreased bone resorption. The number and type of side effects was equal to the rate and types seen in the placebo group.

Even though the rapid gain in BMD observed in the previous study is promising when considering a treatment option for osteoporosis and other conditions with bone loss, there are so far no published studies addressing the question whether treatment with an anti-sclerostin antibody can reduce the number of fractures. However, ongoing phase III studies in postmenopausal women are designed to provide data on whether the increase in BMD previously shown will translate into a reduction of fractures in patients on treatment with romosozumab.

**Anti-sclerostin for enhancement of fracture healing**

The bone formation mechanism that comes into action during fracture healing is a complex biological process that involves both...
local and systemic factors. In most patients traumatic fractures will heal in an uneventful and reasonably predictable way, ending up with the fractured bone being restored with regards to mechanical strength. Still, healing complications are fairly frequent especially in patients with various comorbidities. In order to enhance fracture healing in patients with compromised healing capacity, as well as in patients where a prolonged healing time can be expected due to severe bone loss, the potential benefit of local or systemic administration of bone anabolic substances have been proposed. In a study by Secreto et al. [14] it was suggested that Wnt signaling might play an important role in fracture healing. Through administration of substances that causes an upregulation of the Wnt pathway an enhancement of the fracture healing process have been shown, while inhibition of the Wnt signaling have been shown to inhibit fracture repair [15,16]. Although the exact mechanism by which sclerostin negatively regulates bone formation is not fully known, one hypothesis is that sclerostin inhibits the Wnt signaling thereby reducing the osteoblast differentiation and function. By using anti-sclerostin antibodies an indirect bone anabolic effect will be achieved that theoretically might improve not only bone formation in general but also the important local bone formation that is a prerequisite for successful fracture healing.

In a preclinical study using a closed femoral shaft fracture model in rats that were stabilized with a rod, a comparison of healing was done between animals receiving an anti-sclerostin antibody through subcutaneous injection or placebo [17]. Using μCT to assess local bone formation, significantly more bone formation could be seen at the fracture site in animals receiving the antibody substance compared with the placebo controls. The increase was significant for both bone mineral content and bone volume at the end point at 7 weeks following the fracture being created. For assessment of mechanical strength a three point bending test was done that revealed a significant increase in load at failure as well as in stiffness. Fractures in the animals that were given anti-sclerostin antibody systemically had a mechanical strength that reached 48% of the strength in the intact contralateral femur while the corresponding level for the animals treated with placebo was 27%. In a study on non-human primates healing of a fibular osteotomy in male monkeys was studied [17]. The animals were given 30 mg of anti-sclerostin antibody or placebo through subcutaneous injection every other week for a total of ten weeks. The formation of callus was measured using pQCT at the study end point being 10 weeks. Total callus area as well as total callus BMC was significantly greater in the animals treated with the antibody compound compared with placebo. Mechanical testing using a torsional destructive model revealed that fibula specimens from animals treated with the anti-sclerostin antibody were significantly stiffer while the load at failure also was relatively higher than in specimens from animals receiving placebo although the difference was not statistically significant.

A few years ago a clinical program was initiated where the intention was to explore whether systemic administration of an anti-sclerostin antibody to patients who had suffered a tibial shaft fracture or a hip fracture might shorten time to healing. Enrollment was initiated to phase II studies for both these fracture types but prior to completion of the studies both were prematurely closed.

According to a press release from the companies involved, preliminary interim data in the tibial study suggested that patients treated with either of three doses of the anti-sclerostin antibody, in addition to internal fixation of their fractures, did not differ significantly in time to radiological healing when compared with placebo. In addition, indications from regulators on very high regulatory demands for fracture healing studies with a need for two long-term pivotal studies in each fracture type made the companies close down the fracture healing program prior to completion of the first clinical studies. Whether or not treatment with systemic administration of an anti-sclerostin antibody in humans will reduce time to healing of fractures is therefore at present not known.

Conclusion

Sclerostin is a protein produced by osteocytes that inhibit bone formation. By the use of a sclerostin blocking antibody a pathway for rebuilding bone has been described with potential use for treatment of osteoporosis that presently is being explored. In addition several preclinical studies have shown reduced time to fracture healing following subcutaneously administered anti-sclerostin antibody. Recently phase II studies in tibial and hip fractures were initiated but both were closed prematurely due to high regulatory hurdles for fracture healing studies and due to interim data suggesting lack of efficacy in shortening time to radiological healing in tibial shaft fractures. Whether anti-sclerostin antibody in humans will reduce time to healing of fractures is therefore still not known.

Conflict of interest

The author has no conflicts of interest related to this manuscript.

References

Introduction

Teriparatide [PTH (1–34)] is a genetically engineered analog of human parathyroid hormone that is used to treat osteoporosis in patients who are at a high risk for fracture. This drug is recommended for use in post-menopausal women (PMW), men with hypogonadal osteoporosis, as well as men and women with glucocorticoid-induced osteoporosis. Intermittent (once-daily) doses of teriparatide seem to stimulate osteoblast activity and therefore result in a net increase of bone formation. It is recommended for use in post-menopausal women (PMW), men with hypogonadal osteoporosis, as well as men and women with glucocorticoid-induced osteoporosis. In vivo studies have generated important findings regarding teriparatide’s role in the enhancement of fracture healing. The intention of this article is to review the clinical findings of teriparatide to stimulate fracture healing. The drug was shown in a prospective randomized, double blind study to achieve earlier radiographic cortical bridging of three of four cortices (7.4 weeks) compared to patients who were assigned to the placebo group (9.1 weeks). Another study compared mean time for healing and functional outcome in two groups of elderly women who had suffered osteoporotic pelvic fractures: one group received daily 100 μg parathyroid hormone (1–84) injections, while the other group received no treatment. Patients who received the PTH (1–84) injections accelerated radiographic and clinical fracture healing (7.8 weeks) when compared to patients who received no treatment (12.6 weeks, p<0.001). Numerous case series state the safety and potential benefits of teriparatide use in patients recovering from fractures. In the following scenarios, teriparatide might be considered in patients with osteoporosis and a fracture: (1) patients with severe osteoporosis with use of bisphosphonates for a number of years with a fracture not expected to predictably unite, e.g. atypical femur fracture or open tibia fracture, (2) in cases where an osteoporotic patient has failed fracture healing and is considering surgical treatment e.g. non-union surgery. It seems prudent to reevaluate these patients frequently and reconsider which drug class of osteoporotic drug is best for the patient. Finally, it must be stressed that we do not recommend teriparatide in osteoporotic patients that may be well treated with bisphosphonates and a fracture is expected to heal uneventfully, nor when patients with metabolically normal bone have a fracture.
fracture healing process in monkeys with surgically-induced femur fractures by decreasing the size and increasing degree of mineralization of the fracture callus; thus indicating that PTH (1–34) might also accelerate the healing process in human fractures [4]. In a similar study, researchers examined the effects of intermittent administration of parathyroid hormone on fracture healing in ovariecotomized rats [12]. After inducing bilateral tibial shaft fractures in the rat models and then stabilizing them via intramedullary nailing with Kirschner wires, the animals were given daily doses of either saline, 17-estradiol, or recombinant PTH (1–84) for 30 consecutive days. Morphometric and mechanical analyses of fracture callus were used to assess fracture healing. The results of the study showed that intermittent administration of PTH increased the morphometric and mechanical parameters of fracture callus (callus length, callus diameter, ultimate load, ultimate stiffness, ultimate stress, etc.) [12]. Intermittent administration of PTH (1–34) also seems to have a positive influence on callus formation and mechanical strength of tibial fractures in rats [6]. After daily administration of 60 μg PTH (1–34), 200 μg PTH (1–34), and saline solution, rats that were given daily doses of 200 μg experienced increases in both external callus volume as well as ultimate axial load. Finally, a group of researchers investigated the efficacy of a parathyroid hormone related protein (PTHrP) analog as systemic therapy for impaired bone healing in corticosteroid-treated rabbits [9]. A 1mm defect was created bilaterally in the ulnae of 30 rabbits and delayed healing was induced by administering daily prednisone injections beginning 2 months prior to killing the rabbits and continuing all the way up to the time of killing. At 6 weeks post-injury, 9 out of 10 rabbits in the experimental group had achieved radiographic union and only 2 out of 10 in the control (saline) group had achieved similar results in that same time period. PTHrP was shown to be an effective therapy for preventing impaired bone healing as it caused higher radiographic intensity, larger callus area, increased stiffness, and increased torque.

Teriparatide and bone healing: clinical research

The clinical benefits of teriparatide are likely to extend beyond its approved use as a fracture prevention drug in patients with osteoporosis (Fig. 1). In a prospective randomized, double blind study, Aspenberg et al. compared the effects of 20 and 40 μg/day teriparatide injections versus placebo on the time to radiographic healing of distal radial fractures in postmenopausal women [7]. Radiographic evidence of complete cortical bridging in three of four cortices happened earlier for patients assigned to the teriparatide 20 μg/day group (7.4 weeks) when compared to patients who were assigned to the placebo group (9.1 weeks). Another study compared mean time for healing and functional outcome in two groups of elderly women who had suffered osteoporotic pelvic fractures; one group received daily 100 μg parathyroid hormone (1–84) injections, while the other group received no treatment [13]. Patients who received the PTH (1–84) injections accelerated radiographic and clinical fracture healing (7.8 weeks) when compared to patients who received no treatment (12.6 weeks, p<0.001).

Numerous case series state the safety and potential benefits of teriparatide use in patients recovering from fractures. One such study describes the effects of daily 20 μg teriparatide injections on a cohort of 145 patients who had failed previous fracture healing, i.e. presented some form of nonunion or delayed union, or were high-risk candidates for healing acute fractures [10]. Ninety-three percent of patients demonstrated radiographic and clinical union of their fractures, and only 3% of patients failed to see early progression to clinical and radiographic union. Additional case studies have shown that delayed healing in acute and stress fractures were well treated with daily teriparatide injections along with a Vitamin/ calcium citrate supplement. Raghavan et al. showed that two women with slow healing metatarsal stress fracture healed within four weeks of starting PTH therapy [14]. Likewise, Borges et al. presents the case of an 84-year-old woman with a transstrochanteric femur fracture that showed slow progression of fracture healing one month post-surgery and healed very quickly after initiating daily 20 μg doses of teriparatide [8].

Recommendations for teriparatide usage in fracture patients

The decision to use teriparatide in patients with moderate or severe osteoporosis and a fracture must be considered...
thoughtfully, as there is no cookbook answer for its use in this setting. Despite some promising results in animal studies and positive clinical support, regulatory bodies such as the U.S. Food and Drug Administration do not currently approve teriparatide for the enhancement of fracture healing. There are a few scenarios where teriparatide might be considered in patients with osteoporosis and a fracture. First, it might be considered when a patient with severe osteoporosis has been taking bisphosphonates for a number of years and has a fracture not expected to predictably unite, e.g. atypical femur fracture or open tibia fracture. Teriparatide could be used to treat the osteoporosis, while providing a vacation from bisphosphonates, and potentially providing a trophic effect on fracture healing. The drug also might be considered in cases where an osteoporotic patient has failed fracture healing and is considering surgical treatment e.g. non-union surgery. It seems prudent to reevaluate these patients frequently and reconsider which drug class of osteoporotic drug is best for the patient. Finally, it must be stressed that we do not recommend teriparatide in osteoporotic patients that may be well treated with bisphosphonates and a fracture is expected to heal uneventfully, nor when patients with metabolically normal bone have a fracture.

Research shows that dosing may influence teriparatide's anabolic effect on bone healing [5,7,10,11]; 20 µg per day given subcutaneously appears beneficial for stimulating union, while a 40 µg daily injection has little to no effect on the time to fracture healing. There is some controversy as to how long patients should continue to use teriparatide after their fracture has been treated. While the recommended use is for a maximum period of 2 years, it remains unclear as to whether the patient should take teriparatide only until the fracture has healed or whether he/she should take the drug for the entire two year period. Several factors must be taken into consideration before being able to make this decision.

Contraindications to teriparatide treatment include hypersensitivity to PTH, PTH (1–34), or any of the drug’s excipients. There is also a potential risk of osteosarcoma associated with the use of teriparatide; therefore patients at an increased baseline risk of osteosarcoma (Paget’s disease of bone, pediatric patients with open epiphyses, and prior external beam or implant radiation therapy involving the skeleton) should avoid this form of treatment. Teriparatide injections also pose a significant financial burden, thus the patient’s ability to manage the cost of this therapy is likely to determine how long he/she will receive this treatment. Additionally, one must carry out a thorough assessment of the patient’s response to the drug, making sure that there are no serious side effects threatening the patient’s well-being, both now and in the future. Ultimately, the decision to continue teriparatide treatment up to the recommended 2-year period is a complex one and it involves careful analysis of the patient’s entire medical history. There are currently no known alternatives to teriparatide; however, there are many ongoing studies and clinical trials testing the efficacy of new drugs that may become available in a short period of time.

Conflict of interest

The authors have no conflicts of interest relating to the subject matter discussed in this manuscript. They have received no support of any kind in its creation.

References

Osteoporotic fracture healing – responsiveness to mechanical stimulation?

Osteoporotic fracture is known to have impaired healing capacity and therefore takes longer time to heal, as compared with younger one. The mechanism of impaired osteoporotic fracture healing is multifactorial, where lower responsiveness to mechanical loading is generally believed to be one factor, yet not absolutely confirmed. In recent years, low intensity pulsed ultrasound (LIPUS) is demonstrated to have good efficacy in treating normal fracture healing, as proven by many randomized controlled trials, as well as in vitro and animal evidences. The effects of LIPUS on osteoporotic fracture healing was also validated in an animal study, which revealed that osteoporotic fractured bone of SD rats showed radiologically and biomechanically comparable responses to LIPUS as age-matched normal fracture healing, in terms of callus width, bridging rate, bone volume fraction, and stiffness etc. Gene expression profiling also confirmed that osteoporotic fractured bone responded well to LIPUS very well by upregulating Col1 and BMP2 (osteogenesis) at early phase, VEGF (angiogenesis) at middle phase and RANKL (remodeling) at late phase. These confirm that osteoporotic bones respond well to LIPUS as good as normal bone. These findings may be associated with estrogen receptors (ERs), as estrogen depletion is sensed and relayed by ERs and ERs also function as mechano-sensors. A previous study observed a delayed ERs expression pattern in fracture callus of OVX rats, as compared with SHAM rats, which correlated well with the expression pattern of BMP-2 (callus formation-related gene). Hence, the responses of osteoporotic fractured bone to LIPUS may be related to the local ERs expression at fracture callus that needs further experiments to validate.

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tibial fractures [17] and non-unions of various bones [18] were demonstrated clinically. A few meta-analyses also verified the different extents of positive effects of LIPUS on fracture healing [19,20]. In vivo, LIPUS was shown to increase blood flow around the fracture site [21]. At cellular level, LIPUS was found to increase cellular activities of many cell types, e.g. increased calcium nodule formation and alkaline phosphatase activity in osteoblasts [22], more β-catenin nuclear translocation in osteocytes [23], promoted osteogenesis in mesenchymal stem cells [24] and stimulated proliferation/differentiation in periosteal cells [25], which are helpful to promote fracture healing at various phases. With all these positive scientific evidences, LIPUS is well accepted to be an effective biophysical modality to modulate mechanical micro-environment and blood flow in fracture site for accelerating fracture healing. However, all these animal or clinical evidences are on normal fracture in adults; the effect on osteoporotic fracture was not yet elucidated.

Effects of LIPUS on osteoporotic fracture healing – animal evidence

The first animal study to depict the efficacy of LIPUS on osteoporotic fracture healing was conducted on 120 female Sprague-Dawley (SD) rats divided into four groups – Sham ovariectomy with LIPUS treatment (Sham-T), Sham ovariectomy control (Sham-C), ovariectomy with LIPUS treatment (OVX-T) and OVX control (OVX-C) [26]. Half of the 6-month-old rats were bilaterally ovariectomized for OVX groups (FDA-verified animal model of osteoporosis [27]), while another half was sham operated for Sham groups. All the rats were housed for 3 months to develop osteoporosis and the reduction in BMD was confirmed by peripheral quantitative computed tomography (pQCT, Densiscan 2000, Scanco Medical, Bruttisellen, Switzerland), where ~9.6%, ~4.6% and ~2.3% of BMD were detected at 5th lumbar vertebra, right femoral head and right femoral shaft respectively. They were then created closed fractures at femoral mid-shaft according to Einhorn’s protocol [28]. LIPUS (pulsed 1.5 MHz, 30.0 mW/cm² spatial-averaged temporal-averaged intensity; Exogen 3000+, Smith & Nephew, Memphis, TN, USA) was given 20 min/day and 5 days/week for durations of 2, 4, or 8 weeks, at which radiography, BMD and microarchitecture measurement, histomorphometry and mechanical testing were performed. Results indicated that both the treatment groups (Sham-T and OVX-T) were of significantly enhanced callus formation, faster mineralization and better remodeling than their control groups (Sham-C and OVX-C) [26]. Interestingly, by comparing the results between Sham-T and OVX-T, OVX-T showed comparable healing responses with Sham-T group in most parameters, while OVX groups indicated relatively more significant differences in various assessments than Sham groups. The better healing responses in OVX-T than Sham-T included significantly higher CW (+15.0% at week 4), earlier appearance of callus bridging (week 4.17 vs. week 4.75) and higher percentages of completed healing (66.7% vs. 41.6% at week 4; 100% vs. 83.3% at week 8), higher ratio of increment in BV/TV value (+26% vs. +18.7% from week 2 to 4), faster response of endochondral ossification (faster drop in CW, faster decrease in cartilage area) and a higher stiffness value (+37.4% at week 4 and 36.9% at week 8) [26]. These findings were consistent with a previous study using low-magnitude high-frequency vibration (35 Hz, 0.3 g where g = gravitational acceleration) with the same study design and animal model, which also demonstrated relatively better effects on osteoporotic fracture healing than on the age-matched non-osteoporotic one [29]. Similar results were also found in Rubinacci’s study which OVX non-fractured rats treated with vibration treatment (30 Hz, 3 g) showed significant increase in cortical and medullary areas,
periosteal and endosteal perimeters while Sham animals did not have any effect, which illustrated that ovariectomy may sensitize the cortical bone to mechanical stimulation [30]. All these confirm the efficacies of LIPUS on both normal and osteoporotic fracture healing, as well as osteoporotic bone can well respond to mechanical stimulation as good as age-matched normal bone. Next step is to conduct clinical trials to validate the clinical efficacy of LIPUS on fragility fracture patients.

Molecular gene profiling of osteoporotic fracture healing augmented by LIPUS

The detailed gene profile of osteoporotic fracture healing augmented by LIPUS was further investigated [31]. The results showed that collagen type 1 (Col-1) and bone morphogenetic protein-2 (BMP-2) were significantly upregulated at week 2 post-fracture in LIPUS group than control group (3.11× and 1.95× respectively) in osteoporotic rats; vascular endothelial growth factor (VEGF) was significantly upregulated at week 4 (3.51×); osteoprotegerin (OPG) was upregulated at week 2 post-fracture (1.88×), followed by the significant surge of RANKL expression (1.99×). This gene expression data further confirm the above fracture measurements, indicating the process of callus formation was increased by LIPUS during the early phase; the remodeling phase was made sooner to occur, and that angiogenesis was increased by LIPUS during the osteoporotic fracture repair process [31].

Impaired osteoporotic fracture healing is associated with delayed expression of estrogen receptors

It has been known that the immediate effects of estrogen depletion is sensed and relayed by estrogen receptors (ERs);
also, ERs can function as mechanical signal transduction through its ligand-independent function [32]. In addition, ERs have been reported to localize in fracture callus [33] that indicates the potential roles of ERs in fracture healing. Hence, there is evidence to support that the quantity of ERs may play a role in determining bone formation and fracture healing. When comparing the gene expression of ERs between Sham and OVX groups, it was found that ERs expressions were significantly higher in Sham group at week 2 and later significantly lower at week 8 than OVX group; in other words, OVX group demonstrated an opposite trend (Table 1) [34]. Meanwhile, ER-α correlated well with BMP-2 significantly (r=0.545, p=0.003), where BMP-2 is known to be important for callus formation. ERs protein expressions were further confirmed using immunohistochemistry, which appeared mostly in progenitor cells, osteoblasts and osteoclasts (Fig. 1). These findings revealed that the impaired healing of OVX-induced osteoporotic fracture may be associated with the delayed expression of ERs. The enhancement effects of LIPUS on osteoporotic fracture healing may be partially contributed by the local increase of ERs expression in fracture callus, which becomes more sensitive to mechanical signals leading to promoted fracture healing but this however needs further experiments to verify.

Acknowledgement

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Conflict of interest

The authors have no conflict of interest.

References

Bone graft substitutes and bone morphogenetic proteins for osteoporotic fractures: what is the evidence?

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KEYWORDS
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ABSTRACT
Despite improvements in implants and surgical techniques, osteoporotic fractures remain challenging to treat. Among other major risk factors, decreased expression of morphogenetic proteins has been identified for impaired fracture healing in osteoporosis. Bone grafts or bone graft substitutes are often used for stabilizing the implant and for providing a scaffold for ingrowth of new bone. Both synthetic and naturally occurring biomaterials are available. Products generally contain hydroxyapatite, tricalcium phosphate, dicalcium phosphate, calcium phosphate cement, calcium sulfate (plaster of Paris), or combinations of the above. Products have been used for the treatment of osteoporotic fractures of the proximal humerus, distal radius, vertebra, hip, and tibia plateau. Although there is generally consensus that screw augmentation increased the biomechanical properties and implant stability, the results of using these products for void filling are not unequivocal. In osteoporotic patients, Bone Morphogenetic Proteins (BMPs) have the potential impact to improve fracture healing by augmenting the impaired molecular and cellular mechanisms. However, the clinical evidence on the use of BMPs in patients with osteoporotic fractures is poor as there are no published clinical trials, case series or case studies. Even pre-clinical literature on in vitro and in vivo data is weak as most articles focus on the beneficial role for BMPs for restoration of the underlying pathophysiological factors of osteoporosis but do not look at the specific effects on osteoporotic fracture healing. Limited data on animal experiments suggest stimulation of fracture healing in ovariectomized rats by the use of BMPs. In conclusion, there is only limited data on the clinical relevance and optimal indications for the use of bone graft substitute materials and BMPs on the treatment of osteoporotic fractures despite the clinical benefits of these materials in other clinical indications. Given the general compromised outcome in osteoporotic fractures and limited alternatives for enhancement of fracture healing, clinicians and researchers should focus on this important topic and provide more data in this field in order to enable a sound clinical use of these materials in osteoporotic fractures.

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Introduction
Despite improvements in the treatment of osteoporosis, osteoporotic fractures remain challenging to treat. Osteoporotic fractures have an impaired ability to heal [1,2], and often require more time to heal [3–6]. Since osteoporotic bone is less likely to heal on its own and the degree of comminution is generally high, patients often require surgery to repair the fracture. Poor bone quality, however, may complicate implant fixation. Modern angle-stable plate-screw systems and minimally invasive operative techniques have improved the stability of fixation in osteoporotic bone, but success is still not guaranteed. Due to the high porosity and low mechanical strength of osteoporotic cancellous bone, implants are often augmented with bone void fillers in order to improve outcome. Furthermore, decreased expression of bone morphogenetic proteins (BMPs) in osteoporosis combined with the essential general role of BMPs in fracture healing made BMPs attractive for improvement of impaired molecular and cellular mechanisms in osteoporotic fracture patients [7].

Bone grafts can be used to stabilize the implants and provide a scaffold for ingrowth of new bone. BMPs have the potential of de novo new bone formation due to their osteoinductive capabilities [8].

So, these materials are suitable as bone grafts fill voids, provide support, and may enhance the biological repair of the fracture or the fracture defect. This paper is aimed at providing an overview of available evidence for the use of bone graft substitutes and BMPs for the treatment of osteoporotic fractures.

Bone graft substitute materials
The limitations of autografts and allografts led to the development of bone graft substitutes. Both synthetic and
Bone graft substitute materials provide an osteoconductive matrix, but do not contain osteogenic cells or osteoinductive growth factors. Sufficient porosity, especially the presence of interconnected pores determine the ability of bone graft materials to foster ingrowth and osteointegration. Pore sizes of at least 100 μm are sufficient for osteoid formation and osseous ingrowth [12]. The presence of interconnected pores may be more critical for osseous ingrowth than the pore size per se [13,14]. Most bone graft substitute materials used for treating osteoporotic fractures contain calcium sulfate or calcium phosphate.

Calcium sulfate is a self-setting, biologically inert, moldable, and osteoinductive material that provides a scaffold for osteoblasts. It rapidly dissolves (without cellular influence) in 6–8 weeks. This may be advantageous in some cases, but if it dissolves too quickly, the augmenting effect may be lost too early, causing implant loosening.

Calcium phosphate materials include synthetic tricalcium phosphate, beta tricalcium phosphate, and coraline hydroxyapatite. The osteoconductive matrix allows osteogenic cells to create new bone under the influence of host osteoinductive factors. Calcium phosphate materials degrade at a slower rate than calcium sulfate materials, with hydroxyapatite being relatively inert. Calcium phosphate materials are available as block, granules, or cement. Blocks and granules are highly porous. They provide less initial biomechanical strength, but strength will increase upon ingrowth of new bone. Calcium phosphate cement is injected as a paste and hardens in vivo. They can be injected or molded into small bone defects and provide structural support with low porosity but good initial compressive strength.

**Use of bone graft substitutes for treatment of osteoporotic fractures**

Calcium sulfate and calcium phosphate cement have clear benefits when used for screw augmentation, as described in detail elsewhere [15]. Clinical applications described include osteoporotic fractures of the proximal humerus, distal radius, vertebra, hip, and tibia plateau.

Both calcium sulfate and phosphate cements show promising results in the treatment of proximal humeral fractures. Minimally invasive plate fixation (internal locking system (PHILOS) augmented with calcium sulfate cement (MIIG X3; Wright Medical Technology, Arlington, TN, USA) resulted in fewer complications, less reduction loss, and better joint function than plating alone [16]. MIIG 115 also resulted in fewer failed reductions when injected in the medial metaphyseal junction [17]. Reduction failed in 7.1% (1 of 14) grafted patients versus 13.3% (4 of 30) non-grafted patients. Functional outcome was good in both groups. Unfortunately, treatment allocation was not randomized. Augmentation of severely impacted valgus fractures with Norian, an injectable hydroxyapatite cement, resulted in good functional outcome [18]. Augmentation was used after open reduction with screws or buttress plate fixation. All fractures united within the first year, and no patient showed loss of reductions or osteonecrosis of the humeral head.

Clinical benefit of bone graft substitute material use in osteoporotic distal humerus fractures is undecided, as studies show contradicting results. A biomechanical study showed that cement augmentation increased the biomechanical properties in volar plating. This included significant increase in cycles and load to failure, and construct stiffness at loads >325 N as well as less fracture gap movement and screw cutting distance at the holes of the ulnar column [19]. Augmentation with calcium phosphate cement also maintained fixation of unstable distal radius fractures [20]. Garcés-Zarzalejo et al., on the other hand, stated that bone grafts and bone graft substitutes are not mandatory for the treatment of unstable distal radius fractures with locking compression plates [21]. All 60 fractures in their study (treated without graft), healed uneventfully with no significant loss of reduction. A randomized study also showed that augmentation of metaphyseal defects with calcium phosphate bone cement after volar locking plate fixation offered no benefit over plate fixation alone [22].

Two studies showed increased screw hold in spine after augmentation [23,24]. Bone graft substitutes for the treatment of osteoporotic vertebral fractures have been used for kyphoplasty and vertebroplasty. Although pain and the disability scores decreased after balloon kyphoplasty with injectable calcium phosphate cement (Callos), the augmentation properties also decreased within six months, including progression of vertebral height loss and increase in kyphotic angle [25]. Epidural leakage of the paste into the spinal canal was observed in 48.4% (15 of 26) cases. Vertebroplasty using calcium phosphate cement resulted in immediate pain relief and prevented the vertebral body from late collapse and pseudoarthrosis [26]. All 86 patients (99 vertebroplasties) had complete bone union within six months after surgery. Vertebroplasty using bisphosphonate-loaded calcium phosphate cement gave good results in sheep [27]. Pedicle screw fixation combined with transpedicular bone grafting with demineralized bone matrix (OsteoSet, Wright Medical Technology, TN, USA) restored and maintained vertebral height successfully, and patients reported less pain at three months follow-up than pre-surgery [28].

Two studies reported that cement augmentation can increase the rotational stability and screw pull-out force in osteoporotic femoral heads [29,30]. Augmentation with calcium phosphate cement enhanced the fixation stability of femoral neck and trochanteric fractures [31]. A meta-analysis, however, found no convincing evidence for the use of any orthobiologic bone cement in the augmentation of fractures of the hip [32].

Current evidence does not unequivocally support the need to use bone graft substitutes in the treatment of osteoporotic tibia plateau fractures. A meta-analysis showed that for tibia plateau augmentation, hydroxyapatite granules, tricalcium phosphate, demineralized bone matrix, allografts, and autografts all resulted in uneventful healing in >90% of cases [33]. The rapid degradation of calcium sulfate may be a disadvantage, as 11% of cases treated with calcium sulfate showed subsidence [34]. Injectable calcium phosphate cements allow to support a reduced joint surface using a noninvasive procedure. Cement extrusion into a joint cavity should be prevented as these cements will not dissolve [35].

**Preclinical studies of the role of BMPs in osteoporosis and in osteoporotic fractures**

After the key discovery of the osteoinductive potential of BMPs to form ectopic bone reported by M. Urist in 1965 [8], more than 40 different BMPs have been described in the meantime. M. Urist himself called osteoporosis a “bone-morphogenetic auto-immune disorder” [36] and certain important interactions between BMPs in the pathomechanism of osteoporosis could be identified. Genetic polymorphisms in BMP-2 were found to be responsible for familial osteoporosis [37,38]. The link between BMP-2 and osteoporosis is mainly the role of BMP-2 in the achievement of peak bone mass rather than osteolytic activity during bone loss. Both decreased anabolic activity and reduced gene expression of BMP-2 have been reported in aged rats and naturally occurring products are available. Each has its specific composition, which determines its biological and biomechanical behavior [9–11]. As such, each product will have its unique clinical indication(s).
reduced expression of BMP-2 was confirmed in mesenchymal stem cells obtained from confirmed in ovariectomized rats [39,40]. Pountos et al. [41] could show a positive effect of BMP-2 and BMP-7 on the osteogenic differentiation of mesenchymal stem cells obtained from patients with lower extremity fractures underscoring the hypothesis to stimulate fracture healing in these patients by application of BMPs.

Several studies were carried out to look at the therapeutic effect of BMPs to reverse bone loss in osteoporosis. Phillips et al. (2006) [42] looked at the effects of locally applied BMP-7 with different carriers into defects of ovine vertebrae bodies. BMP-7 showed a positive trend in improving mechanical strength and histomorphometric parameters of osteopenic vertebra without statistical significance, despite the absence of consistent change in BMD. Turgemann et al. (2002) [43] applied exogenous BMP-2 intraperitoneal into mice with type I and II osteoporosis and reported an increase of trabecular bone strength combined with an increase in the number of adult mesenchymal stem cells, increase of their osteogenic activity and proliferation as well as a decrease in apoptosis. Similar results were published for the i.v. application of BMP-6 applied in aged O VX rats [44]. Significantly increased bone volume and mechanical characteristics of both the trabecular and cortical bone, the osteoblast surface, serum osteocalcin and osteoprotegerin levels, and decreased the osteostat surface, serum C-telopeptide, and interleukin-6 were found. Bone mineral density maintained gains for another 12 weeks after discontinuation of BMP-6 therapy.

The preclinical literature on the effects of BMPs on osteoporotic fracture healing is poor. One animal study evaluated the effects of BMP-2 in a segmental tibia defect of ovariectomized vs. sham-operated rats. The BMP-2 treated animal exhibited higher fracture healing score, including callus formation, bone union, marrow changes and cortex remodeling compared to the sham group after 8 weeks [45].

Clinical evidence for the use of bone morphogenetic proteins

Only BMP-2 and BMP-7 have been licensed for the clinical use in patients. Open tibia fractures and lumbar spinal interbody fusion are official indications for BMP-2 (InductOs®, Medtronic, Tolochenaz, Switzerland; Infuse®, Memphis, USA) and BMP-7 is licensed for tibial non-unions (Osigraft®, Olympus Biotech; in the meantime withdrawn from the market). There are statements in the Summary Product Characteristics (SPC) both of InductOs® and of Osigraft® stating that the “Safety and efficacy of InductOs have not been demonstrated in patients with metabolic bone diseases” and “Osigraft must not be used in patients that have a non-union resulting from pathological fractures, metabolic bone disease (or tumors)”. This limits their official use in osteoporotic fracture patients if osteoporotic fractures are defined as pathological fractures. This is mainly due to the lacking data of the use of BMPs in osteoporotic patients and not due to documented adverse effects in this entity. Despite the theoretical benefits for improvement of fracture healing in osteoporosis, there are no published clinical trials, case series or case studies of BMP-2, BMP-7 or other BMPs in patients with osteoporotic patients. Therefore, it must be stated that there is complete absence of clinical evidence for BMP application in patients with osteoporotic fractures.

Conclusion

Given the generally compromised outcome in osteoporotic fractures and limited alternatives for enhancement of fracture healing, it should be assumed that bone graft substitute materials BMPs have been extensively studied for this entity. Therefore, it is more than disappointing that there is only very limited clinical data available on this indication that do not allow for an evidence-based algorithm. With a growing elderly population and limited treatment alternatives, the tremendous challenge of treating patients with osteoporotic fractures will become increasingly important and both bone graft materials and BMPs are still a viable option. Researchers and clinicians should grasp the opportunity to contribute towards this important topic and seriously evaluate the potential benefits and harms of these materials in osteoporotic fractures.

Conflict of interest

Volker Alt serves as a research member of the Osteosynthesis and Trauma Care Foundation, which is supported by the Stryker Corporation. He is a paid consultant for Medtronic and has been involved in speakers bureau/paid presentations for aap Implantate and Heraeus. Esther Van Lieshout has no conflict of interest.

References


Stem cell therapy: is there a future for reconstruction of large bone defects?

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\begin{abstract}
Large bone defects caused by fracture, non-union and bone tumor excision has been a major clinical problem. Autogenous bone grafting and Ilizarov method are commonly performed to treat them. However, bone grafting has limitation in volume of available bone, and Ilizarov method requires long periods of time to treat. Accordingly, there is need for stem cell therapy for bone repair and/or regeneration. Mesenchymal stem cells (MSCs) hold the ability to differentiate into osteoblasts and are available from a wide variety of sources. The route of “intramembranous ossification (direct bone formation)” by transplantation of undifferentiated MSCs has been tested but it did not demonstrate the success initially envisaged. Recently another approach has been examined being the transplantation of “MSCs pre-differentiated in vitro into cartilage-forming chondrocytes” into bone defect, in brief, representing the route of “endochondral ossification (indirect bone formation)”. It’s a paradigm shift of Stem Cell Therapy for bone regeneration. We have already reported on the healing of large femur defects in rats by transplantation of “MSCs pre-differentiated in vitro into cartilage-forming chondrocytes”. We named the cells as Mesenchymal Stem Cell-Derived Chondrocytes (MSC-DCs). The success of reconstruction of a massive 15-mm femur defect (approximately 50% of the rat femur shaft length) provides a sound foundation for potential clinical application of this technique. We believe our results may offer a new avenue of reconstruction of large bone defect, especially in view of the their high reproducibility and the excellent biomechanical strength of repaired femora.

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\end{abstract}
ossification involve an initial condensation of mesenchymal stem cells and the eventual formation of calcified bone [6]. However, intramembranous bone formation accomplishes this directly, whereas endochondral ossification incorporates an intermediate step in which a cartilaginous template regulates the growth and patterning of the developing skeletal element [6].

Intramembranous ossification

Intramembranous ossification gives rise to the flat bones that comprise the cranium. Intramembranous ossification follows four steps. (1) Undifferentiated MSCs differentiated into osteoprogenitor cells to group clusters, and ossification centers form. (2) Secreted osteoid traps osteoblasts, which then become osteocytes. (3) Trabecular matrix and periosteum form. (4) Compact bone develops superficial to the trabecular bone, and crowded blood vessels condense into red marrow.

Endochondral ossification

Endochondral ossification gives rise to long bones that comprise the appendicular skeleton, facial bones, vertebrae amongst others. Endochondral ossification follows five steps. (1) Undifferentiated MSCs differentiate into chondroblast. (2) Chondroblast secretes matrix to form the cartilage template of the future bony skeleton and the perichondrium forms around the cartilage template. (3) Capillaries penetrate cartilage and perichondrium transforms into periosteal bone

Fig. 1. Masquelet’s induced membrane reconstructive technique. A lot of cancellous bone needed for reconstruction.

Fig. 2. Distraction osteogenesis (Ilizarov technique) for reconstruction of limb length discrepancy.
collar. (4) Osteoblasts secrete bone matrix, replacing cartilage matrix to form the primary ossification center. (5) Cartilage and chondrocytes continue to grow at ends of the bone. Secondary ossification centers develop.

**Bone tissue engineering**

*Critical-sized bone defect (CSBD) in animal model*

The use of animal models plays an important role in basic biomedical research of bone regeneration in the context of tissue engineering. Long bone segmental defect models have widely used in some animal species, for the research of bone healing and regeneration. Table 1 is a summary of defect size that was created in the femoral diaphysis of several species [7–20]. However, there is a lack of clear evidence that the model was actually a critical defect.

Originally, critical-sized bone defect (CSBD) was defined as the smallest size bone defect that will not heal spontaneously during the lifetime of the animal [21,22]. More recently, a CSBD is considered as the smallest size bone defect in a particular bone and species of animal that will not heal spontaneously during the experimental period. We created exact bone defect models at 1 mm intervals, and revealed that externally fixed defects of 4 mm and larger in rat femur failed to heal within the 8-week time frame [23]. Therefore, we started to use 5 mm bone defects as a CSBD model in rat femur.

**Traditional approach of bone tissue engineering**

Mesenchymal stem cells (MSCs) are multipotent cells with a high capacity for self-renewal and the potential to differentiate into several cell types, including bone (osteoblasts), cartilage (chondrocytes) and fat (adipocytes) [24–28]. In the field of bone tissue engineering, the employment of MSCs to elicit bone regeneration for the repair of bone defects has received much attention [29]. The route of “intramembranous ossification (direct bone formation)” has been preferred for the many studies. However, the use of adult bone marrow stromal cells in order to achieve bone and cartilage formation and repair, has been associated with less success and more problems than expected [30,31]. Even for the small animal model, success rate of reconstruction of a CSBD by this route was not more than 5% in our preliminary study in rat.

**Shift the paradigm from intramembranous ossification to endochondral ossification**

Endochondral ossification elicited by transplanting MSCs pre-differentiated to the chondrogenic pathway has been the focus of a number of studies in recent years [29,32–34]. Farrell E et al. summarized the several hypotheses that this route of bone formation would be more successful than intramembranous ossification [31]. Firstly, chondrocytes normally reside in an avascular tissue and as a result are “designed” to function in a low oxygen environment, similar to what they would encounter upon implantation into an unvascularised region [31,35]. Secondly, MSCs under in vitro conditions always become hypertrophic when cultured chondrogenically, the next step in the endochondral ossification pathway [31,36,37]. Thirdly, the release of factors from primed chondrogenic cells progressing along the endochondral route would be much more complex and controlled spatiotemporally than any growth factor combination one could devise in order to improve in-vivo vascularization and bone formation [31,32]. The strategy of implantation of MSCs pre-differentiated in vitro into cartilage-forming chondrocytes has been the focus of several recent studies for bone tissue engineering.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimentally produced femoral segmental defects in several species</th>
</tr>
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<tbody>
<tr>
<td>Species</td>
<td>Size (mm)</td>
</tr>
<tr>
<td>Rat [22–27]</td>
<td>4–10</td>
</tr>
<tr>
<td>Rabbit [28,29]</td>
<td>10–15</td>
</tr>
<tr>
<td>Goat [33,34]</td>
<td>20–25</td>
</tr>
<tr>
<td>Sheep [35,36]</td>
<td>25</td>
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Fig. 3. Chondrocytic phenotype of 3D-cultured MSC-DCs. Differentiation of 3D-cultured MSCs to MSC-DCs is demonstrated by positive dye staining (Alcian blue, safranin O and toluidine blue), detection of collagen type II, and non-detection of collagen types I and X. Bar = 100 μm. (Reprinted from Ref. [29] with permission of Elsevier).
Regeneration of 15 mm bone defects by implantation of MSC-DCs (MSC-derived chondrocytes)

We reported on the healing of both 5 and 15 mm femur defects in rats by implantation of MSCs pre-differentiated in vitro into cartilage-forming chondrocytes (Fig. 3) [29]. We named the cells as Mesenchymal stem cell-derived chondrocytes (MSC-DCs) [29,38]. Healing of both critical-sized (5 mm) and massive (15 mm) full-thickness femur defects in rats by implanting a uniquely fabricated PLGA (D,L-lactic-co-glycolic acid) scaffold seeded with MSC-DCs.

In femora with MSC-DC implants (both 5- and 15-mm), new bone formation is already evident at 2 weeks, and by week 4 the osteotomy gap is bridged with bony tissue. At 8 weeks, dense, newly formed cortical bone has created bone union. By the end of the study period at 16 weeks, the density of the new cortical bone has further intensified. (Reprinted from Ref. [29] with permission of Elsevier).

Fig. 4. Regeneration of 15-mm full thickness segmental bone defect by implantation of MSC-DCs block. In femora with MSC-DC implants, new bone formation is already evident at 2 weeks, and by week 4 the osteotomy gap is bridged with bony tissue. At 8 weeks, dense, newly formed cortical bone has created bone union. By the end of the study period at 16 weeks, the density of the new cortical bone has further intensified. (Reprinted from Ref. [29] with permission of Elsevier).

Origin of newly formed bone tissue: host or donor?

What is the origin of newly formed bone tissue in implantation of MSC-DCs? We reported in vitro implantation of human MSC-DCs in SCID mouse. Immunohistochemistry using mouse and human specific COL1A1 antibodies identified the newly formed bone as mouse specific, indicating that chondrocyte formed matrices are replaced by host derived osteogenic cells and that they function as extremely effective inducers of bone formation [38].

Conclusion

In summary, the etiology and treatment modalities of non-union and CSBD continue to be a subject of great interest to clinicians [39–45]. Stem cell therapy could be an option to manage the treatment of large bone defects in the future and to resolve the several problems associated with the current surgical procedures such as the Masquelet technique and the Ilizarov technique. Paradigm shift from intramembranous ossification to endochondral ossification occurred in the field of bone tissue engineering. Pre-differentiation towards the chondrogenic pathway to induce endochondral bone regeneration after transplantation is an innovative strategy for bone tissue engineering. Advancement of this strategy may open a new avenue of reconstruction of large bone defects.

Conflict of interest

The authors declare no conflict of interest associated with this manuscript.

References


Effects of macroporous, strontium loaded xerogel-scaffolds on new bone formation in critical-size metaphyseal fracture defects in ovariectomized rats

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Introduction

As one implication of over aging societies in industrial countries, osteoporosis is an increasing disease with high costs for social systems. Even low energy trauma as falls from height result in complex fractures with bony defects mainly located in the metaphyseal area of the long bones. To investigate new bone substitute materials for this specific application, it is necessary to reliably simulate the clinical fracture defect situation in osteoporotic patients. We recently established a clinically relevant metaphyseal fracture defect model in ovariectomized rats with osteopenic bone status in which different biomaterials can be tested [1]. In the next step, we showed that strontium (II) modified calcium phosphate cement can stimulate bone healing in this model compared to plain calcium phosphate cement [2].

KEYWORDS

metaphysis
fracture defect
biomaterial
scaffold
strontium
osteoporosis

ABSTRACT

New bone formation was studied in a metaphyseal fracture-defect in ovariectomized rats stimulated by a plain and a strontium-enriched macroporous silica/collagen scaffold (ScB30 and ScB30Sr20) and a compact silica/collagen xerogel (B30). 45 female Sprague-Dawley rats were randomly assigned to three different treatment groups: (1) ScB30 (n=15), (2) ScB30Sr20 (n=15), and (3) B30 (n=15). 12 weeks after bilateral ovariectomy and multi-deficient diet, a 4 mm wedge-shaped fracture-defect was created at the metaphyseal area of the left femur. A 7-hole T-shaped plate at the lateral aspect of the femur stabilized the bone and the defect was filled with ScB30, ScB30Sr20 or B30 subsequently. After six weeks, histomorphometrical analysis revealed a statistically significant higher bone volume/tissue volume ratio in the ScB30Sr20 group compared to ScB30 (p=0.043) and B30 (p=0.0001) indicating an improved formation of new bone by the strontium-enriched macroporous silica/collagen scaffold. Furthermore, immunohistochemical results showed increased expression of BMP2 and OPG and a decreased RANKL expression in the ScB30Sr20 group. This was further confirmed with the gene expression analysis where an increase in prominent bone formation markers (ALP, OCN, Runx2, Col1a1 and Col10a1) was seen. No material remnants were found in the scaffold group indicating an almost complete degradation process of the biomaterials. This is confirmed by ToF-SIMS analysis that did not detect any strontium in the ScB30Sr20 group neither in the defect nor in the surrounding tissue. Taken together, this study shows the stimulating effects of strontium through increased bone formation by up regulation of osteoanabolic markers. This work also indicates the importance of material porosity, geometry and biodegradability in bone healing.

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Strontium has been shown to have a two-fold action on bone formation, enhancing bone forming osteoblasts on one hand and inhibiting bone resorbing osteoclasts on the other [7,8]. Studies on healthy animals also show local administration of strontium, in the form of strontium ranelate stands better in relation to the oral administration [9,10]. Systems that aid the local release of strontium include: strontium substituted hydroxyapatites [11,12], surface modified metal oxide layers with strontium salts [13], strontium containing titanium implants [14,15], strontium loaded nanotube arrays [16,17] and strontium substituted bioactive glasses [18].

We recently developed a compact xerogel and a macroporous scaffold based on a two-phase construction of silica and collagen [5,6,19]. Additionally, we loaded the scaffold with strontium to enhance bone formation. The intention of this work is to analyze new bone formation processes in the above-described animal model induced by macroporous scaffolds based on a silica/collagen matrix as well as strontium-modified scaffolds. Therefore, the first intention of the current work is to determine the effects on bone formation in presence of a strontium-modified scaffold (ScB30Sr20) in comparison to a strontium-free scaffold (ScB30), and a compact xerogel (B30) in the above-mentioned clinically relevant fracture-defect model [1]. Second aim is the detection of the released strontium in the surrounding tissue by time of flight secondary ion mass spectrometry (ToF-SIMS) analysis and the investigation of the degradation process of the biomaterials.

**Materials and methods**

*Ethics statement and animal study*

After approval of the animal application by the local authorities according to the Protection of Animals Act (Reference number: V 54 – 19 c 20-15 (1) G1 20/28 Nr. 108/2011), 45 female Sprague-Dawley rats were used: 8 for histological and ToF-SIMS analysis and 7 for molecular analysis (per group). Each rat was randomly assigned to three different treatment groups: (1) silica/collagen xerogel scaffold (ScB30) (n=15), (2) strontium-modified silica/collagen xerogel scaffold (ScB30Sr20) (n=15), and (3) silica/collagen compact xerogel (B30) (n=15). First, we induced an osteopenic bone status by bilateral ovariectomy combined with a multi-deficient diet as described previously [1]. Briefly, under general anesthesia with intraperitoneal injection of ketamine (62.5 mg/kg bodyweight, Hostaket®, Hoechst) and xylazine (7.5 mg/kg bodyweight, Rompun®, Bayer) bilateral ovariectomy was performed. The animals were allowed two weeks of recovery and were then fed with calcium-, phosphorus- and vitamin D3-, soy- and phytoestrogen-free diet (10 mm pellets, Altromin-C1034, Altromin Spezial futter GmbH, Lage, Germany) for three months. Bone status was ensured through measurement of bone mineral density (BMD g/cm²) by means of Dual-energy X-ray absorptiometry (DXA) using DXA scanner (Lunar prodigy, GE Healthcare, Germany). Subsequently a 4 mm defect in the distal femur metaphysis was generated, stabilized with a T-shaped mini-plate [1]. The defect was filled either with ScB30, ScB30Sr20, or B30 (Fig. 1). After six weeks, femurs were harvested for detailed investigations. In case of plate fixation failure, e.g. breakage or loosening, specimens were not taken to further analysis.

*Preparation of compact xerogels and macroporous scaffolds*

Compact silica/collagen xerogels were prepared as follows. The collagenous component was prepared by dialysis (MWCO 12–14 kDa, Roth, Germany) of bovine tropocollagen type I (GFN, Germany) against deionized water followed by fibrillation in 30 mM neutral sodium phosphate buffer solution, lyophilisation (Christ Alpha 1–4 laboratory freeze-dryer, Germany), and resuspension in 0.1 M TrisHCl pH 7.4 (Roth) to obtain a homogeneous 30 mg/ml suspension [6]. The silica component was prepared by 1 h hydrolysis of tetraethoxysilane (TEOS, 99%, Sigma, Germany; molar ratio TEOS/water = 1/4) under acidic conditions (0.01 M HCl) to obtain silicic acid followed by cooling in a fridge. In the
next step vigorous stirring of calculated volumes of silicic acid and collagen suspension to obtain a final composition of 70% silicic acid and 30% collagen (sample label: B30) resulted in the formation of 800 μl hydrogels. Strontium carbonate was introduced as a third phase by being previously added (sample label: B30Sr) to the collagen suspension. In this case the final gel composition was 50% silicic acid, 30% collagen, 20% strontium carbonate. Mixtures of B30 or B30Sr were transferred to molds and gels were stabilized for 3 days followed by gel drying for 7 days in an Espec SH-221 climate chamber (Japan) at 37°C and 95% relative humidity and finished by applying a climate ramp to achieve ambient conditions. The resulting disc-like compact xerogel samples (diameter: 5 mm, height: 3 mm) were ground and classified according to the particle size. On the one hand B30 xerogel particles <0.250 mm were compacted using a custom-made pressing tool to obtain monolithic B30 xerogel samples exhibiting exactly the above described bone defect shape. On the other hand B30 or B30Sr xerogel particles <0.120 mm were added to a 30 mg/ml collagen suspension adjusting a xerogel/collagen weight ratio of 1/1. These xerogel particle/collagen suspensions were transferred to custom-made silicon molds to again obtain the above described bone defect shape, cooled at 0.5 K/min to -20°C (Espec SH-221 climate chamber, Osaka, Japan) followed by freeze-drying (Christ Alpha 1-4 lab freeze-dryer, Osterode, Germany). The scaffolds were chemically cross-linked by immersing in 1 wt% N-((3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC)/N-hydroxysuccinimide (NHS) (Sigma) in 40% ethanol for 24 h. Finally, the scaffolds were rinsed in deionized water and freeze-dried again. According to the xerogel particle composition used, scaffold sample labels are ScB30 or ScB30Sr20, respectively. All xerogel or scaffold samples were gamma-sterilized at 25 kGy before used for implantation.

Animal surgery

10 weeks old female Sprague-Dawley rats were obtained from Charles River (Sulzfeld, Germany). Followed by an acclimatization period of four weeks, the animals were randomly assigned to the three treatment groups. Induction of an osteopenic bone status was then performed by bilateral ovariectomy using a dorsal approach and a low calcium-, phosphorous- and vitamin D3-, and soy- and phytoestrogen-free multi deficient diet (Altromin-C1034, AltrominSpezialfutter GmbH, Lage, Germany) for 12 weeks as described earlier [1]. After 12 weeks, a wedge-shaped fracture-defect with a length of 4 mm and a medial gap of 0.35 mm at the distal metaphysis of the left femur using an ultrasound bone saw (Piezosurgery® 3, Saw blade OT7S-3, Metcron, Köln, Germany) was created. The fracture-defect was stabilized by a T-shaped 7-hole mini-plate (Leibinger® XS-miniplate, Styrek, Schönkirchen, Germany) as described by Alt et al. [1]. The fracture-defect was subsequently filled either with ScB30, ScB30Sr20, or B30 (Fig. 1A–C) according to the randomization protocol. Multi-deficient diet was continued until 6.4 × 1012 1/cm². Mass spectra from 100 × 100 μm² areas (300 scans, 128 × 128 pixel) were taken 6 weeks after surgery.

Sample processing, staining procedures and histomorphometry

The femurs were harvested after six weeks and fixed in phosphate-buffered 4% paraformaldehyde for 48 hours at 4°C until processing. Samples were then embedded in Technovit® 9100 NEU according to the manufacturer’s protocol (Heraeus Kulzer, Hanau, Germany) and were sectioned into 5 μm thick slices with the aid of Kawamoto’s film (Section-Lab Co. Ltd., Japan) in order to avoid loss of biomaterials. Qualitative and quantitative morphological analyses were done on sections stained with movat-pentachrome and von-Kossa/van Gieson as described earlier [20,21].

For the histomorphometric analysis of new bone formation, osteoid formation, macrophage count and implant retention, the original ROI comprising the initial wedge shaped osteotomized defect area was used (Fig. 1D). With the help of Adobe Photoshop CS6, the measurements for ROIs, area of bone, implant, osteoid and the void (sectioning artifacts) were made respectively to determine the ratio of bone volume versus tissue volume (BV/TV), osteoid volume versus tissue volume (OV/TV) and implant retention in the defined ROI. Count for ED1 positive cells per trabecular area (Macrophage/Tb.Ar) was also performed. The measurements were done blind folded concerning the test groups. The consecutive sections were then used for immunohistochemical and ToF-SIMS analysis.

Immunohistochemistry

Immunohistochemistry was carried out with the following antibodies: Rabbit Anti-BMP2 Polyclonal Antibody (AP20597PU-N; Acris), Rabbit Anti-Osteoprotegerinen Polyclonal Antibody (250800; Abbiotec), Rabbit Anti CD254/ RANKL Polyclonal Antibody (AP30826PU-N; Acris), Monoclonal Mouse Anti-Human Muscle Actin (M063S; Dako) and Mouse Anti-Rat Monocytes/Macrophages Polyclonal Antibody ED1 (MAB1435; Chemicon), respectively.

Goat Anti-Rabbit (BA-1000, Vector) was used as a secondary antibody for BMP-2, OPG and RANKL followed by a Vectastain ABC kit (Elite PK-6100, Standard, Vector Laboratories, Burlingame, CA, USA). Finally visualization was done using Nova Red (SK4800, Vector Laboratories, Burlingame, CA, USA) and hematoxylin (Shandon Inc., Pittsburgh, USA) as a counterstain. For ED1 and ASMA, antigen identification was done using DakoEnvision+System-HRP (DAB) for use with Mouse Primary Antibodies (Dako, K4006).

Images were taken using Axioplan 2 Imaging system (Carl Zeiss, Germany) with a Leica DC500 camera (Leica, Bensheim, Germany), acquired with Leica IM1000 software and processed using Adobe photoshop CS6.

ToF-SIMS measurements

5 μm thick slices of the embedded bone were deplastified with 2-methoxyethylacetate (MERCK, Germany). 3 samples of each group were measured with a ToF SIMS 5–100 machine (ION-TOF Company, Münster, Germany) using Bi3+ in positive polarity. The samples were scanned 3 times (10 shots/pixel and 10 frames per patch) with a resulting total dose density of 6.4 × 1012 1/cm². Mass spectra from 100 × 100 μm² areas (300 scans, 128 × 128 pixel) were taken with Bi3+ in positive polarity ((Bi3+) = 0.38 pA). Data evaluation was done with Surface Lab 6.3 software (ION-TOF Company, Münster, Germany). Details of the applicability of ToF-SIMS for bone imaging are previously described [22].

mRNA preparation and gene expression analysis

The left femur was obtained 6 weeks after material implantation. Samples were snap frozen in RNA later® RNA Stabilization Solution (Ambion, CA, USA) and stored at -80°C until RNA isolation. The gene expression analysis was carried out for the following target genes:
(A) New bone formation: 1. Alkaline phosphatase (ALP), an osteoblast marker indicating bone mineralization; 2. Osteocalcin (OCN), a non-collagenous protein secreted by osteoblasts, which plays a role in mineralization and calcium ion homeostasis; 3. Collagen type1 alpha1 (Col1a1), a hypertrophic chondrocytes marker; 4. Runt-related transcription factor 2 (Runx2), an essential protein for osteoblastic differentiation; 5. Collagen type I alpha1, a major component of type I collagen, (Col1a1).

(B) Bone resorption: 1. TNFSF11 gene (RANKL, RANK ligand as a member of the tumor necrosis factor (TNF) cytokine family, ligand for osteoprotegerin and a key factor, which regulates osteoclast differentiation and activation; 2. TNFRSF11B gene (osteoprotegerin; OPG), a decoy receptor for RANKL which works by neutralizing its function in osteoclastogenesis; 3. Carbonic anhydrase, an osteoclast marker involved in bone matrix dissolution. β2-microglobulin (B2M) was used as a reference gene. The primer pairs are provided in the Supplemental Table S1.

RNA isolation (confined to the entire defect region) of each sample was carried out using Lipid Tissue Mini Kit® (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. RNA quality and quantity was measured using Nanodrop2000® (Thermoscientific, Schwerte, Germany) and Qubit 2.0 fluorometer (Invitrogen, Darmstadt, Germany). 0.5 µg of RNA was reverse-transcribed to cDNA with the Quantitect® Kit (Qiagen, Hilden, Germany) as per the manufacturer’s protocol. Primers were designed to amplify 72-191 bp long amplicons within the coding sequences of the above described target genes.

Quantitative polymerase chain reaction (qPCR) analysis for Runx2-ALPL-, OCN-, Col1a1-, Col10a1-, RANKL- and OPG-primers and B2M reference gene primers was performed using the LightCycler detection system (Roche, Mannheim, Germany) in combination with the Quantifast SYBR Green PCR Mastermix® (Qiagen, Hilden, Germany). Roche LightCycler Kit® was used for Car2-primers and B2M- reference gene-primers. The real time analysis was done in a total reaction volume of 10 ml comprising of 1 ml cDNA, 5 ml Quantifast SYBR Green PCR Mastermix or 2 ml Roche 5× PCR Mastermix and 0.1 ml of each primer (20 nM). The final volume of 10 ml was achieved with RNase free H₂O.

The thermal cycling program with Quantifast Mastermix® consisted of an initial denaturation step of 5 min at 95°C, followed by 40 cycles of 10 s at 95°C and 30 s at 60°C. Finally, the product’s specificity and identity was verified by a melting curve analysis.

qPCR analysis using Roche PCR Mastermix® comprised of a two-step cycling protocol consisting of an initial denaturation step of 10 minutes at 95°C and a combined cycling protocol which includes a denaturation step at 95°C for 5 s and an annealing step at 60°C for 5 s followed by a final extension at 72°C for 5 s carried out for 40 cycles. A melting curve analysis was finally done in order to determine the product’s specificity. All analyses were done in duplicate and the mean was used for further calculations. The following were used as controls: every sample processed without reverse transcription (−RT); as a control for contamination with genomic DNA and RT-PCR runs without template (H₂O). Specificity of amplification was confirmed by melting curve analyses and 2% agarose gel electrophoresis. The amplification efficiency for the tested primer pairs ranged from 1.93 to 2.00 which is ideal for the genes thus compared.

**Statistical analysis**

The data were checked for normal distribution and homoscedasticity. One-way ANOVA along with Tukey’s multiple comparison tests was adopted to determine the variation between the groups in histomorphometrical analyses. If the requirements were not complied nonparametric Mann-Whitney U test was performed. All the statistical analysis was done using SPSS V. 20.0 (SPSS Inc., USA). The asterisks indicate the significance level *p<0.05, **p<0.01 and ***p<0.001 respectively. P-values of less than 0.05 were chosen to indicate significance.

**Results**

**General information**

In vivo BMD measurements using DXA reflected osteopenic bone status of the femur, statistical analysis showed a BMD decrease from the day of ovariectomy to euthanasia (p=0.026, Supplemental Table S2). Out of 45 rats, 4 animals were lost during anesthesia and 2 died shortly after ovariectomy resulting in n=12 in the ScB30 group, n=13 in the ScB30Sr20 group, and n=14 in the B30 group. Regular wound healing was observed At the time of euthanasia, plate failure was seen in one rat of ScB30 group and five rats of B30 group. Plate breakage comparison was higher in B30 than ScB30Sr20 group (p=0.02). No significant difference was seen in ScB30 vs. ScB30Sr20 (p=0.30) and ScB30 vs. B30 (p=0.10). Instable specimens were not used for further analysis.

**Histology and histomorphometry**

Descriptively, material retention was more in B30 fracture gap compared to the other groups, which showed negligible material remnants. B30 group showed debris, vacuoles and foam cells beside neutrophil granulocytes (Supplemental Fig. 2). Furthermore, an increased mineralized tissue formation along with enhanced chondrocyte and cartilage distribution in ScB30 and ScB30Sr20 when compared to B30 group was seen.

Histomorphometric analysis showed a statistically significant increase in bone formation (BV/TV expressed as [mean±SD; minimum: maximum]) in the entire defect region in ScB30Sr20 [0.122±0.026; 0.1: 0.15] when compared to ScB30 [0.089±0.009; 0.08 :0.1] (p=0.043) and B30 [0.034±0.005; 0.027: 0.039] (p=0.0001), respectively (Fig. 2D). A significant increased osteoid volume (OV/TV) was seen in B30 [0.123±0.017; 0.105: 0.147] when compared to ScB30 [0.032±0.005; 0.023: 0.035] (p=0.0001) and ScB30Sr20 [0.026±0.004; 0.021: 0.030] (p=0.0001) (Fig. 2E).

B30 degradation was slow, showing a statistically lower implant retention of ScB30 and ScB30Sr20 in the defect area [2.01±0.005; 2.0: 2.01] (p=0.0001) (Fig. 3B). More ED1 positive cells were seen in ScB30 group [11.9±0.455; 11.5: 12.5] than in B30 group [9.285±0.227; 9.1: 9.6; p=0.0001] and the least in the ScB30Sr20 group [5.4±0.391; 5.0: 5.9] (p=0.0001; Fig. 3A).

Detailed histology at 6 weeks post operation exhibited increased mineralized tissue replacement of ScB30 and ScB30Sr20 materials in comparison to a highly retained B30. Around the non-degraded B30 were abundant neutrophil granulocytes (Supplemental Fig. 2). Furthermore, an increased mineralized tissue formation along with enhanced chondrocyte and cartilage distribution in ScB30 and ScB30Sr20 when compared to B30 group was seen.

**Immunohistochemical analysis**

Immunohistochemical staining revealed higher BMP-2 and OPG signals in the ScB30Sr20 group than in ScB30 group. BMP-2 and OPG signals were hardly detected in B30 group (Fig. 4 A–C). Analysis of the RANKL signal intensity was highest in ScB30, slightly less in B30 and lower in the ScB30Sr20 (Fig. 4 G–I). a-SMA

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Fig. 2. Histomorphometrical analysis of movat pentachrome stained technovit-embedded sections of ScB30, ScB30Sr20 and B30 groups. Pictorial representation of movat pentachrome staining depicting the highest bone formation (A–C) and Von Kossa/van Gieson stained sections showing osteoid formation (A’–C’). Were ScB30Sr20 (A and A’), ScB30 (B and B’) B30 (C and C’). Quantification of the bone volume over tissue volume (BV/TV) shows statistically significant increase in the bone formation in the whole defect region in the ScB30Sr20 and ScB30 group in comparison to the B30 group alone (D). A statistically significant increase in osteoid volume over tissue volume (OV/TV) in B30 group when compared to ScB30 and ScB30Sr20 groups, respectively (E). The asterisks indicate *p<0.05, **p<0.01 and ***p<0.001 respectively. Specific regions are labeled as follows: b, bone; m, material; Os, osteoid.

Fig. 3. Macrophage count was done using ED1 antibody on undecalcified plastic sections of ScB30, ScB30Sr20 and B30 groups. Quantification of the count for macrophages over trabecular area (Ma/Tb.Ar) confirms significant increase in the ED1 positive cells in the entire defect region of the ScB30 group followed by B30 and ScB30Sr20 groups respectively (A). Photomicrographs of ED1 stained sections showing an elevated distribution of brown stained cells in ScB30 group (C) when compared to ScB30Sr20 (D) and B30 groups (E). Graphical representation of the implant retention in the entire defect region showing highest retention in case of B30 group, which is almost negligible in ScB30 and ScB30Sr20 groups (B). The asterisks indicate *p<0.05, **p<0.01 and ***p<0.001 respectively.
detection of revascularization revealed a comparatively higher number of vessels in the ScB30Sr20 and ScB30 group respectively compared to B30, which showed scarce revascularization in the biomaterial vicinity. These vessels were comparatively fewer and smaller when compared to the other two groups (Fig. 4 J–L).

### Molecular biology

qPCR expression analysis results showed differences between the ScB30Sr20 and ScB30 groups (Fig. 5) Relative expression of osteoblast markers ALP (p=0.035), OCN (p=0.049), Runx2 (p=0.030) and Col1a1 (p=0.030) were higher in the ScB30Sr20 group when compared to the B30 group (Fig.5 A–D ). Additionally gene expression analysis indicates an up-regulation of Col10a1, marker for hypertrophic chondrocyte in the ScB30Sr20 group when compared to the B30 group (p=0.035; Fig. 5E).

Interestingly, increased expression of ALP (p=0.022), OCN (p=0.022), Runx2 (p=0.027), Col1a1 (p=0.018) and Col10a1 (p=0.036) were seen in B30 when compared to ScB30 (Fig 5 A–E). In addition an increased expression of OCN (p=0.044) and Col1a1 (p=0.044) was seen in B30 compared to ScB30Sr20 group respectively (Fig. 5 B and D). However no differences in the catabolic genes were seen in between the groups (Fig. 5 E). Although a trend was seen with a decreased catabolic effect exerted by the ScB30Sr20 group when compared to ScB30 and B30 groups respectively (p=0.052).
ToF-SIMS analysis

Almost no significant amounts of Ca⁺ can be found inside the defect area in all implanted materials (Fig. 6 A–C). Silicon is distributed heterogeneously over the sample (Fig. 6 A and B). No compact remaining biomaterial is visible any more. In contrast, the remaining compact xerogel is clearly found in the mass image (Fig. 6 C). The bone section with the ScB30Sr20 material shows orange spots (Ca⁺) inside of the defect area, which indicate few mineralized areas (Fig. 6 B).

To confirm the Sr loading of the scaffold we analyzed the mass spectra of ScB30Sr20 before implantation (Fig. 6 B2: green line) and registered early a Sr signal with more than 100 counts. To determine the distribution of released strontium from the material six weeks after implantation, the mass spectra of the Sr signal in the specimen treated with ScB30Sr20 was performed (Fig. 6 B1, 2). Almost no strontium was detected in the implant area six weeks after surgery (Fig. 6 B2: orange line). Furthermore, an increased Sr signal outside the defect area was not found either.

Discussion

Bone loss and decreased bone quality is the hallmark of osteopenic and osteoporotic bone. The rat model reproduced in this study was recently characterized [23]. DXA analysis showed decreased BMD in response to treatment. This study aimed at enhancing healing of osteoporotic metaphyseal fractures by using collagen scaffold with and without silica and strontium modification.

Diaphyseal healing in osteoporotic fractures was compared extensively to healthy bone. In general studies showed compromised biomechanical competence and discrepancies in cellular composition in osteoporotic fractures [24]. Likewise, clinical studies on osteoporotic patients showed healing delay in intramedullary fixed femoral diaphyseal fractures. Interestingly, this diaphyseal fracture delayed healing was rarely reported to develop into a non-union [25]. Although, osteoporotic fractures at the metaphyseal region are more frequent than diaphyseal fractures [26], less information is available because until recently [1] no preclinical models were available. Healing requires precursor cells as in the periosteum, which are less available in metaphyseal fractures than diaphyseal fractures [27]. Therefore; osteoporotic diaphyseal fracture models utilized an osteoconductive approach rather than an osteoinductive one to enhance healing. Food and Drug Administration (FDA) guidelines [28] advised ovariectomized rats to study osteoporosis. The OVX+Diet rat model study was extensively characterized [23] and was utilized previously to study material-aided bone healing [2]. Furthermore, this study considered the nutritional aspects that reflect on bone healing, as osteoporotic patients usually treated with nutritional supplemenations for prevention and during fracture healing [29,30]. The lack of such nutrients such as vitamins D and K which were reported to protect bone after ovariectomy by reducing the induced bone loss resulting from estrogen deprivation [31-35], show the maximum effect of the described biomaterials on healing.

Clinically, establishment of a stable callus formation at the early stage of healing is detrimental; therefore a comparable time point of 6 weeks post op was investigated in this study. Our aim is to emerge the influence of different bone substitute materials, amongst others enriched with strontium carbonate, on this early stage of fracture healing in alliance with a reduced stress on the animal. This is in accordance with the recommendation of Garcia et al. [36] who suggest an observation period of 15 weeks in total, but with separate time-points after 2, 5 and 10 weeks after fracture induction. Recently, we have described metaphyseal bone healing in union (3 mm) and non-union (5 mm) osteoporotic and non-osteoporotic metaphyseal fractures. The 3 mm sized defect showed a bony consolidation and woven bone filling the gap in the controls were osteoporotic animals, despite the bony cortical bridging, showed lower biomechanical competence, cartilage remnants and unmineralized tissue that indicated delayed healing [2]. In the 5mm sized fracture gap, both osteoporotic and non osteoporotic animals showed no bony consolidation or cortical bridging, cartilaginous remnants and unmineralized...
tissue were abundant in both groups [1]. The 4 mm sized-fracture defect described here still showed no biomechanical competence at the early stage of healing (Supplemental Table S3).

To study enhanced healing in a clinically relevant metaphyseal fracture-defect in osteopenic rat bone silica/collagen scaffold used as a carrier to strontium and was tested with and without strontium modification. The strontium-loaded scaffold ScB30Sr20 resulted in a significantly higher bone formation (BV/TV) in the defect area in comparison to ScB30 and the compact xerogel B30. This accords with IHC analysis that showed higher signal intensity of BMP-2 and OPG in the ScB30Sr20. Moreover, RANK-L expression was lower in the ScB30Sr20 group than the other two. Both observations suggest an effect of strontium that enhances bone formation through inhibiting RANK/RANK-L pathway [7].

OPG acts as decoy receptor for RANKL and thereby neutralizes its function in osteoclastogenesis. It inhibits the activation of osteoclasts and promotes osteoclast apoptosis. Therefore, local OPG/RANKL ratio indicates bone homeostasis and whether resorption is initiated or termination is triggered. The relative expression was not different in any of the groups. This result was expected as the 6 weeks time point represents the early healing stage of bone and none of the materials could have enhanced osteoporotic bone healing into the remodeling stage. Intriguingly the collagen group (B30), showed higher expression of anabolic genes when compared to the ScB30 and sometimes higher than ScB30Sr20. This increase however exhibits an imbalanced cellular composition of the fracture gap, suggesting that the presence of collagen influences the anabolic gene expression and cartilage formation leading to the increased osteoid area and smaller cartilage portion seen in B30 histomorphometrical evaluation. Type X collagen (Col 10a1) is considered a hypertrophic chondrocyte marker and its increased expression in the ScB30Sr20 confers the increased mineralized portion area in the same group in comparison to the ScB30 group. Osteoblast precursors and ultimately mature osteoblasts synthesize a matrix of osteoid composed mainly of type 1 collagen [37], that was increased in the ScB30Sr20 when compared to ScB30. Mature osteoblasts mineralize the osteoid matrix and are subject to many solubility factors such as Runx2...
[38], which was also higher in the ScB30Sr20 group. This along with the higher expression of osteocalcin, a bone extracellular matrix marker; and ALPL as a mineralization marker indicated an enhanced bone formation process.

In support to the data above, generated osteoanabolic enzymes were also detectable by IHC. We recently could show this strontium associated positive effect on osteogenesis with strontium-modified calcium-phosphate cement (SrCPC) [2] as well as Li et al. in a study on strontium-substituted hydroxyapatite coatings [10] or Vestermark et al. with a strontium-enriched hydroxyapatite bone graft extender [39]. Hott et al. showed an increased bone formation especially in the cancellous bone in the metaphyseal area in rats after treatment with strontium [40]. Comparing the strontium-free silica/collagen scaffold ScB30 with the solid xerogel B30, we detected a statistically significant increased BV/TV ratio in the scaffold group. This strontium-independent positive effect might be explained by the macroporous structure of the scaffold (Supplemental Fig. S1) that showed enhanced osteoconductive properties. The stimulating impact of the scaffolds is also visible in the immunohistochemical analyses. Furthermore, revascularization is an essential factor in bone regeneration and an increase in vessel numbers in the silica/collagen scaffolds might be due to their higher porosity (Supplemental Fig. S1) making them favorable for blood vessels formation. This effect was clearer seen in the strontium-doped scaffold because of its ability to induce angiogenesis [41].

The B30 group exhibited an increased presence of non-degradable implant material particles, segmented neutrophil granulocytes along with elevated levels of ED1 protein expression, debris, vacuoles and foam cells indicating a debris-induced inflammation mediated by macrophages. The highest count of ED1 positive cells was seen in the ScB30 group, which could be attributable to the active remodeling in this group. The fact that remnants of the implanted biomaterial were only seen in the B30 and not in the ScB30Sr20 or in the ScB30 group shows a fast degradation process with complete degradation of both scaffold materials six weeks after implantation. This is confirmed by ToF-SIMS analysis. Strontium counts could not be detected anymore in the ScB30Sr20 group indicating a complete degradation of the strontium-loaded scaffold. On the other hand, strontium was recently reported to antagonize NFκ-B activation of macrophages as osteoclast precursors [42]. Furthermore, strontium was correlated to lower RANKL expression [43] that enhanced osteoblast formation [44]. Both reports accord with our findings in lower macrophage numbers and lower RANKL expression and signal in the ScB30Sr20 group. However, the osteoanabolic effects of the released strontium were still seen by the increased BV/TV ratio, BMP-2 and OPG intensity (immunohistochemical analysis), as well as ALPL, OCN, Runx2, Col1a1 (gene expression analysis) as seen in the ScB30Sr20 group. However, a prolonged degradation process could potentially further improve new bone formation by better Sr-release kinetics and conservation of the osteoconductive architecture.

Plate failure in the B30 group is a clear demonstration of clinical challenge of non-healing fractures in osteoporotic patients. The inferior bone formation in the B30 was indicated by a less BV/TV ratio in comparison to the scaffold groups. The lack of Sc and Sr reflected on tissue regeneration in the B30 animals where no bony bridging was enhanced and resulted in compromised stability leading to over load the plate until breakage. A similar effect was seen in the comparable strontium-CPC study in which plate failure rate was slightly less in the SrCPC-group compared to pure CPC or the empty defect due to better new bone formation in the SrCPC group [2].

Conclusion

The current study confirms the potential of strontium in enhancing bone healing in diseased bone. Although, the study describes one time point for the analysis; different bone regeneration outcomes could be identified. Nonetheless, pathway or cellular contribution shall be validated in future studies. Interestingly, under conditions of inferior bone mineral density and mal-nutrition combined with estrogen deficiency the study highlights the importance of strontium and material porosity in biomaterial-aided bone healing. The gained knowledge should be utilized to explore functional and molecular alterations in the healing process to optimize materials geometry and strontium release to the most favorable time points. Therefore, more considerations to strontium role in inhibiting macrophages proliferation and RANKL expression are required so that the later remodeling phase of bone healing is not affected. However, we could anticipate the geometrical material properties (porosity) contributed to the osteoconductivity of the material and that the strontium interfered with bone resorption caused by estrogen deficiency and malnutrition. Nevertheless, underlining molecular and cellular changes of the inflammatory and remodeling phases of healing should be investigated before the material could be translated into clinical application in a more efficient impact on bone regeneration.

Conflict of interest

All authors confirm that they do not have any financial or personal conflict of interest in relation to this article.

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References


Medical management of osteoporosis and the surgeons’ role

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ABSTRACT

Osteoporosis is a worldwide public health issue and with the aging population the resultant increase in fragility fractures has generated a significant socioeconomic impact. Robust scientific research has increased our knowledge of the endocrine mechanisms and pathophysiology of osteoporosis. This information has led to Level 1 randomized clinical trials which demonstrate the beneficial effects of appropriate regimens in reducing the fracture risk and the coincident mortality. Despite these contributions the public health problem remains and has stubbornly failed many public awareness campaigns by governmental and private professional organizations. Effectiveness in delivering the message is greatly enhanced following the sentinel fragility fracture whether it be distal radius, hip, or spine. The treating orthopedic surgeon has the full attention of the injured patient who can be steered into osteoporosis screening programs and ultimately treatment. Studies in Canada have shown that if the surgeon initiates the process by so much as ordering the bone densitometry exam the patient is more likely to get treatment for their underlying disease than if it is just suggested that the patient see their medical doctor at some future date. The patient takes the cue from the surgeon. Patient compliance goes up and the treatment is instituted.

We as surgeons must be part of the solution. This has been emphasized in the worldwide efforts in orthopedic surgery such as the “Bone and Joint Decade” and “Own the Bone” programs. This commitment to bone health and restoration is important. Our patients deserve no less.

Introduction

The incidence of osteoporosis around the globe continues to increase in almost epidemic proportions, as life expectancy continues to increase [1–3]. In the Unites States, it is estimated that osteoporosis affects >10 million individuals over the age of 50 years and an additional 33.6 million individuals suffer from osteopenia. In addition, the prevalence of osteoporosis increases from 19% among women aged 65–74 years to over 50% in women aged over 85 years. As our population continues to age, the number of individuals aged over 50 years with osteoporosis is expected to increase to nearly 14 million by 2020 [4]. Consequently, the rate of osteoporotic or fragility fractures, defined as fractures that occur from low-energy trauma such as falls from a standing height or less as a result of poor bone quality and low bone mineral density (BMD), are also increasing. Fragility fractures are associated with higher rates of hospitalizations, a decrease in functional status, ability to live independent, and pose a high mortality rate [2–6]. In addition, these fractures result in an economic burden being placed not only on patients but on the whole healthcare system; In the United States alone, osteoporotic fractures are responsible for approximately 500,000 hospitalizations, 800,000 emergency room visits, 2.6 million physician visits, and 180,000 nursing home placements each year [4].

Fragility fractures can occur in any bone but vertebral body, distal radius, and hip fracture are the most common [2,7]. Of these, hip fractures have become an international marker of osteoporosis since they are strongly correlated with low BMD, they cause significant disability, and are associated with an increased risk in mortality. Several studies have demonstrated an increased risk in mortality following a hip fracture. Most notable, is an increased 1 year mortality rate of 10–20% in women, which is almost twice as high than seen in men [2,4,8–10]. These key figures coupled with the risk of a subsequent fragility fracture being increased 1.5 to 9.5 times after a fragility fracture [11–14], highlights a unique opportunity for orthopedic surgeons to promote osteoporosis awareness and influence patient care at the time of initial fracture care.

Although fragility fractures are associated with considerable healthcare costs and dramatic consequences, only 20% of patients with a hip or fragility fracture receive medication to treat their osteoporosis [15,16]. The reasons for which the majority of patients suffering from a fragility fracture are lacking appropriate osteoporosis treatment are multifactorial, and a thoughtful coordinated multi-disciplinary approach is needed to fully address this problem. As orthopedic surgeons are typically involved in the care of these patients during their initial fragility fracture, they can play a pivotal role in initiating patient osteoporosis screening, diagnosis, treatment, and follow-up.
However, there are several barriers that need to be addressed before a broad impact on osteoporosis management can be achieved. Primarily, orthopaedic surgeons feel undertrained to treat osteoporosis and feel the primary care physician (PCP) should lead this effort [17–19]. In a recent study surveying 2,910 orthopedic surgeons in regards to their knowledge about managing fragility fracture, less than 10% of respondents included a BMD scan when evaluating patients with a fragility fracture, 32% prescribed appropriate dosages of calcium and vitamin D, and approximately 30% would refer to the respective team to manage falling [20]. The authors concluded that the majority of orthopedic surgeons questioned lacked knowledge, in several areas, to manage fragility fractures.

Second, there is a lack of understanding and association, by patients and physicians, between osteoporosis and fracture risk [21–24]. One study surveying patients who sustained a fragility fracture highlights the lack of patient education and awareness of osteoporosis as being the cause of their fracture. This study showed that while 91% of patients were aware of osteoporosis in general prior to their fracture, only 30% of patients believed their current fracture was caused by osteoporosis; on the other hand, respondents who were previously diagnosed and treated for osteoporosis were more likely to believe that osteoporosis was the cause of the fracture sustained in the fall. Of note, elderly patients were also reluctant to add more medications to their often long list [25]. From the physicians perspective, barriers to treatment initiation included: orthopaedic surgeons feeling that osteoporosis management is not their responsibility, the cost of therapy, the time and cost of diagnosing osteoporosis, confusion about the medications available for osteoporosis and their side effects, the perception that their effectiveness is unproven, and the perception of an onerous burden to initiate secondary prevention measures added to the complex management of the elderly patients’ co-morbid medical conditions [19,21–27].

Despite these data highlighting the difficulties in getting patients into treatment following sentinel fragility fractures in the elderly there are several simple and effective strategies to improve this which can be initiated by the treating orthopedic surgeon which can significantly reduce subsequent fracture rates, patient outcomes, and mortality. Randomized controlled trials have demonstrated that treatment of osteoporosis in patients with fragility fractures can reduce the risk of subsequent fractures by up to 50% and mortality rates by up to 30% [28–30]. Since orthopaedic surgeons are central to the treatment of these fragility fractures, it is logical and efficient for them to be involved and lead in initiating diagnostic measures which are heeded by the affected patients with greater compliance [24,31]. Several studies have shown that when the orthopaedic surgeon suggests the osteoporosis screening and continues to be engaged in the treatment regime better outcomes result [25,31–33]. Additionally, studies have shown that when osteoporosis treatment starts at the time of sentinel presenting fracture the rate of secondary fractures are reduced [34,35].

Osteoporosis is one of the “silent” conditions which is discovered at the time of hip, wrist, or spine fracture. Despite its recognition it continues to be formally underdiagnosed and treated. In order to improve the short- and long-term outcomes of these patients, a coordinated multidisciplinary team approach is required. The steps by raising patient and physician awareness of osteoporosis. This must be followed by a structured educational efforts focused on primary care physicians and orthopaedic surgeons. The goal is to forge effective communication in the co-management and long range care of osteoporotic patients.

Conflict of interest

The authors have no conflict of interest to declare.

References


How do bisphosphonates affect fracture healing?

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ABSTRACT

Bisphosphonates (BPs) have been in use for many years for the treatment of osteoporosis, multiple myeloma, Paget’s disease, as well as a variety of other diseases in which there is reduced bone mineral density. Given that bisphosphonates inhibit bone resorption, an important stage of fracture healing; this class of compounds has been widely studied in preclinical models regarding their influence on fracture healing. In animal models, bisphosphonate treatment is associated with a larger fracture callus, coincident with a delay in remodeling from primary woven bone to lamellar bone, but there is no delay in formation of the fracture callus. In humans, de novo use of bisphosphonate therapy after fracture does not appear to have a significant effect on fracture healing. Rarely, patients with long term use of Bisphosphonates may develop an atypical fracture and delay in fracture healing has been observed. In summary, bisphosphonates appear safe for use in the setting of acute fracture management in the upper and lower extremity in humans. While much remains unknown about the effects on healing of long-term bisphosphonates, use prior to “typical” fracture, in the special case of atypical fracture, evidence suggests that bisphosphonates negatively influence healing.

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Introduction

Bisphosphonates have been in clinical use since 1968 (etidronate), and use of these compounds has increased in prevalence after the Food and Drug Administration approved alendronate for use in September of 1995. As such, millions of doses of bisphosphonates (BPs) have been taken worldwide and we have now begun to collect and analyze critical data regarding acute, as well as long-term consequences of their use. Herein, we will examine the current best evidence regarding the effects of bisphosphonate use on fracture healing, including evidence from animal models, as well as human studies, with the aim to inform the reader about the implications of bisphosphonate use on bone healing.

Fracture repair is a complex, multi-staged process, of which the end goal is the return of the damaged bone to a functional and biomechanically sound state. Immediately post fracture, bleeding occurs and this is followed by the formation of a hematoma at the fracture site. This creates an inflammatory environment that recruits mesenchymal stem cells (MSCs) to the site of healing, followed by expansion of these cells and their differentiation into either osteoblasts or chondrocytes. Via intramembranous ossification, the osteoblast cells form new bone on the existing bone surface, flanking the fracture site, generating the hard callus. In the center, over the site of fracture, which is a more hypoxic environment and one that is less mechanically sound, chondrocytes form a cartilaginous or soft callus via endochondral ossification. As the chondrocyte population expands, these cells become hypertrophic and the cartilage tissue is mineralized. The callus is then invaded by the vasculature, allowing for infiltration by osteoclasts that in turn remove the mineralized cartilage allowing for the ultimate bridging of the fracture by woven bone. This is followed by a secondary and more prolonged remodeling phase, which includes resorption by the osteoclasts, which converts the woven bone to lamellar bone, and remodeling of the original fractured bone below the callus, yielding bone that mechanically and anatomically matches the pre-fractured bone [1,2].

All bisphosphonates (BPs) are analogues of inorganic pyrophosphate, wherein a carbon, in place of the natural oxygen, connects the two phosphates. As a result, BPs have two side chains that can be modified to modulate their pharmacological properties. Clinically used BPs can be divided into non-nitrogen containing compounds such as etidronate, clodronate, tiludronate and nitrogen containing BPs such as pamidronate, alendronate, ibandronate, risedronate and zoledronate. All BPs have a high affinity for calcium and in the body, they concentrate in the skeleton at sites of active bone remodeling. Both classes of BPs become embedded in new bone during the anabolic phase of remodeling by binding to the hydroxyapatite of bone, where they remain inert. When bone containing a BP is resorbed the BPs are released in the acidic lacuna created by the osteoclast, and are taken up by these cells. The non-nitrogen containing BPs induce apoptosis in the osteoclast by incorporating into...
ATP and thereby reduce resorption by decreasing the number of active osteoclast cells on the bone surface. The more widely used nitrogen containing BPs inhibit farnesyl pyrophosphate synthase (FPPS), a key enzyme in the mevalonate pathway. This results in cytoskeletal changes in the osteoclast, which inhibit the activity of the osteoclast and or may induce apoptosis of these cells. Similar to the non-nitrogen containing BPs, the net result is a decrease in osteoclastic bone resorption. Because these compounds become entombed in the bone, they reside in the body long after treatment cessation and indeed, the calculated half-life of elimination of BPs from the skeleton is up to 10 years [3]. This is substantiated by the observation of detectable levels of pamidronate in the urine of patients 8 years after they had ceased treatment [4]. Given that resorption of bone by the osteoclast is a key component of fracture repair, concerns have been raised regarding BP associated inhibition of the repair process, both in situations where there has been past use of these compounds and it is known that BPs have been retained in the skeleton and in cases of acute treatment after fracture.

Materials and methods

A literature search of Medline, Google Scholar and PubMed was performed for articles addressing the subject of bisphosphonates on fracture healing and this literature search yielded 275 citations (search conducted in December, of 2014). Included among our search findings were two recent reviews of meta-analyses of human studies and these papers were not considered as a primary reference, yielding 273 papers for consideration [5,6]. Several of the articles identified addressed the topic of preclinical fracture healing with bisphosphonates. In addition to database queries, the reference lists of potentially relevant articles were also examined to identify additional relevant studies. Inclusion criteria for consideration of studies for this literature review included: 1) papers were written in English, 2) publication of the study findings in peer-reviewed journals and 3) in vitro and in vivo studies that evaluated the impaction of Bisphosphonates on fracture healing. We used the following exclusion criteria: 1) articles using languages other than English and 2) letters, reviews, expert opinion publications or other articles that were not primary reports of findings. Articles meeting the above mentioned criteria were retrieved and all of the studies related to these were extensively reviewed.

Results

The search strategies yielded a total of 273 potential articles. During the selection process, the articles were excluded by title and or by abstract, because they were clearly irrelevant to the study question. The papers meeting our inclusion criteria are described in greater detail below. These papers could be distributed into two main categories. The first category was comprised of studies which investigated the effects of BPs in animal models and the second category included the studies which examined effects of BPs use on human fracture healing.

Preclinical animal models

Several animal models have been used to examine the effects of BPs on fracture healing including mice, rats, rabbits, dogs and ovine models. Using these models, the impact of bisphosphonate administration on indirect fracture healing (healing with callus) has been extensively examined and the results have been remarkably consistent, but direct fracture healing has been less well studied. Overall, these studies of indirect healing suggest that BP administration appears to decrease the remodeling of fracture callus with a concomitant increase in fracture bridging and or retained cancellous bone structures within the callus [7,8] but it is apparent that BPs do not interfere with the formation of the callus itself [8–15]. As a result, there is a delay in the conversion of the woven bone at the fracture to mature lamellar bone [16].

Indirect fracture healing

Fu and colleagues studied fracture callus properties in ovariectomized rats using alendronate long-term and found a larger fracture callus formed in the treated animals. However, despite the observation of a delayed conversion of woven bone to lamellar bone in the intervention group, the mechanical properties of the callus were similar to control animals [16]. Manabe et al. found similar findings using ibandronate. In this study, the authors noted that extending the dosing interval could mitigate the delay of conversion of woven bone to lamellar bone in the callus [17].

Kidd and colleagues studied the effects of either a high or low dose of risedronate on stress fracture healing in a rat ulna model. In the animals treated with the higher dose (1.0 mg/kg, twice the normal dose for osteoporosis treatment), they found a delay in healing. Specifically they noted a reduction in bone resorption and in new bone formation along the fracture line at 6 and 10 weeks post fracture. This delay in healing was not observed in the animals treated with a low dose (0.1 mg/kg). Regardless of the dose of risedronate used, they observed no interference with callus formation [18]. Sloan and colleagues had similar findings in which the effects of alendronate were examined [19].

Yu and colleagues noted that early in fracture repair, there was a delay in cartilage hypertrophy and in angiogenesis and later in the repair, there a delay in remodeling of the callus, cartilage and bone in mice treated with zoledronate. This effect was more pronounced in mandible fracture healing than tibia fracture healing in their study [20]. It must be noted that this is in stark contrast to studies in rabbits, in which use of zoledronic acid was observed to accelerate mandible fracture healing [21].

Bosemark et al. used a combination of zoledronate and BMP7 in an autograft healing model in rats. While they did observe an effect of just BMP7 alone, the combined therapy resulted in a substantial increase in callus volume and a four-fold increase in mechanical strength at the healed fracture site as compared to the repair observed in the controls. The impact of the combined therapy was nearly double that for the BMP7 treatment alone with regards to callus volume and ultimate force at failure at the fracture site [22]. In a follow up study, this same group examined the combination of BMP7 and zoledronate on healing in an allograft model and found a very similar result [23]. Doi and colleagues examined the impact of zoledronic acid plus BMP2 in a rat femoral fracture model and determined that healed fractures from rats treated with either zoledronic acid alone or zoledronic acid combination with BMP2 showed greater ultimate load at failure and greater stiffness than either the control treated animals or the animals treated with BMP2 alone. The authors further concluded that the combination of BMP2 and zoledronic acid enhanced fracture fusion [24].

Another group studied low-intensity, pulsed ultrasound combined with alendronate in a rat osteotomy model, wherein the fracture was fixed with intramedullary pin. An increase in bone mineral density at the osteotomy site was observed in the ultrasound treatment alone group, the alendronate treatment alone group and in the combined treatment groups, with the greatest effect seen in the combined treatment animals. However, no mechanical testing of the healed bone was conducted in this study [25].
Direct fracture healing

Direct bone healing is typically seen with rigid internal fixation such as compression plating of a transverse fracture undergoing direct osteonal healing. Using rats wherein an osteotomy was rigidly fixed with a compression plate, Savardias and colleagues examined the impact of ibandronate given three weeks prior to fracture. They showed impairment in progression to fracture union, a reduction in mean stress at failure as assessed by 4 point bending and reduction of bone mineral density at the osteotomy site in the treated animals versus controls. Lastly, they observed the presence of cartilage-like tissue and undifferentiated mesenchymal tissue at the osteotomy site [26].

Human studies of acute fracture healing after short term and long term use of BPs

Bisphosphonates are the most commonly used therapy in humans for osteoporosis treatment. Like what was found using pre-clinical models, most studies report that fracture callus formation is larger but that healing is not impaired in patients treated with bisphosphonates compared with untreated patients [27,28]. Indeed, a large meta-analysis of 8 randomized controlled trials using BPs with fracture healing as an endpoint concluded there was no delay in callus formation or healing [5].

In general, the finding of no impact of BPs on fracture healing was observed in studies focused on specific types or sites of fractures, but this was not a universal observation. For example, the use of zoledronic acid give to the patient after fracture, irrespective of the timing post fracture, has not been shown to affect fracture healing in two separate studies of hip fracture patients [6,29]. In the upper extremity, bisphosphonates have been studied for many different types of fractures and likewise, most authors report no significant delay in union in these fractures with BP use immediately after the fracture, but an increase in risk of fracture non-union was reported. Conversely, fracture healing in patients already on BP at the time of fracture was reported to be slightly delayed, but there was no difference in non-union incidence [30]. Similarly, Rozental and colleagues report that BP use prior to distal radius fracture resulted in <1 week of delay in healing, but in a separate study, the early administration of BPs after distal radius fracture did not result in a delay in healing [31,32]. One key instance in which many authors report delayed healing to occur is with long-term use of bisphosphonates and development of atypical femoral fractures [33,34]. Unfortunately, a delay in healing was reported to occur in 26% of cases of these rare fracture [35].

Discussion

Considerable study of the effects of BPs on fracture healing has been done using animal models and essentially all species studied exhibit formation of larger bony callus and delayed remodeling of woven bone into lamellar bone with use of this class of compounds. Despite this deviation from the normal pattern of healing, this larger callus size seen in BP treated animals was usually reported to be mechanically equivalent to but not superior to that found in the control animals. The increases in biomechanical strength may be due to the retention of trabecular elements in the callus and or increased fracture bridging. It should be noted that the timing of BP dosing relative to the time of fracture does appear to influence this delay in healing.

Comparatively speaking, there are fewer human studies that have been performed with BPs used around the time of fracture healing. In all of the studies reported to date, there has been no significant delay noted in upper extremity or lower extremity fractures when BP therapy was initiated right after fracture. Conversely, in patients already on long-term BP therapy who sustain an atypical femoral fracture (a clinically rare event), a delay in bone union was observed an estimated 26% of the time [35]. At this time, there is insufficient data regarding any delay in healing in patients using long-term BP who suffer more typical fractures and as such this clinical situation is not fully understood. Similarly, there is a paucity of information about the benefits of “drug holidays” during long-term BP use upon suffering of fracture.

Conclusion

Animal models of BP use show no delay in periosteal healing but a significant effect on fracture remodeling was consistently observed. BPs do not appear to delay human fracture healing when the use of these compounds is initiated following the acute fracture, regardless of the timing of the initiation of treatment during the healing period. Conversely, there is some evidence to suggest that BP therapy should be stopped in patients that have been already treated long-term with BPs and then suffer an atypical fracture. Cessation of BP use (drug holiday) at the time of fracture may also be prudent after long-term treatment with this class of drugs, however this remains a topic of investigation.

Conflict of interest

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