New mechanisms and targets in the treatment of bone fragility

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ABSTRACT

Bone modelling and remodelling are cell-mediated processes responsible for the construction and reconstruction of the skeleton throughout life. These processes are chiefly mediated by locally generated cytokines and growth factors that regulate the differentiation, activation, work and life span of osteoblasts and osteoclasts, the cells that co-ordinate the volumes of bone resorbed and formed. In this way, the material composition and structural design of bone is regulated in accordance with its loading requirements. Abnormalities in this regulatory system compromise the material and structural determinants of bone strength producing bone fragility. Understanding the intercellular control processes that regulate bone modelling and remodelling is essential in planning therapeutic approaches to prevention and treatment of bone fragility. A great deal has been learnt in the last decade. Clinical trials carried out exclusively with drugs that inhibit bone resorption have identified the importance of reducing the rate of bone remodelling and so the progression of bone fragility to achieved fracture reductions of approx. 50%. These trials have also identified limitations that should be placed upon interpretation of bone mineral density changes in relation to treatment. New resorption inhibitors are being developed, based on mechanisms of action that are different from existing drugs. Some of these might offer resorption inhibition without reducing bone formation. More recent research has provided the first effective anabolic therapy for bone reconstruction. Daily injections of PTH (parathyroid hormone)-(1–34) have been shown in preclinical studies and in a large clinical trial to increase bone tissue mass and reduce the risk of fractures. The action of PTH differs from that of the resorption inhibitors, but whether it is more effective in fracture reduction is not known. Understanding the cellular and molecular mechanisms of PTH action, particularly its interactions with other pathways in determining bone formation, is likely to lead to new therapeutic developments. The recent discovery through mouse genetics that PTHrP (PTH-related protein) is a crucial bone-derived paracrine regulator of remodelling offers new and interesting therapeutic targets.

INTRODUCTION

The significance of osteoporosis as a public health problem in women and in men lies in the morbidity and mortality associated with fragility fractures [1]. The most commonly recognized cause of bone fragility is sex-hormone deficiency in women and in some men, but a number of other factors are recognized that contribute to fracture susceptibility in both sexes [2].

Until recently, prevention and treatment of bone loss was dependent entirely on the use of drugs that inhibit bone resorption, a mechanism that does not restore

Key words: bone formation, bone mineral density, bone remodelling, bone resorption, osteoblast, osteoclast, osteoporosis.

Abbreviations: ARF, absolute risk for fracture; BMU, basic multicellular unit; BMD, bone mineral density; CA II, carbonic anhydrase II; HRT, hormone replacement therapy; OPG, osteoprotegerin; PTH, parathyroid hormone; PTH1R, PTH receptor 1; PTHrP, PTH-related protein; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; TNF, tumour necrosis factor.

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Figure 1  Mechanism of remodelling in BMUs 
(A) Remodelling foci or BMUs within the cortex and on the surfaces of trabeculae and cortex adjacent to the marrow cavity. (B) The remodelling sequence: (1) quiescent state with flat osteoblast lining cells and an adjacent micro-crack; (2) osteoclastogenesis involving precursors from the circulation and the marrow; (3) reversal phase; (4) bone formation by osteoblasts; and (5) filling of resorption space with new bone. (C) Stromal and haematopoietic relationships, with RANKL binding to RANK to promote osteoclastogenesis. OC, osteoclast.

Bone structure or bone that has already been lost [3]. Now, means are available to promote the formation of new bone, and recent discoveries show promise of leading to new molecular targets for anabolic therapies, as well as several new approaches to inhibition of bone resorption. For both antiresorptive and anabolic targets, some are at an early stage of research, whereas others are more advanced in development. Newer understanding of mechanisms of the cellular and molecular control of bone resorption and formation underpin these approaches.

BONE REMODELLING

In order to maintain its material and structural strength, bone is continuously resorbed and reformed at approx. 1–2 million microscopic remodelling foci per adult skeleton. Within each of these ‘basic multicellular units’ (BMUs), focal resorption is carried out by haematopoietically derived osteoclasts and takes approx. 3 weeks per site, whereas the refilling of lost bone by osteoblasts, derived from bone marrow stromal cells and circulating precursors, takes approx. 3–4 months [4] (Figure 1).

In the adult human skeleton, approx. 5–10% of the existing bone is replaced every year by bone remodelling. The remodelling process, which continues throughout adult life, is an integral part of the calcium homoeostatic system and provides the mechanism for resorptive removal of microdamage, repair by bone formation and adaptation to mechanical stress. The cellular sequence is initiated when mechanical deformation or microcracks in old bone provoke signalling that leads to osteoclast development and bone resorption (Figure 1). This sequence of events is initiated asynchronously throughout the skeleton, at sites that are geographically and chronologically separated from each other. Both bone resorption and bone formation occur at the same place in these BMUs, so that there is no change in the shape of the bone [4]. A volume of trabecular bone is more rapidly turned over than the same volume of cortical bone because it is fashioned with more surface, upon which remodelling occurs. It is not known how the differing turnover of the two types of bone relates to bone strength, or how this might determine antiresorptive drug action.

In addition to remodelling, bone modelling on its periosteal surface is characterized by bone formation without prior bone resorption. This process, so vigorous during growth, establishes the adult size and shape of bone. At the completion of linear growth with closure of the epiphyses, periosteal apposition continues but markedly less so [5].

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Tight regulation of these processes is essential for the achievement and maintenance of skeletal strength. Modelling and remodelling during growth achieves peak bone strength and continued remodelling during adulthood maintains the mechanical integrity of the skeleton. Circulating hormones contribute, but the key influences are locally generated cytokines that are the signals mediating information transfer among osteoblasts, osteoclasts, immune cells and constituents of the bone matrix.

**COUPLING OF BONE FORMATION TO RESORPTION**

The maintenance of trabecular and cortical bone mass and architecture in accordance with loading requirements requires that bone resorption and formation be coordinated, such that a high or low level of focal resorption is usually associated with a similar, but not necessarily identical, change in the level of focal bone formation in the BMU. The theory that resorption is followed by an equal amount of formation has come to be known as ‘coupling’ (Figure 1B).

Reduced bone formation plays a central role in age-related decline in bone mass [6,7]. During growth there may be a positive BMU balance, because the amount of bone deposited in each BMU exceeds the amount of bone resorbed. After completion of longitudinal growth, with the attainment of peak bone strength, the need for a positive BMU balance declines. It is possible that there is a progressive decline in bone formation in the BMU, so the BMU balance becomes less positive, zero in young adulthood and then less than the volume of bone resorbed. The BMU balance becomes negative, marking the onset of bone loss.

Bone loss remains slow before menopause because the remodelling rate is low. After menopause when remodelling rate increases, each remodelling event produces bone loss and structural decay. This negative balance may worsen if oestrogen deficiency reduces the life span of osteoblasts and increases the life span of osteoclasts [8]. This produces double jeopardy: high remodelling with many resorption sites and an increasingly negative BMU balance. Although an increase in resorption depth is reported after menopause, some studies suggest resorption depth declines as age advances [9].

Concepts of the pathogenesis of bone fragility have developed that focus on changes in the number of bone cells, either overproduction of osteoclasts or underproduction of osteoblasts. In the latter, this is either through inadequacy of precursors or failure in differentiation. In either case, the effect is similar in quantitative terms; there is less bone. In qualitative terms, structural differences may be produced since a reduction in bone formation produces focal thinning of trabeculae while excessive resorption may produce loss of trabecular connectivity and so greater fragility [10].

**OSTEOCLASTS AND BONE RESORPTION**

The only cell capable of resorbing bone is the osteoclast, which is generated from haematopoietic precursors in the monocyte/macrophage lineage. The bone marrow microenvironment plays an essential role as a source of cytokines, including the TNF (tumour necrosis factor) and interleukin families, as well as PTHrP [PTH (parathyroid hormone)-related protein] that regulate osteoclast formation. As the receptors for these factors are expressed in cells of the osteoblast lineage, bone-resorbing agents must first act on cells of the osteoblast lineage to produce their effects [11]. Despite the several different signalling mechanisms that these agents use upon their target cells, they converge to a common mechanism in promoting osteoclast formation. The discovery of OPG (osteoprotegerin), a soluble member of the TNF receptor superfamily, revealed it as a very effective inhibitor of osteoclast formation. This led to identification and cloning of RANK (receptor activator of nuclear factor κB) and RANKL (RANK ligand), and its recognition as the common factor mediating osteoclast formation in response to all known stimuli [12].

Osteoblasts/stromal cells are also the source of M-CSF (macrophage colony-stimulating factor), which plays a crucial role in osteoclast formation by promoting the proliferation of precursors. The critical communication between the osteoblastic/stromal cell and haematopoietic lineages results from RANKL binding to its receptor, RANK, on the haematopoietic lineage (Figure 1B), and the process is inhibited by the decoy receptor OPG, which binds RANKL and prevents it from promoting osteoclast formation.

All of these discoveries have been validated by studies in genetically altered mice, as follows: (i) overexpression of OPG results in mice with osteopetrosis because of failure to form osteoclasts, whereas genetic ablation of OPG leads to severe osteoporosis; (ii) genetic ablation of RANKL results in osteoporosis because RANKL is necessary for normal osteoclast formation; and (iii) genetic ablation of RANK also leads to osteopetrosis because it is the receptor for RANK. As this signalling pathway is also functional in immune cells, RANK-null mice have severe abnormalities in that system, with failure of lymph node development and impaired immune responses [12].

Osteoclastic resorption takes place in a sealed-off microenvironment. The most prominent ultrastructural feature of osteoclasts is the deep folding of the plasma membrane, the so-called ruffled border, in the area facing the bone matrix [11]. This structure is surrounded by a peripheral ring tightly adherent to the bone matrix, thus sealing off the subosteoclastic resorbing compartment. The mechanism of bone resorption requires acidification of the resorption space through the active transport of protons driven by a V-type H+-ATPase.
At the same time, a passive transport of chloride through the chloride channel preserves electroneutrality, and is mediated through the chloride channel ClC-7. This results in dissolution of bone mineral, thereby exposing the organic matrix to proteolytic enzymes, particularly cathepsin K, which are responsible for the degradation of organic matrix. Inactivation of any of these (V-type H\textsuperscript{+} ATPase, ClC-7 channel and cathepsin K) by genetic or pharmacological means results in failure of osteoclasts to resorb bone. Each is therefore a target for drug development [13].

**PATHOGENIC AND STRUCTURAL BASIS OF BONE FRAGILITY AND THE RATIONALE FOR TREATMENT OF OSTEOPOROSIS**

Not all patients with reduced BMD (bone mineral density) or fractures have the same underlying pathogenesis or structural abnormalities responsible for the bone fragility. Some patients with fractures have reduced tissue mineral density others do not [14], some have reduced osteocyte density others do not [15]. Although most women with fractures may have high remodelling, normal or low remodelling rates are also well documented [16–18]. Some women with fractures have a negative BMU balance due to reduced bone formation, increased resorption, or both; others have no negative BMU balance at all, so presumably the low BMD is due to attainment of a low peak BMD during growth [19]. Yet others may have reduced periosteal apposition during growth or ageing [19,20].

**EARLY EFFECTS OF ANTIRESORPTIVE AGENTS ON BONE FRAGILITY**

In most postmenopausal women, the remodelling rate is high: large numbers of BMUs excavate cavities on the three components of the endosteal envelope, on the endocortical surface producing cortical thinning, within the cortex producing intracortical porosity and on trabecular surfaces producing trabecular thinning and loss of connectivity (Figures 2A and 2E).

When an antiresorptive agent, such as alendronate, risedronate or raloxifene, is given, the steady state is perturbed, and there are no longer equal numbers of remodelling units being created and completed [21,22]. The birth rate of new BMUs decreases quickly and down to a level determined by the potency of the drug. Meanwhile, the many BMUs at varying stages in the remodelling cycle go to completion by depositing a volume of new bone that partly refills the excavated site (Figure 2B).

This newly deposited bone mineralizes and BMD increases because new BMUs are now not being created as rapidly. New excavated sites are appearing during therapy, but there are less of them and they are more shallow. Morphologically, the completion of remodelling by bone formation is seen as a reduction in cortical porosity, whereas the appearance of fewer and more shallow resorption cavities during therapy results in a slowing of cortical and trabecular thinning. Bone formation in each BMU eroding a trabecula reduces thinning locally, presumably preventing microdamage and progression towards perforation.

The slowing of the remodelling rate also allows more time for tissue mineral density to increase, because mineralized bone that would be removed during high remodelling is not removed and continues to become more completely mineralized (Figures 2C and 2D). Thus the early effect of drugs that inhibit bone resorption appears to be driven by the reduction in bone remodelling rate, which allows completion of the formation phase of the remodelling cycle in the many remodelling sites present before treatment was initiated. There is more complete secondary mineralization of existing bone, reduction in the appearance of new excavated sites (preventing further structural decay) and so a progressive restoration of tissue mineral density, reduced intracortical porosity, and a slowing of the progress of cortical and trabecular thinning and perforation [23–25]. In this way, bone strength is maintained and possibly partly restored, perhaps accounting for the early reduction in fracture risk in clinical trials during the treatment relative to the placebo group. There is no experimental evidence in animals or human subjects to verify this at present. New non-invasive high-resolution pQCT (peripheral quantitative computer tomography) may help address the issue.

**BMD AND BONE MARKERS AS PREDICTORS OF FRACTURE RISK REDUCTION**

The increase in BMD produced by the filling of the remodelling sites active before treatment and the rise in tissue mineral density takes place during the first 6–18 months and accounts for most of the increment to be seen during 3–4 years of antiresorptive treatment. Therefore most of the increase in BMD is a function of the level of baseline remodelling before treatment. The greater the numbers of excavated sites, the greater will be the rise in BMD, as there are more sites that are filled with new bone which is then mineralized. For example, the increase in BMD using the same drug and same dose is greater in persons with high baseline remodelling than with low remodelling [26–28] (Figure 3). The potency of the drug to suppress the birth rate of new BMUs and the drug dose will also contribute.

These factors, and other concerns, account for the observation that only a small proportion of the fracture risk reduction can be explained by the rise in BMD and suppression of remodelling markers [29]. A greater increase in BMD or a greater suppression in bone
Figure 2  Schematic representation of the morphological basis for the increase in BMD during antiresportive treatment
The central graph depicts the increase in BMD with alendronate, risedronate and raloxifene, and the decline in placebo, as a function of duration of treatment. The surrounding cartoons depict the morphological basis for the increase in BMD. (A) Many resorption cavities are present eroding trabeculae before treatment (arrows). (B) When treatment is started, resorption pits partly fill with new bone (arrows). (C) This bone mineralizes producing the early rise in BMD. (D) Continued slower rise in BMD is the result of more complete secondary mineralization of the existing bone in the face of now slow remodelling (see text). Microradiographs show the homogeneously mineralized bone after alendronate (upper panel) and normal bone (lower panel), with densely mineralized older bone and dark grey newly deposited less densely mineralized bone. (E) Without treatment (placebo), trabeculae thin and become disconnected as the same number of resorption pits resorb bone from an ever-decreasing amount of bone. yrs, years.

Figure 3  Effect of alendronate on high and low remodelling
The graph shows the greater increase in BMD with alendronate in persons with high baseline remodelling compared with those given the same drug and dose with low remodelling. Adapted from Calcified Tissue International, 65, 1999, 359–364, Bone turnover and the response to alendronate treatment in postmenopausal osteoporosis, S. Gonnelli, C. Cepollaro, C. Pondrelli, S. Martini, A. Montagnani, R. Monaco and C. Gennari: with kind permission of Springer Science and Business Media. The cartoons on the left depict the mechanism. Higher baseline remodelling has more resorption sites that partly fill with bone, resulting in a greater increase in BMD. When remodelling is slow, there are less sites to complete remodelling with new bone so the rise in BMD is less. yrs, years.
remodelling combining two drugs compared with one [30], or one drug compared with another [30,31], cannot be relied upon as surrogate measures of greater antifracture efficacy [31]. Indeed, individuals who lose bone during treatment with raloxifene or risedronate still have a reduction in risk for fracture compared with placebo [32–34]. Moreover, individuals treated with risedronate who gain over 3–5% have the same fracture risk reduction as those who gain less BMD [33,34].

These data do not support the use of surrogate measures of antifracture efficacy as a means of deciding whether one drug is more efficacious than another. Greater efficacy of one drug over another requires the demonstration of greater antifracture efficacy in concurrently conducted randomized trials. If this were otherwise, these costly and protracted randomized trials would no longer be required. Indeed, controls could also be dispensed with. Given this, there is no scientific reason to repeat a BMD measurement in an individual.

The structural basis for the early reduction in fracture risk during therapy is uncertain [35]. The term ‘reduction’ in risk is the net effect of two processes: (i) the continued structural decay in the placebo group, and (ii) the prevention or partial reversal of this decay in the treated group. Purportedly, what is important is a reduction in intracortical porosity, slowing of cortical thinning, maintenance (but not restoration) of trabecular thickness and connectivity and restoration of tissue mineral density [23]. These changes may not be captured with sufficiently high sensitivity and specificity using BMD or remodelling markers. Clarification of which of these processes are most important must await further research using new non-invasive techniques.

**LESSONS FROM THE SPECIAL CASE OF STRONTIUM RANELATE**

Although the morphological changes described above are an appealing explanation for the early fracture risk reduction, recent evidence using strontium ranelate challenges this view. This drug has been shown convincingly to produce a reduction in risk of vertebral and non-vertebral fractures within the first 6–12 months [36,37].

Based on limited available data published in abstract form [38], there is no reduction in remodelling rate and so there is no interruption of steady state. Completion of remodelling in excavated sites present before treatment is matched by the same numbers of excavated sites appearing because remodelling rate is not suppressed.

The mechanism responsible for the slowing of progression of bone fragility and lower fracture incidence is not apparent. Although the antiresorptives suppress both markers of bone resorption and bone formation (Figure 4), strontium ranelate appears to produce a dissociation between markers of bone resorption, which are suppressed, and bone formation, which increase modestly. We propose that there may be a reduction in the depth of the excavation cavities being formed while the volume of bone deposited in each of the more shallow cavities remains unchanged. Although the number of excavated sites appearing on the endosteal bone surface is unchanged, the BMU balance in each will be less negative than before treatment. Bone architecture will continue to decay but less rapidly than in the placebo-treated subjects.

This might account for the early and sustained reduction in fracture risk relative to controls. If the volume of bone formed in each BMU is increased, perhaps by an increase in the life span of osteoblasts, the net effect of a reduction in volume resorbed plus a larger volume deposited may produce a positive BMU balance. In this scenario, the high remodelling rate is an advantage, as each remodelling event will deposit a small moiety of bone. This might produce focal thickening on trabeculae, focal cortical thickening and a reduction in cortical porosity. No evidence for these morphological changes is available at this time, but research continues to explore these possibilities. Suggestions of a ‘dual action’ in stimulating bone formation and suppressing bone resorption have not yet been confirmed experimentally.

**THE LATER EFFECTS OF ANTIRESORPTIVE AGENTS: ARE BENEFITS SUSTAINED?**

With higher tissue mineral density, reduced porosity and slightly improved bone strength, a new steady state is established during treatment with the antiresorptive agents. Now the remodelling rate is slow and the depth of each excavated site is likely to be more shallow. If the BMU balance remains negative, each remodelling event will produce bone loss and structural decay.
However, each of the now fewer remodelling events removes only a small volume of bone. As bone loss is driven more by remodelling rate than the magnitude of the negative BMU balance, while remodelling is suppressed, trabecular and cortical thinning will proceed very slowly. At the same time, there is a progressive increase in tissue mineral density of the remaining and slowly diminishing bone tissue mass. The net effect as measured by densitometry is a continued increase in BMD (as it only 'sees' mineral) while bone tissue mass slowly declines, the ever so slowly diminishing bone tissue is becoming progressively more densely mineralized (Figures 2C and 2D).

Fractures continue (these drugs do not eliminate fracture risk, they reduce it by 30–60 %), but the fracture rate is likely to remain lower than in the untreated patients in whom rapid remodelling and the negative BMU balance persist, so that the same high remodelling intensity removes the same total volume of bone from an ever decreasing total bone volume (like a melting ice block shrinking in the face of a constant melting rate), so fragility increases in controls.

LONG-TERM EFFECTS OF ANTIRESORPTIVE AGENTS: COULD BONE FRAGILITY INCREASE?

The purpose of modelling and remodelling during growth is to establish the skeleton’s peak bone strength; its purpose in adulthood is to maintain it [39]. Bone, like roads, buildings and bridges, develops fatigue damage during repeated loading. Only bone has the mechanism to detect the location and remove it. Bone resorption is not during repeated loading. Only bone has the mechanism to detect the location and remove it. Bone resorption is not. Bone resorption is not.

Therefore prolonged suppression of remodelling may do harm for several reasons. In the normal skeleton, adjacent regions of bone vary in their tissue mineral density. In younger, more recently remodelled, bone the tissue is less densely mineralized. Adjacent to this is older more densely mineralized tissue (Figure 2). This heterogeneity in tissue densities produces a composite structure that prevents extension of microdamage, because energy (from mechanical stress) is needed for cracks to progress. This energy is dissipated since a crack has more difficulty in progressing through tissue of lower density.

As remodelling is slowed during treatment, more time is available for secondary mineralization of the new tissue and more complete mineralization of older tissue (that would have been removed if remodelling was high). So adjacent regions of bone become more similar and more homogeneous in their tissue density. This homogeneity offers less obstruction to crack progression and lengthening. In addition, the higher tissue density increases tissue stiffness (brittleness), predisposing to increased microdamage production, whereas the reduced remodelling may also reduce removal of existing microdamage [24,25,40–43].

Microdamage and increased bone brittleness (reduces toughness or the ability to absorb energy by deforming) occurs in animals given high doses of bisphosphonates, but these doses are well above those used clinically. A great deal more research is needed regarding the long-term effects of bisphosphonates in human subjects. Convincing evidence of a deleterious effect in humans is lacking even though uncontrolled case reports of impaired fracture healing with alendronate are documented [44,45]. The 10-year follow-up of patients taking alendronate contained an inappropriate control group, so that the claim of no deterioration in antifracture efficacy lacks credibility [46].

It remains to be established whether drugs that greatly suppress remodelling are more appropriate in persons with high remodelling and low tissue mineral density, but deleterious in persons with lower remodelling and normal tissue mineral density (in whom further suppression may predispose to microdamage).

NEW PATHWAYS AND NEW ANTIRESORPTIVES IN EARLY AND LATE CLINICAL DEVELOPMENT

Although the last decade has brought great advances in osteoporosis treatment with antiresorptive agents that have been studied in careful thorough clinical trials, their real and potential limitations are sufficient to warrant the continued search for new approaches. The aims would be to improve the fracture risk reduction from the current 30–50 %, to avoid the possibility of long-term effects on bone structure, to find drugs whose effects reverse with cessation of therapy and drugs that inhibit resorption without inhibiting bone formation.

The discovery of RANKL, RANK and OPG and the effects of manipulating their genes in mice have revealed powerful physiological control mechanisms for osteoclast development and activity. The most obvious drug candidate at first was OPG, which is so very effective at inhibiting osteoclast formation and activity in vitro and in vivo by binding RANKL and preventing it from signalling through RANK.

In rodent models of osteoporosis, treatment with OPG as protein or as gene therapy was effective, and OPG lowered blood calcium in cancer-induced hypercalcaemia, as well as preventing the growth of experimental bone metastases [12]. Development of OPG therapy was terminated in favour of a fully human monoclonal antibody against RANKL, subcutaneous injection of which inhibits bone resorption in human subjects [47]. A single subcutaneous injection reduces
bone resorption for several months, as assessed by assay of markers of bone resorption. Anti-RANKL is currently being investigated in a controlled clinical trial, the results of which should be available within a year. Whereas this approach is effective by virtue of its powerful inhibition of osteoclast formation, the following avenues have the potential to arrive at drugs that inhibit resorption by blocking the activity of mature osteoclasts.

A relatively selective osteoclast-specific structure that seems to play a rate-limiting role in osteoclast activity is the integrin αvβ3 receptor, which is produced in osteoclasts as well as in budding blood vessels and leucocytes. Treatment of rats with the disintegrins, echistatin or kistrin, which bind with high affinity to integrin αvβ3, inhibits bone resorption stimulated by PTH or oestrogen deficiency. Moreover, small-molecular-mass compounds that mimic the tripeptide RGD sequence, recognized by the integrin, were shown to have similar effects [48]. The target selectivity of such a compound would have to be determined in toxicological and clinical studies.

An essential component of osteoclastic bone resorption is acidification of the resorption lacuna, which reduces the pH to approx. 4 in order to dissolve the bone mineral. A vacuolar H⁺-ATPase in the osteoclast membrane plays a key role in this process by mediating the active transport of protons. Inhibitors of this enzyme, for example bafilomycin, have been shown to inhibit osteoclastic bone resorption in vitro and in vivo. A relatively osteoclast-selective H⁺-ATPase inhibitor has been shown to inhibit ovariectomy-induced bone loss in rats [49]. Passive transport of chloride through chloride channels preserves electroneutrality in the course of the acidification process, and preventing chloride transport will lead to a rapid hyperpolarization of the membrane, inhibiting further secretion of protons and thus resulting in an inhibition of further bone resorption.

Several chloride channels have been characterized through analysis of mutations leading to genetic disorders and generation of knockout mice. Mutations in the CLCN7 gene are responsible for various forms of osteopetrosis [50]. An interesting feature of the bone phenotype resulting from blockade either of acidification or the chloride channel is that bone resorption is inhibited without any resulting inhibition of bone formation. Early data with an orally delivered CLCN7 inhibitor showed that it inhibited bone loss in ovariectomized rats without inhibiting bone formation [51]. It is possible that such inhibitors of resorption could be more readily combined with anabolic therapy than those resorption inhibitors (e.g. bisphosphonates) that lead to inhibited bone formation.

Cathepsin K is a lysosomal cysteine proteinase, expressed selectively and at high levels in osteoclasts, with enzymatic properties suited for degrading type I collagen. Cathepsin K inhibitors with appropriate pharmaceutical properties could potentially be used as antiresorptive therapeutics [52]. Other enzymes involved in acidification are CA II (carbonic anhydrase II) and the sodium bicarbonate exchanger in the basolateral membrane, which help maintain the neutral pH inside the cell. Genetic mutations of CA2 in patients produce osteopetrosis, but also renal acidosis and mental retardation [53]. Inhibition of CA II has been considered for suppression of osteoclastic resorption; however, to our knowledge, it is low in priority due to potential side effects and lack of tissue specificity.

Thus, although effective inhibitors of osteoclast activity are currently known and in clinical use, additional ones are likely to be developed and used if they are better suited for particular indications or provide greater efficacy or convenience.

**PROMOTING BONE FORMATION: RECONSTRUCTING THE SKELETON**

Antiresorptive agents do not reconstruct the skeleton, but until recently no therapeutic approaches were available to restore bone once it had been lost. That situation has changed with the development of PTH-(1–34) (teriparatide) as a highly effective anabolic therapy for the skeleton, despite its best known action as a resorptive hormone [54]. Intermittent PTH treatment stimulates periosteal apposition in growing animals and endosteal bone formation, so the cortex thickens on its internal and external surfaces, while new bone is also deposited on trabeculae producing trabecular thickening and perhaps increased trabecular connectivity [55].

**PTH: MECHANISMS OF ANABOLIC ACTION**

The anabolic effectiveness of PTH requires that it be administered intermittently, and this has been achieved with the use of daily injections that rapidly achieve a peak level in blood which is not maintained [56]. When PTH is administered in this intermittent mode, processes are initiated in bone which result in increased bone formation, presumably as a result of activation of genes responding specifically to a transiently activated signalling system that requires a rapid increase in PTH, with a rapid decline to pre-existing levels. On the other hand, if PTH is infused or is administered in such a way that elevated plasma levels are maintained, the dominant effect is stimulation of osteoclast formation and bone resorption [56], to the extent that these over-ride any anabolic response.

Two general mechanisms are favoured to explain the anabolic action of PTH. One is that PTH promotes differentiation of committed osteoblast precursors in the bone marrow and possibly also in lining cells, without stimulating proliferation of precursor cells [57]. The second is that PTH increases the lifespan of mature
osteoblasts and osteocytes by preventing apoptosis [58]. The transcription factor Runx2 is essential for development and maintenance of the osteoblast phenotype [59], and at least two ways are proposed for its mediation of the anabolic effect of PTH. Enhanced expression of Runx2 in response to PTH has been shown in osteoblasts [60], and Runx2 has been proposed as a mediator of the anti-apoptotic effect of single injections of PTH [61]. On the other hand, when PTH is given continuously by infusion, proteosomal degradation of Runx2 is enhanced and the anti-apoptotic effect of PTH is lost [62].

Recent research has shed new light on the control of bone formation, and the effect of PTH treatment is such that it is a matter of some importance to find out whether any of the newly recognized pathways play any part in PTH action. Such studies are at a very early stage, but some interesting interactions are emerging. For example, the importance of Wnt signalling in bone has been established from both human and mouse genetics with recognition of high-bone-mass syndromes in activating mutations of the Wnt signalling pathway [63], and osteoporotic syndromes with the reverse [64]. Binding of Wnts to their receptor complexes leads to activation of the TCF/LEF (T-cell factor/lymphoid enhancer factor) family of transcription factors [65]. PTH treatment of osteoblasts has been shown to be capable of activating the pathway in vitro [66], but it is not known whether this is significant in vivo.

Genetic studies have also revealed the protein sclerostin as a powerful inhibitor of bone formation, an inactivating mutation of which results also in a high-bone-mass syndrome. The protein acts by inhibiting both BMP (bone morphogenetic protein) and Wnt signalling [67]. Sclerostin production is rapidly inhibited by treatment with PTH [68], suggesting a further anabolic mechanism for PTH, removing a physiologically effective inhibitor. Much remains to be learned about the molecular mechanisms of PTH action and its interactions with other important pathways. Understanding these could lead to new approaches to therapy or to useful modifications of existing treatments.

**EFFECTS OF ANABOLIC AGENTS ON BONE FRAGILITY: FOCUS ON PTH**

Neer et al. [54] reported that treatment with intermittent PTH reduced the risk of vertebral and non-vertebral fractures during 18 months. Most of the increase in cortical and trabecular thickness induced by PTH is due to bone formation on the endocortical surface of the cortex adjacent to marrow and on either side of trabeculae. Bone histomorphometric evidence of periosteal apposition following PTH in adult human subjects is not available [69]. If there is a lack of periosteal apposition in adult subjects in response to PTH, this may reflect the limited cellular activity on this surface and particularly the need for some resorptive activity for the anabolic effect of PTH [70,71].

There are more questions than answers concerning the comparative, combined or sequential use of resorption inhibitors with PTH. Sequential or combined use of a bone-forming agent to combat reduced bone formation, with an antiresorptive to combat increased bone resorption, has appeal. However, comparator trials with structural analysis and antifracture efficacy are unavailable. Thus whether PTH is more efficacious than antiresorptives or whether antiresorptives given prior, during or after PTH is better (or worse) than either agent alone is unknown.

Surrogates of structural strength or antifracture efficacy are no substitute for structural analysis and antifracture efficacy; the BMD increase after an antiresorptive is a poor predictor of fracture risk reduction [29]. Although the increases in BMD with PTH treatment are greater than with antiresorptive drugs, the mechanisms differ. Antiresorptives increase tissue mineral density while PTH deposits new bone, increasing the thickness of trabeculae and cortex. Whether the greater increase in BMD after PTH or combined treatment equates with a greater fracture risk reduction than the lesser increase in BMD after bisphosphonates is unknown. Although the decrease in remodelling markers with antiresorptives do predict fracture risk reduction [32], markers increase with PTH, so that the usefulness of marker changes in combined therapy as surrogates of antifracture efficacy is questionable.

Prior alendronate treatment, but apparently not other antiresorptives, e.g. raloxifene, delays BMD and remodelling marker responses to PTH [72–75], raising the question of whether the anabolic response is partly mediated by factors produced by osteoclasts or products of the resorbed matrix [70,76]. When PTH is stopped, antiresorptives prevent the BMD decline [77], but whether the reported residual protection against fractures after stopping PTH is greater with or without an antiresorptive is unclear. Thus only the use of PTH followed by an antiresorptive has an evidence base, albeit limited to preservation of BMD. There is no evidence in human subjects to support the use of combined therapy. Prior antiresorptive use does not seem to influence the eventual response to PTH, at least in terms of BMD or markers of remodelling.

**WHAT ANABOLIC TREATMENTS MIGHT FOLLOW PTH?**

PTHrP was discovered as the cause of hypercalcaemia in cancer and, because of its structural similarity to PTH in its N-terminal region, it shares with PTH the ability to
act with molar equivalence upon a common receptor, the PTH1R (PTH receptor 1) [78]. Although the full-length PTHrP molecule is capable of a number of other effects [78,79], it is not surprising that injectable preparations of truncated forms of PTHrP exert actions similar to those of PTH. Thus PTHrP-(1–34), -(1–36) and -(1–74) have all been shown to be anabolic in studies with rodents [80]. Although the only form of PTH marketed in several countries is injectable PTH-(1–34), other approaches are either in clinical trial or at earlier development stages. These include PTH-(1–84) and PTHrP(1–36) [81], both by injection, as well as nasal delivery of PTH-(1–34).

Efforts are also being directed towards development of low-molecular-mass mimics of PTH action through its receptor, and of calcilytic agents, which are calcium receptor antagonists that work by substituting for calcium on the calcium receptor of the parathyroid cell, which responds with a burst of PTH secretion [82]. Indeed, the anabolic effect of PTH is so impressive that its mechanistic pathway should provide many opportunities for either development of new treatments or improvement of old. Particularly intriguing possibilities arise out of recent discoveries concerning PTHrP.

**PTHrP IN BONE DEVELOPMENT**

The discovery of PTHrP production in bone raised the possibility that this molecule has important local actions in bone, perhaps even being the primary ligand for the receptor it shares with PTH (PTH1R). Targeted disruption of the genes for PTHrP (Pthlb) or the common PTH/PTHrP receptor (Pthrl) in mice resulted in death in the perinatal period with gross skeletal abnormalities consistent with chondrodysplasia [83], with further investigation showing that PTHrP plays a central role in endochondral bone formation.

It seems likely that PTHrP is also involved in intramembranous bone formation. In rabbits, cells of the osteoblast lineage expressed PTHrP mRNA and protein throughout the entire sequence of intramembranous bone formation, with prominent production by cuboidal actively synthesizing osteoblasts and weaker expression in lining cells on the mineralized trabeculae [84]. Together with the finding of PTHrP mRNA in rat and mouse cells of the osteoblast lineage [85], this supported a role for PTHrP in the differentiation of mesenchymal precursors to mature cells of the osteogenic lineage.

Further investigations of PTHrP-mutant mice provided evidence to suggest that PTHrP is equally important for the orderly commitment of precursor cells to the osteogenic lineage and subsequent maturation and/or function. Although Pthrbp<sup>-/-</sup> mice die around birth, Pthrbp<sup>+/−</sup> mice are phenotypically normal at birth, but by 3 months of age exhibit a form of osteopenia that is characterized by a marked decrease in trabecular thickness and connectivity [83,86]. This bone deficit associated

with PTHrP haplo-insufficiency pointed to the likelihood of PTHrP playing a significant part in the maintenance of normal bone. Proof of this came recently when specific ablation of Pthrbp in osteoblasts resulted in mice with impaired bone formation, both in vitro and in vivo [87], thus identifying a central physiological role for PTHrP in the regulation of bone formation. The results of the mouse genetic experiments favour the idea that there is a critical role for PTHrP in bone remodelling.

What are the ways in which PTHrP as a paracrine/autocrine factor in bone can contribute? In order for PTHrP to stimulate bone formation by enhancing osteoblast differentiation and reducing osteoblast apoptosis, control mechanisms must exist to ensure that only short-lived high levels of PTHrP are available to local targets in order to favour bone formation, as persistently increased local PTHrP levels would favour increased osteoclast formation through stimulation of RANKL production. PTHrP release needs to be exquisitely regulated in terms of concentration, location and time, so that it is presented only briefly to these target cells. On the other hand, the spatiotemporal controls might be such that excessive osteoclast formation is much less likely under these conditions in comparison with when PTH is presented systemically to the whole skeleton. Possible mechanisms could involve cytokine-mediated and/or neural control of PTHrP gene transcription, mRNA stability or proteolytic processing of the protein. This presents itself as a target for drug development.

**ANTIFRACTURE EFFICACY**

*Which patients with osteoporosis to treat and when to do so?*

The most important factor determining whom and when to treat is an individual's ARF (absolute risk for fracture) [88]. In clinical trials of patients with osteoporosis, the ARF in the placebo arm varies enormously, but it often is approx. 1–3 in 100 persons/year, i.e. 1% annually. Say the ARF is 2 in 1000 persons/year, as occurs in early postmenopausal women, if a drug halves fracture risk, as most of these drugs do, then one event is averted, one woman will sustain a fracture despite treatment and 998 who were not going to fracture were exposed to drug treatment. Thus one fracture is prevented but 999 women/year are treated without benefit. If the ARF is ten times higher, as occurs in patients typically recruited into clinical trials, 2 in 100 women/year and the drug still halves the risk, one fracture is prevented but only 99 women are treated. Higher fracture risks (10–14%) are seen in nursing home residents, where one event is averted for every 10 or so persons exposed to treatment.

Thus some idea of an individual's absolute risk is central in decision making. A higher absolute risk and so a greater imperative to treat exists with advancing age,
lower BMD, a prior fracture and high remodelling rate; each of these contributes independently to fracture risk. With newer methods of investigation, higher sensitivity is likely to be achieved by defining other independent risk factors for fracture such as microdamage burden, porosity, cortical thickness, thinning of trabeculae and loss of their connectivity [89].

Approx. 85% of fractures occur in women over 60 years of age. An important signal for the need to treat is a prior vertebral or non-vertebral fracture. The risk for further fractures increases 3–5-fold as the number or severity of prevalent vertebral deformities is increased. In a person with osteoporosis, an incident fracture (with or without a prevalent fracture at baseline) increases the absolute risk of a further incident fracture to 30–40% within 3 years. Thus the evidence of antifracture efficacy is strongest in patients with a baseline vertebral or non-vertebral fracture. It is optimal to treat fewer older persons (>60 years) at high risk rather than many younger persons at low risk. This ensures that those likely to respond to treatment receive it, and those at low absolute risk and thus unlikely to benefit remain untreated.

What drug?
Despite the histological and biochemical heterogeneity seen in patients with fractures, the prevailing approach to reducing fracture risk is the use of antiresorptive agents. There have been several well-designed and executed clinical trials that provide a strong evidence base for antifracture efficacy of several agents. However, there have been no comparator trials in human subjects using antifracture efficacy as an endpoint of: (i) one antiresorptive compared with another or with an anabolic agent, (ii) two antiresorptives compared with one; or (iii) antiresorptive compared with antiresorptive plus an anabolic agent.

Thus the decision to use a drug or use one drug in favour of another, whether this be an antiresorptive or anabolic agent, has no evidence base. The decision is based solely on the quality of the design and execution of the clinical trial of that agent. Table 1 summarizes the criteria required to fulfill these rules of evidence-based medicine. Although no trials fulfill the above criteria completely, the studies of alendronate, risedronate, raloxifene, ibandronate, PTH and strontium ranelate largely fulfill these criteria and can be regarded as efficacious [3]. Studies of etidronate, calcitonin, fluoride, calcium, vitamin D and its analogues, such as calcitriol, do not. These drugs may be efficacious, but compelling evidence for this is lacking.

Table 1 Evidence-based guidelines for the design and execution of trials

<table>
<thead>
<tr>
<th>Evidence</th>
<th>Clarity</th>
<th>Confusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized</td>
<td>Unrandomized</td>
<td></td>
</tr>
<tr>
<td>Double-blind</td>
<td>Open label</td>
<td></td>
</tr>
<tr>
<td>Placebo-controlled</td>
<td>Part-time controls</td>
<td></td>
</tr>
<tr>
<td>Large samples</td>
<td>Small sample</td>
<td></td>
</tr>
<tr>
<td>Few drop-outs</td>
<td>Large drop-outs</td>
<td></td>
</tr>
<tr>
<td>Prolonged observation</td>
<td>Short duration</td>
<td></td>
</tr>
<tr>
<td>Intention to treat</td>
<td>Post-hoc analysis</td>
<td></td>
</tr>
<tr>
<td>Count patients with fractures</td>
<td>Count fractures</td>
<td></td>
</tr>
<tr>
<td>Replication</td>
<td>Isolated study</td>
<td></td>
</tr>
<tr>
<td>Consistency</td>
<td>Contradictory</td>
<td></td>
</tr>
<tr>
<td>Aledronate, risedronate, raloxifene, PTH, ibandronate, strontium ranelate</td>
<td>Etidronate, calcitonin, fluoride, active vitamin D and its analogues, calcium supplements</td>
<td></td>
</tr>
</tbody>
</table>

Rigorously studied drugs reported to reduce spine fractures in women with osteoporosis include alendronate, risedronate, raloxifene, ibandronate and strontium ranelate. Antivertebral fracture efficacy is also reported for the anabolic agent PTH-(1–34) (teriparatide) and for PTH-(1–84) [3]. In general terms, these drugs reduce the risk of symptomatic (clinical) and asymptomatic (morphometric) single vertebral fractures by approx. 40–50%. The benefits appear to persist during follow-up, but attrition of patient numbers calls the validity of this inference into question. HRT (hormone replacement therapy) and etidronate have also been reported to reduce vertebral fracture rates, but the studies are less rigorous. The level of evidence for antivertebral fracture efficacy of calcitonin and vitamin D metabolites is insufficient for inferences to be made with any confidence.

Evidence for antivertebral fracture efficacy is less compelling in women with osteopenia, but evidence does exist with raloxifene for patients with osteopenia at the hip, not spine [90], and for strontium ranelate for women with osteopenia at the spine, hip or both (E. Seeman, unpublished work). All of these studies suffer from the same problem. They were not designed specifically to exclude patients with local osteophytes, exostoses or sclerosis of the intervertebral disc so that osteopenia at the spine may be osteoporosis. Nevertheless, finding a reduction in fracture risk in patients with femoral neck osteopenia partly circumvents this problem.

Vertebral fracture risk reduction
Only general statements will be made here. Readers are directed to a comprehensive review and bibliography concerning these agents ([3] and see Figure 5). The most
over 80 years of age with established osteoporosis using strontium ranelate [37]. There is no evidence for a reduction in risk for hip fractures with ibandronate, etidronate, raloxifene or PTH. Calcium and vitamin D reduce the risk of hip fractures in institutionalized women, but not in community dwellers. Use of HRT has also been reported to reduce the risk of hip fractures in community dwelling women in the Women’s Health Initiative.

Non-vertebral fracture risk reduction is reported with risedronate, strontium ranelate and PTH. Post-hoc pooling of high-risk individuals from the two pivotal trials of alendronate resulted in a significant reduction in non-vertebral fractures. Raloxifene has not been reported to reduce the risk of non-vertebral fractures, except in a post-hoc sub-analysis. Calcium plus vitamin D and hip protectors have been reported to reduce hip fractures in nursing-home residents and institutionalized elderly.

**The future**

Although advances in therapeutics are occurring, there are many problems that need to be addressed. Treatment of high-risk individuals is needed and rational approaches to drug therapy involve the better selection of individuals at high risk to minimize exposing persons unlikely to fracture to the cost and side effects of drugs. Half of all of the fractures occurring in the community do so in women and men without osteoporosis, and ways of identifying these individuals are needed. Advances in non-invasive technology are likely to provide insights into the effects of these therapeutic agents on bone structure. Increasingly accurate information concerning the structural heterogeneity of bone fragility from patient to patient [16–19] may improve the sensitivity of fracture risk prediction [91]. Evidence for this is available; patients with low bone mass, high remodelling and a prevalent fracture have a higher fracture risk [92].

**CONCLUSIONS**

The purpose of modelling and remodelling throughout life is to adapt bone’s material composition and structure to prevailing loads. Advancing age is accompanied by accumulating abnormalities in this process. Abnormalities in the balance and rate of remodelling, and limits to periosteal apposition, compromise bone’s material composition and structural design. The solution to the problem of structural failure requires the study of the mechanisms at the molecular, cellular, tissue and structural levels, as each opens another opportunity for therapeutic intervention. New methods of drug treatment are being developed, but their successful application will be

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**Figure 5** Antifracture efficacy (relative risk reduction and 95% confidence intervals) for the main clinical trials

<table>
<thead>
<tr>
<th>Vertebral Fx</th>
<th>Non-Vertebral Fx</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLX 60 (MORE)*</td>
<td>RLX 60, 120 (MORE)***</td>
</tr>
<tr>
<td>RLX 60 (MORE)**</td>
<td>ALN 5/10 (FIT1)*</td>
</tr>
<tr>
<td>ALN 5/10 (FIT1)*</td>
<td>ALN 5/10 (FIT2)**</td>
</tr>
<tr>
<td>ALN 5/10 (FIT2)**</td>
<td>RIS 5 (VERT-NA)*</td>
</tr>
<tr>
<td>RIS 5 (VERT-NA)*</td>
<td>RIS 5 (VERT-MN)*</td>
</tr>
<tr>
<td>RIS 5 (VERT-MN)*</td>
<td>RIS 2.5/5 (Hip Study)***</td>
</tr>
<tr>
<td>CT 200 (PROOF)*</td>
<td>CT 200 (PROOF)*</td>
</tr>
<tr>
<td>Teriparatide*</td>
<td>Teriparatide*</td>
</tr>
<tr>
<td>Strontium ranelate (SOTI)*</td>
<td>Strontium ranelate (SOTI)***</td>
</tr>
<tr>
<td>Strontium ranelate (SOTI + TROPOS)**</td>
<td>Strontium ranelate (SOTI)***</td>
</tr>
</tbody>
</table>

*With prevention of vertebral fracture(s); **without prevention of vertebral fractures; ***with or without prevention of vertebral fractures. Abbreviations: ALN 5/10, 5 or 10 mg of alendronate per day; CT 200, 200 international units of calcitonin per day; FIT, Fracture Intervention Trial; Fx, fracture; HIP, Hip Intervention Program; PROOF, Prevent Recurrence of Osteoporotic Fractures; RIS 2.5/5, 2.5 or 5 mg of risedronate per day; RLX 60, 120, 60 or 120 mg of raloxifene; RR, relative risk; SOTI, Spinal Osteoporosis Therapeutic Intervention; Strontium ranelate, 2 g of strontium ranelate per day; Teriparatide, 20 µg of Teriparatide per day; TROPOS, Treatment Of Peripheral Osteoporosis Study; VERT-MN, Vertebral Efficacy With Risedronate Therapy-Multinational; VERT-NA, Vertebral Efficacy With Risedronate Therapy-North America. The Figure was drawn using data presented in [3].

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heavily dependent upon how much we continue to learn of the dynamics of bone remodelling and remodelling, and how cellular mechanisms influence these processes and therefore bone strength.

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