MECHANISMS OF ACTION OF ANTIRESORPTIVE THERAPIES OF POSTMENOPAUSAL OSTEOPOROSIS

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In the treatment of osteoporosis, the aim of the antiresorptive therapy is to restore bone density by decreasing bone remodeling. The process of bone remodeling plays a role in plasma calcium homeostasis and serves to modify bone architecture in order to meet changing mechanical needs, to maintain osteocyte viability, and to repair microdamage in bone matrix. Estrogen deficiency results in a number of detrimental effects on bone, including suppression of osteocyte survival as well as impairment of osteoblast response to mechanical stimuli and repair of ageing bone. In this review, effects of available antiresorptive therapies on endocrine regulations of bone metabolism in postmenopausal osteoporosis are compared.

The aim of antiresorptive treatment is to ensure adequate bone remodeling, reparation of microdamage of bone, and increased bone strength. Ideally, this effect should be maintained long-term. Several agents are approved for the treatment of osteoporosis. Calcitonin transiently inhibits osteoclast activity without decreasing osteoblast collagen synthesis. Aminobisphosphonates decrease bone remodeling by decreasing osteoclast activity and by inducing osteoclast apoptosis. This allows more time for secondary mineralization to proceed to completion in the existing bone tissue mass, so increasing the mechanical resistance of bone to loading. Estrogens and raloxifene (a selective estrogen receptor modulator that acts as an estrogen agonist in bone) suppress bone remodeling to the premenopausal range, maintaining the function of osteoblasts and osteocytes. In the placebo-controlled osteoporosis treatment trials, all the above treatments reduced the risk of fractures. Raloxifene therapy was also associated with a favorable or neutral effect in the cardiovascular system, and a reduced incidence of breast cancer. Selection of appropriate drug for treatment of postmenopausal osteoporosis should take into account the long-term effect of the antiresorptive agent on bone. Moreover, the effects on other tissues ++should also be considered, and this encompasses both safety concerns, as well as the potentially beneficial effects on other tissues. Further investigation is needed to evaluate the different modes of action of these agents, and their long-term effects on bone and other tissues.

Key Words: Apoptosis - Bisphosphonates - Calcitonin - Cytokines - Estrogen - Osteoblasts - Osteoclasts - SERM - Raloxifene

Bone remodeling

The aim of antiresorptive therapies for osteoporosis is to restore the bone density by decreasing remodeling of bone. Bone remodeling is important in maintaining plasma calcium homeostasis. It also serves to adjust bone architecture in order to meet changing mechanical needs. It helps to maintain os-

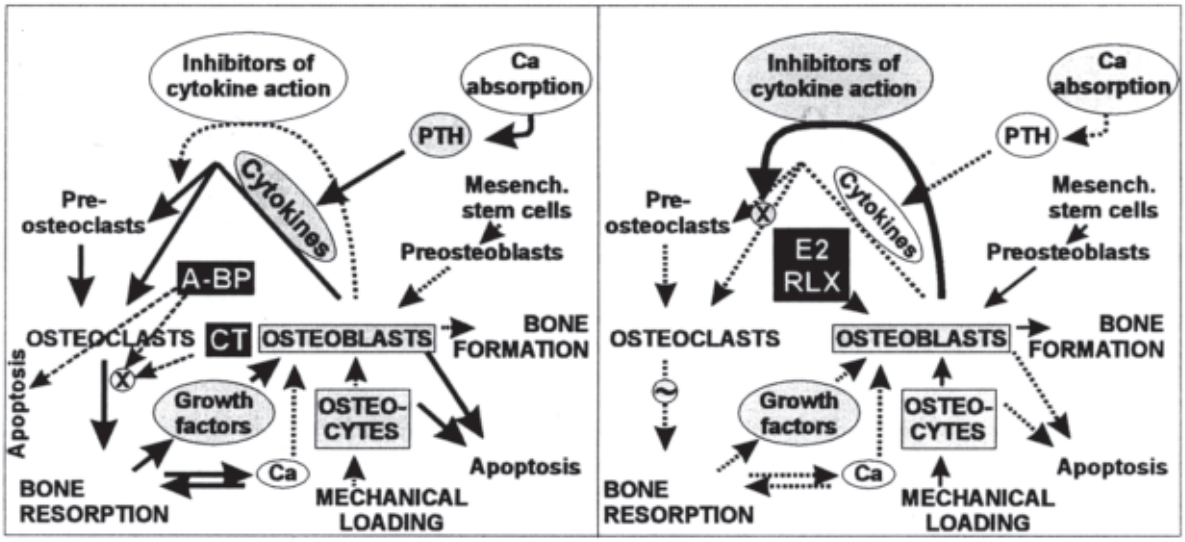

Fig 1 Left Estrogen deficiency up-regulates the production and action of several cytokines, such as interleukins 1 and 6, macrophage-colony-stimulating factor (M-CSF), tumor necrosis factor alpha, and ligand of receptor activating nuclear factor kappa B (RANKL). RANKL binds to its receptor, and along with the M-CSF activates osteoclastic bone resorption. Estrogen deficiency also down-regulates the local production of insulin-like growth factors, and transforming growth factor beta, and inhibitors of cytokines action, such as osteoprotegerin (OPG). Estrogen deficiency suppresses survival of osteocytes and impairs response of osteoblasts to mechanical stimuli, detection of microdamage and repair of aged bone. Aminobisphosphonates (A-BP) inhibit osteoclast recruitment, reduce the osteoclast lifespan, and directly inhibit the osteoclast activity. Calcitonin (CT) transiently inhibits osteoclast activity. Estradiol (E2) and raloxifene (RLX) (right) adjust the local production of cytokines and growth factors, decrease the formation of osteoclasts, and extend osteoblast and osteocyte lifespan.

teocyte viability, and to repair microdamage in bone matrix preventing the accumulation of old bone (PARFITT 2002). In healthy adults, bone mass is maintained by a balance between bone resorption and formation performed by osteoclasts and osteoblasts, respectively. The amount of aged bone removed by the osteoclasts is replaced by an equal amount of new mechanically competent tissue at the same location. The osteoblast-synthesized osteoid undergoes mineralization in two consecutive steps: a primary mineralization on the calcification front followed by a slow process of secondary mineralization progressively adding about 50-60 % of the mineral content in bone matrix (MEUNIER and BOIVIN 1997). In this way, in healthy adults, the whole skeleton is continuously being regenerated every 10 years through periodic cycles of destruction of aged bone (MANOLAGAS 2000). The aim of this paper is to review the mechanisms of action of different antiresorptive agents in bone remodeling in postmenopausal women with osteoporosis.

Consequences of estrogen deficiency

After the menopause, the increased bone remodeling results in an accelerated loss of predominantly cancellous bone, particularly on the endosteal surface of bone. The accelerated phase gradually decreases over 4-8 years into a subsequent slow phase of postmenopausal bone loss (STEPAN et al. 1997).

Estrogen deficiency (Figure 1) increases **osteoclast** recruitment and birth rate of new bone units that undergo a remodeling cycle (activation frequency), and extends the bone resorption phase by reducing osteoclast apoptosis (MANOLAGAS 2000). Estrogen deficiency up-regulates the production and action of several cytokines, such as interleukins 1 and 6 (IL-1, IL-6), macrophage-colony-stimulating factor (M-CSF), tumor necrosis factor alpha (TNF α), and receptor activating nuclear factor kappa B ligand (RANKL) (PFEISCHFLER et al. 2002). The cytokines can have multiple effects on the osteoclast lineage, including the induction of osteoclast differentiation

and maturation, stimulation of inactive osteoclasts to resorb bone and inhibition of osteoclast apoptosis. This is exemplified by the action of RANKL. RANKL is expressed on the cell surface of osteoblasts/stromal cells and binds to RANK (its receptor), expressed on the cell surface of hematopoietic osteoclast precursor cells and mature osteoclasts. Upon RANK activation a cascade of signaling events and gene expression is triggered, that leads to differentiation and maturation of osteoclast precursors, to activation of mature osteoclasts, and protection of osteoclast progenitors and mature osteoclasts against apoptosis (Pfeilschifler et al. 2002). M-CSF is identified as an important permissive factor for the action of RANKL. Simultaneously, estrogen deficiency down-regulates the local production of osteoprotegerin (OPG), insulin-like growth factors (IGF I, IGF II) and transforming growth factor beta (TGF β). OPG acts as a decoy receptor for RANKL, thereby blocking the interaction of osteoblasts/stromal cells and the osteoclast compartment.

The high-turnover phase, due to excessive osteoclast activity, results in deep resorption cavities, trabecular plate perforation, wide separation and disconnection of trabeculae, and enlargement and coalescence of subendocortical spaces. The eroded reversal surface is 5- to 10-fold greater than the active resorption surface indicating a delay between the end of resorption by osteoclasts and the onset of bone formation.

During the subsequent slow phase of the postmenopausal bone loss, an insufficiency of bone formation is unable to keep pace with bone resorption by osteoclasts (Stepan et al. 1987). In addition to estrogen deficiency, a number of other age-related factors contribute to the slow phase of bone loss, (i) secondary hyperparathyroidism caused by age-related decrease in the ability to adapt to a lower calcium intake by increasing intestinal calcium absorption, (ii) decrease in renal calcium conservation, (iii) impaired vitamin D metabolism and (iv) impaired osteoblast recruitment and function. In clinical practice, the loss of bone mineral can be identified and quantified by dual energy X-ray absorptiometry (DXA) and is one of major risk factors for fractures (Kanis and Gluer 2000). The measurement of biochemical markers of bone resorption (type I collagen degradation products, such as CTX or NTX, in serum or urine, or serum osteoclastic acid phosphatase (ACP), and markers of bone formation (serum concentrations of bone alkaline phosphatase (ALP), or type I collagen synthesis product, PINP), allows to differentiate between high- and low-turnover processes (Delmas et al. 2000).

In aging adults, the number of osteoblasts recruited to erosion surfaces is decreased as well as their functional capacity, resulting in a decreased rate of **bone formation.** The number of osteoblasts is determined by the rate of replication of progenitors and the life span of mature cells, reflecting the timing of death by apoptosis. The activity of osteoblasts, and replicative life span of osteoblasts decrease with loss of estrogen (Weinstein and Manolagas 2000) and also with age (Yudoh et al. 2001). The impaired osteoblast activity due to a decrease of systemically available sex hormones, as well as by a decreased local effectiveness of cytokines and growth factors results in a further trabecular thinning (Parfitt 2000). Consequently, wall thickness (the depth of bone structural units on bone surfaces after the completion of bone formation) decreases in women after menopause indicating that each erosion cavity is refilled with a smaller than normal volume of bone (Schaffler et al. 1995).

Estrogen deficiency also suppresses survival of **osteocytes** (Weinstein and Manolagas 2000) and impairs the response of osteoblasts to mechanical stimuli, the detection of microdamage and therefore the repair of aged bone (Figure 1). Osteocytes sense changes in the interstitial fluid flow produced by mechanical forces, and through cell processes communicate to lining osteoblasts on the bone surface the need for focal repair of the microdamage/microcracks. The lining cells secrete collagenase, and by partial degradation of the mineralized surface prime the bone surface, enabling osteoclasts to resorb bone.

With increasing age in women as well in men, bone microdamage, evidenced as microcracks, accumulates more rapidly than intrinsic processes can repair (Schaffler et al. 1995). This has important consequences such as microarchitectural deterioration, and decreased bone strength and stiffness which lead to increased bone fragility and fracture associated with aging and osteoporosis. Thus, both estrogen deficiency and insufficient perception of biomechanical strain are the major mechanisms contributing to

alteration in the ability of the bone to perceive and react to microdamage. Moreover, the cancellous, endosteal, intracortical, and periosteal bone may change differentially during bone loss.

Differences in the biological effects of the drugs on bone remodeling

Estrogen/SERMs. The bone preserving and antiresorptive action of **estrogen** is mediated by estrogen receptor alpha and/or estrogen receptor beta (ERα and ERβ) in osteoblasts (VIDAL et al. 1999). These two receptors do represent distinct proteins generated by separate genomic elements. They display a differential distribution pattern during development, and in various adult tissues. Within a distinct tissue, receptor expression may depend on the differentiation state of the different cells. Estrogen receptors can form heterodimers as well as homodimers, the later with a potentially different activity profile. Both receptors were able to inhibit bone remodeling when acting alone. The antiresorptive action of estrogen is predominantly explained through a regulatory effect of the hormone on factors and cytokines produced by the osteoblasts and affecting osteoclast number, osteoclast activity, and the life span of the osteoclast in a paracrine manner. In addition, estrogen also affects bone resorption by acting directly on osteoclast progenitors. Estrogen decreases the responsiveness of the osteoclast progenitor cells to RANKL, thereby preventing the formation of osteoclasts (SRIVASTA et al. 1999). Consequently, estrogen supplementation in postmenopausal subjects decreases the formation and activity of osteoclasts and reverses the activation frequency to premenopausal level.

The classical genotropic pathway of estrogen action involves binding of estrogen to the ER in the cell cytoplasm. Estradiol binds equally well to both receptors whilst the two 3-hydroxy-metabolites of tibolone (but not the parent molecule) bind and activate preferentially the ERα (de GOOYER et al. 2002). This binding releases the chaperone proteins previously complexed to the ER, allowing the ligand-receptor complex to translocate to the nucleus. The two estrogen-ER complexes dimerize and together with co-activators and co-repressors (factors that influence access to nuclear genetic machinery) regulate the transcription of nuclear genomic elements that contain an estrogen response element (ERE) (KATZENELLENBOGEN et al. 2000). In this genotropic pathway, estrogen down-regulates the local production and action of cytokines (IL-1, M-CSF, TNFα, RANKL, and prostaglandin E_2).

Some of the genomic responses to the receptor-ligand complex may be mediated by a non-ERE pathway, such as the regulation of IL-6 production. Interference of ER with the binding of proteins to the NFkappa-B site of the IL-6 promoter, results in the impaired induction of IL-6.

In addition to the classical genotropic bone protective actions of estrogen, nongenomic effects of sex steroids are demonstrated to alteration of gene expression by means of a biochemical cascade independent of the nuclear transcription properties of the dimerized receptor-ligand complex. The downstream modulation of transcriptional activity involves activation of the Src/Shc/ERK signaling pathway. This pathway has been implicated in the attenuation of apoptosis in several cell types including osteoblasts, osteocytes, embryonic fibroblasts, and HeLa cells. ERα, ERβ, or the androgen receptor can transmit these signals with similar efficiency, irrespective of whether the ligand is an estrogen or an androgen. Applying synthetic ligands such as estren (4-estren-3α,17β-diol), the increase in both bone mass and bone strength can be dissociated from the transcriptional activity of the receptor on reproductive tissues (KOUSTENI et al. 2002). In contrast to the tissue selective properties of SERM compounds, estren may exert its tissue selective profile based on a mechanism-specific mode of action.

Besides the effects of estrogen on osteoclast differentiation and function, the hormone is also reported to affect the osteoblast compartment. Estrogen stimulates osteoblast cell proliferation and diminishes their apoptosis, affects gene coding for enzymes, bone matrix proteins, hormone receptors, transcription factors, and up-regulates the local production of OPG, IGF I, IGF II and TGF β. The anabolic effects of estrogen observed in different model systems include an increased osteoblast proliferation, differentiation, and function, and an extended osteoblast lifespan (MANOLAGAS 2000). In a longitudinal follow-up study of estrogen replacement therapy in older postmenopausal women, a substantial rise in bone

mineral density both at the lumbar spine and proximal femur, and a histological evidence for an increase in cancellous bone volume and wall thickness has been demonstrated (KHASTGIR et al. 2001).

The estrogen response profile has been shown to vary among different target tissues depending on the nature of the estrogen receptor ligand and the ligand dependent conformational change of the receptor. The multiple ER subtypes, the presence of multiple co-regulatory factors, and the estrogen response element profile determine the response of a tissue to an estrogen receptor ligand (BRYANT 2001). Agonists and antagonists for the ER induce distinct structural changes in the receptor that influence its ability to interact with other proteins (e.g., co-activators or co-repressors) critical for the regulation of target gene transcription.

With limitations in current understanding of estrogen's precise bone mechanism of action, **raloxifene**, a selective estrogen receptor modulator (SERM) and estrogen agonist on bone, decreases bone resorption presumably acting on the remodeling cells through the ER and it's binding to the estrogen response element in target DNA. The final common pathway involves inhibition of cytokine release from osteoblasts (TARANTA et al. 2002). Depending on the tissue, SERMs can act as ER agonists, partial agonist or antagonists (BRYANT 2001). SERMs enable the dissociation of bone protective effects from effects on reproductive tissues, thereby offering advantages over estrogens. SERM selectivity may reflect the diversity of ER forms and co-regulators, cell type differences in their expression, and the diversity of ER target genes in the different tissues. Both tamoxifen and raloxifene, two non-steroidal estrogen receptor ligands, induce the recruitment of co-repressors to target gene promoters in mammary cells. In endometrial cells, tamoxifen, but not raloxifene, acts like estrogen by stimulating the recruitment of co-activators to a subset of genes.

Similarly to estrogen, several preclinical studies indicate that raloxifene can exert an anabolic effect on bone. A concentration-dependent increase in the proliferation of osteoblasts derived from neonatal mice calvariae was reported in the presence of raloxifene. This effect was blocked by the estrogen-receptor antagonist ICI 164,384. Raloxifene also increased the osteoblast-specific transcription factor Cbfa1/Runx2 and α2 procollagen type I chain mRNAs, with a pattern that partially coincided with that of 17β-estradiol (TARANTA et al. 2002). Raloxifene and estrogen produce similar increase of bone TGFβ-3 mRNA in ovariectomized rats.

Bisphosphonates bind to the calcium hydroxyapatite through their phosphate groups but are resistant to catalytic hydrolysis by endogenous pyrophosphatases. Their potency is determined by their side chains. The active side chain of alendronate, ibandronate, pamidronate, risedronate, and zoledronate contains an amino group. Nitrogen-containing bisphosphonates (aminobisphosphonates, N-BP) reduce osteoclast function by inhibiting farnesyl diphosphate synthase, an enzyme in the cholesterol biosynthesis pathway. Depletion of farnesyl diphosphate or geranylgeranyl diphosphate levels limits the prenylation of small GTP-containing proteins (e.g. Rho, Rac, cdc42, and Rab), resulting in the impairment of the function of the osteoclast. At the cellular level, N-BP inhibit osteoclast recruitment, inhibit osteoclast adhesion to the mineralized matrix, reduce the osteoclast life span by activation of pro-apoptotic caspases, and directly inhibit the osteoclast activity by alteration in the cytoskeleton, including cell morphology, integrin signaling, and disruption of the ruffled border and trafficking of the endosomes (BENFORD et al. 2001). Ibandronate inhibits also squalen synthase activity. In murine osteoblast and osteocytes cultures, bisphosphonates prevented the induction of apoptosis (PLOTKIN et al. 1999). However, treatment with alendronate and risedronate at concentrations that are achieved on the bone surface caused a total inhibition of calcifying colony forming units (STILL et al. 2003).

Etidronate, tiludronate and clodronate, which lack nitrogen in the side chain, are metabolized within the osteoclasts and macrophages to form toxic methylene-containing analogs of ATP (FRITH et al. 2000).

Statins, which are inhibitors of the 3-hydroxy-3-glutaryl-coenzyme A (HMG-CoA) reductase, also inhibit osteoclastic bone resorption. Compared to the aminobisphosphonates, statins inhibit the mevalonate pathway upstream from the farnesyl diphosphate synthase. However in both cases, the production of farnesyl diphosphate or geranylgeranyl diphosphate is impaired (Figure 2). The ability of several statin analogues to inhibit bone resorption in the fetal rat

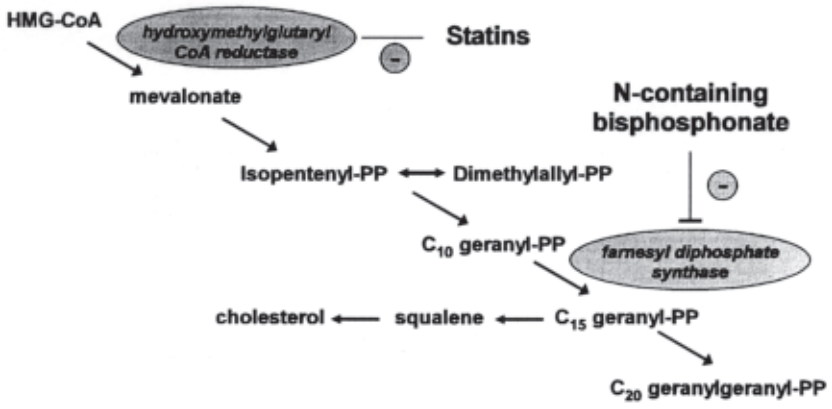

Fig 2 Inhibition of the mevalonate pathway by N-containing bisphosphonates and statins.

long bone explants in vitro is directly correlated with the potency of these compounds for the inhibition of HMG-CoA reductase (Staal et al. 2003). Cerivastatin was shown to inhibit Rap1A protein prenylation in osteoclasts isolated from bone marrow after in vivo treatment.

Calcitonin (CT) is a 32 amino acid polypeptide secreted by C-cells. Physiologically, CT lowers serum calcium concentrations by decreasing bone resorption and increasing urinary calcium excretion (Zaidi et al. 2002). Calcitonin may have an anabolic effect on osteoblasts. However, deletion of the calcitonin gene is accompanied by an increased bone mass (Hoff et al. 2002). Binding of CT to its receptor on osteoclasts results in a fast (minutes) loss of the ruffled border, cessation of motility and pseudopodial and margin retraction and inhibition of osteoclast secretion of proteolytic enzymes and the proton pump (Zaidi et al. 2002). The cessation of the osteoclast bone resorption is dose limited and is not accompanied by a decreased activity of osteoblasts (Zikan and Stepan 2002).

Consequences of differences in mechanisms of action of various drugs at the tissue level

Bone histomorphometry is the method of choice to study the consequence of drugs with distinct mechanism of action at the tissue level. Histomorphometric analysis measures static parameters reflecting the bone structure and microarchitecture in cancellous and compact bone, bone remodeling (resorption and formation) and dynamic parameters such as the rate of newly formed bone by the osteoblast using double tetracycline labeling. Histomorphometric analysis enables to assess important parameters of bone quality, such as texture of bone matrix (lamellar or woven bone), microarchitecture of the trabecular network and presence of mineralization defects.

Menopause affects the activation frequency and results in a decrease of the lifetime of the basic structure units, i.e. osteons in cortical bone or cancellous packets (BSUs). Consequently, new BSUs are resorbed before they have fully completed their secondary mineralization, as proven by the presence of numerous incompletely mineralized BSUs and a low mean degree of mineralization of bone (DMB). The antiresorptive agents (bisphosphonates, estrogen, SERMs) cause a reduction in the activation frequency, prolong the lifetime of the BSUs, allow a more complete secondary mineralization, and finally provoke an increase in DMB (Boivin et al. 2000, 2002; Boivin and Meunier 2002a,b). However, various antiresorptive agents affect the activation frequency and increase in DMB to a different degree.

Examination of bone biopsy specimens from **estrogen as well as raloxifene** treated women revealed normal bone quality and showed similar patterns in comparison with values in young healthy normal women including premenopausal patients (Ettinger et al. 1999; Delmas et al. 2002; Eriksen et al. 1999; Prestwood et al. 2000). Increases in DMB were not statistically different from calcium and vitamin D. Experimentally, raloxifene and estrogen produced equivalent beneficial effects on biomechanical properties of vertebrae and femoral neck. Bone forma-

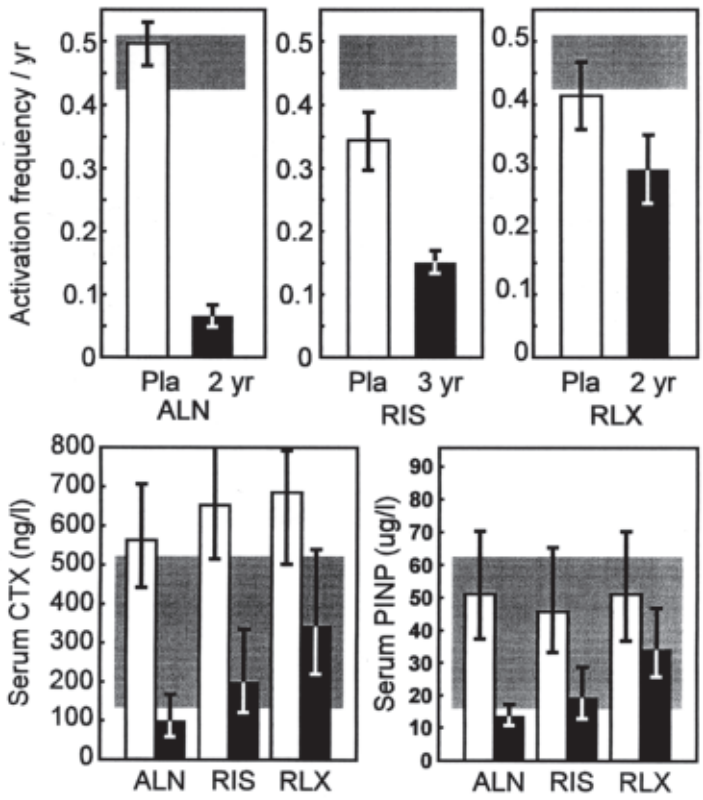

Fig 3 Upper Distribution of the activation frequency (% /year) in cancellous bone from human post-menopausal osteoporotic patients treated with alendronate (ALN 10 mg/day), or risedronate (RIS 5 mg/day), value at the end of the treatment compared with value in placebo treated women, and with raloxifene (RLX, 60 or 120 mg/day for 2 years), value at the end of the treatment compared with value at baseline in the same patients. The shaded area indicates mean and 1 SE range in premenopausal women (BOIVIN et al. 2002).

Bottom Serum concentrations of C-terminal telopeptide of type I collagen (CTX) and the aminoterminal propeptide of type I collagen (PINP) (mean and 1 SD range) in untreated women with postmenopausal osteoporosis and after 1 year of treatment with alendronate (10 mg/day, 65 patients), risedronate (5 mg/day, 20 patients), and with raloxifene (60 mg/day, 50 patients). All patients received calcium (500 mg/day) and vitamin D (800 IU/day). The shaded area indicates mean and 2 SD range for 65 healthy premenopausal women (STEPAN et al. 2002).

tion rate at the tissue level and activation frequency in women treated with raloxifene and/or estrogen were lower than the average for premenopausal women but within the expected range (BOIVIN et al. 2002; KHASTGIR et al. 2001) (Figure 3). The anabolic effect of estrogen is observed when higher doses are administered. Especially, subcutaneous estradiol implants increase BMD substantially more than other routes of administration (KHASTGIR et al. 2001).

The effects of **alendronate** (ALN) on bone quality and remodeling have been assessed in 231 postmenopausal women with osteoporosis treated with oral doses of 5, 10 or 20 mg/day, after 2 and 3 years of treatment (CHAVASSIEUX et al. 1997). Mineral apposition rate was unaffected by ALN treatment. Osteoid thickness, osteoid volume and osteoid surface significantly decreased. Whatever the dose, mineralizing surfaces and activation frequency significantly decreased at each time point (by 92% after 2 years and 96% after 3 years at 10 mg daily dose) (Figure 3). ALN dose-dependently and markedly decreased the rate of bone turnover. It was devoid of adverse effects on bone mineralization. Similar effects of ALN on the bone turnover have also been observed in patients with corticosteroid-induced osteoporosis, after 1 year of treatment. Interestingly, after 3 years of risedronate treatment which is accompanied by a significant reduction in both verte-

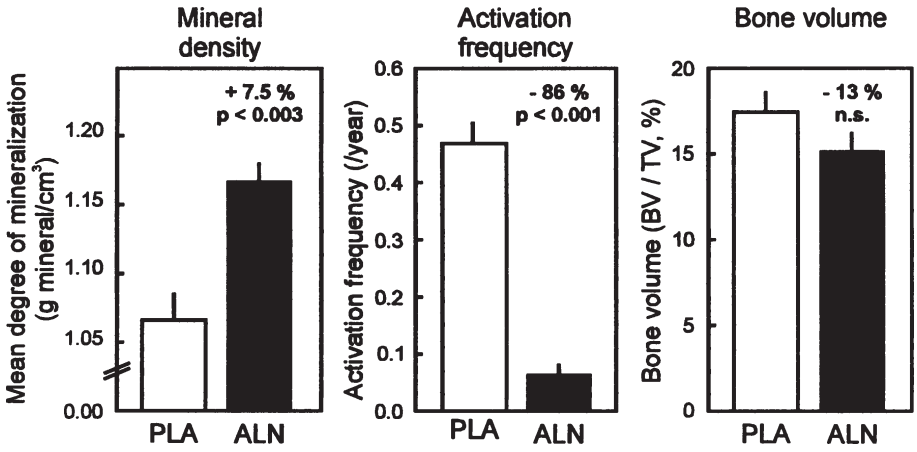

Fig 4 Mean degree of mineralization of total (compact and cancellous) bone, activation frequency, and trabecular bone volume after 2 years on alendronate treatment (ALN, 9 women) or placebo (PLA, 15 women) (mean and 1 SE) (Boivin et al. 2000).

bral and nonvertebral fractures (Cranney et al. 2002), the activation frequency in patients with postmenopausal osteoporosis was decreased only by 47% at 5 mg dose (Eriksen et al. 2002).

The results obtained using bone histomorphometry are in a good agreement with changes in biochemical markers that provide precise information on bone remodeling at the total skeleton level. Within 6 months of treatment with ALN (10 mg/day), raloxifene (60 mg/day), or nasal calcitonin (200 IU/day), both biochemical markers of type I collagen degradation (serum CTX) and synthesis (serum PINP) decreased below the premenopausal range, to the premenopausal mean values, or remained unchanged, respectively (Figure 3) (Stepan et al. 2002).

The bone biopsy specimens have been used for the evaluation of DMB by microradiography (Boivin et al. 2000; Boivin and Baud 1984; Meunier and Boivin 1997; Boivin and Meunier 2002a). In adult bone, the mean DMB depends on the rate of turnover. In postmenopausal women treated for 2 and 3 years with 10 mg/day of ALN, the mean DMB in cancellous bone was increased by 7.3% and 11.4% ($p < 0.001$), respectively, when compared to the placebo group (Boivin et al. 2000; Meunier and Boivin 1997; Boivin and Meunier 2002b) (Figure 4). After 2 and 3 years of ALN, and compared with the corresponding placebo, the distribution of the degree of mineralization in compact and cancellous bone showed a clear shift toward the highest mineralization values and a decrease in the number of bone structural units having low values of mineralization. The between-group differences in mean DMB were similar to those of BMD at the lumbar spine (+8.7% after 2 years and +9.6% after 3 years, respectively), suggesting that mean DMB augmentation probably accounted for the majority of the increase in BMD seen with ALN. However, the mean bone volume (BV/TV, %) did not change significantly, which is in good agreement with biochemical evidence of highly reduced synthesis of type I collagen in patients treated with ALN (Figure 3).

The relationship between the degree of mineralization and the mechanical properties of bone is complex. Again, it may have different consequences at the cancellous and cortical bone. The reduction of bone turnover induced by ALN followed by a prolonged secondary mineralization improved the bone strength as proven by the reduction in fracture rate after up to 4 years treatment with ALN (Black et al. 1996; Pols et al. 1999; Cummings et al. 1998; Liberman et al. 1995). Similar observations have been performed in corticosteroid-induced osteoporosis treated with ALN. However, the continuous profound decrease of the bone formation rate may have a negative effect. Damaged bone must be promptly removed to restore mechanical integrity. This bone remodeling targeted towards damaged sites is initiated by altered osteocyte morphology. It has been hypothesized that about 30% of bone remodeling is targeted to repair of microdamage (Burr 2002). Suppression of bone remodeling and microdamage repair allows microdamage to accumulate, it underlies the development of stress fractures, and plays a role in the increased bone fragility (Burr

et al. 1997). Suppressed bone turnover by high doses of bisphosphonates is associated with an increased microdamage accumulation and reduced bone toughness in the cortical bone of the dog rib, a highly mechanically loaded bone (Mashiba et al. 2000). In this model, one year treatment with RIS or ALN significantly decreased trabecular remodeling in vertebrae (ALN, 95%) and ilium (ALN, 90%) without impairment of mineralization, and significantly increased microdamage accumulation in all skeletal sites measured. It should be emphasized that in studies performed in human treated with ALN (doses lower than in animals), an increase in the number or extent of microdamage have never been reported. The normalized toughness, i.e., the ability of bone to resist the progression of microdamage, of the L-4 vertebra was reduced by 21% in both RIS and ALN groups. When the two bisphosphonates groups were pooled, this reduction in toughness reached statistical significance ($p = 0.02$) (Mashiba et al. 2001). The data are in agreement with observations that resistance of bone to development and progression of microdamage declines as the tissue mineral content (percent volume of bone that is mineral ash) exceeds about 65 %.

In patients treated with nasal **calcitonin** 200 IU daily, the erosion depth was significantly lower than in the placebo group after 12 months, whereas bone volume and activation frequency did not differ between the groups (Thamsborg et al. 1996). Subcutaneous administration of 50-100 IU salmon calcitonin results in an inhibition of bone resorption lasting 8-16 hr. However, inhibition of bone resorption after the nasal administration of 200 IU calcitonin does not exceed 4 hr, and the area under curve of decrease in serum marker of bone resorption (C-terminal telopeptide of type I collagen) after nasal administration of 200 IU is similar to that after the subcutaneous administration of 2 IU salmon calcitonin (Zikan and Stepan 2002). For obtaining the same hypocalcemic effect with nasal calcitonin, up to 30 times higher dosages of salmon calcitonin must be administered.

Consequences of differences in mechanisms of action of different drugs and the day-to-day care of patients

All of the above antiresorptive drugs, regardless of their nature, share a similar feature, to reduce bone remodeling. The physiological equilibrium between bone resorption and bone formation within the premenopausal range can be achieved using estrogens, raloxifene and partly also calcitonin. This enables for adequate repair of microdamage of bone, and increase in bone strength. Decreased bone remodeling results in reduction in plate perforation and plate connectivity disruption, and in a slight increase in BMD (Dempster 2002). Deep decrease of bone resorption and bone formation below the premenopausal range induced by therapeutic doses of bisphosphonates, namely by the ”more potent” ALN, allows more time for secondary mineralization to proceed to completion in the existing bone tissue mass. Consequently, the mineral content (BMD) increases and the entire skeleton gets harder and mechanically more competent. However, if remodeling remains deeply decreased for years, bone may become more brittle and prone to structural failure, but no histomorphometric data, including the measurement of the degree of mineralization of bone, after long-term treatment are today available.

Accordingly, three years of treatment with 10 mg/day ALN resulted in the pooled estimate of the difference in percentage change of BMD in comparison with placebo at the lumbar spine of 7.5%, and 5.6% at the hip (Black et al. 1996; Liberman et al. 1995; Cummings et al. 1998; Cranney et al. 2002). The mean pooled relative risk for vertebral fractures in women treated with 5 mg or more of ALN was of 0.52%, and was comparable to that of women treated with 60 mg or more of raloxifene (0.60%) despite a twice lower increase in the lumbar spine BMD in the raloxifene treated patients (Cranney et al. 2002). Thus, the magnitude of increases in BMD with antiresorptive therapies differs greatly, yet the vertebral fracture risk reductions are similar. Interestingly, maximal vertebral fracture reduction has also been reported after short duration of antiresorptive therapy, before full increments in BMD have been achieved.

With a long-term ALN treatment, lumbar spine BMD, but not femoral neck BMD, continued to increase (Tonino et al. 2000) when the incremental gain in BMD (treatment over control) decreased progressively with each year. The pooled relative risk for vertebral fractures in women treated for 3 years with 5 mg or more of ALN was decreased by 48%. The relative risk of nonvertebral fractures in patients giv-

en 10 mg or more per day was decreased by 49% (Cranney et al. 2002). However, when the original 3-years study with ALN in postmenopausal women (Liberman et al. 1995) was extended, the observed rate of new clinical vertebral fractures was at least three times higher during the year 6 and 7 compared to the rate during the first three years of treatment. It was evident despite the continuous BMD increase in the lumbar spine (Tonino et al. 2000). The rates were also higher than predicted from the data in the fracture intervention trial (FIT) study.

The increased mineralization of bone and the resulting increase in bone stiffness may be advantageous in preventing fractures of cortical bone that tolerates greater load per unit area when compared with trabecular bone. By contrast, the vertebral bodies consist mainly of trabecular bone that tolerates much greater peak strains (deformations) than cortical bone, but much lower loads per unit area. Therefore, an increased mineralization of bone may explain the ability of bisphosphonates to prevent hip fractures within relatively short time of treatment in very elderly but not preventing fractures in younger women without low BMD (Cummings et al. 1998). By contrast, raloxifene prevents vertebral fractures in patients with both osteopenia and osteoporosis, but does not prevent nonvertebral fractures in younger women within three years of treatment (Ettinnger et al. 1999).

Hormone replacement therapy (**HRT)** is an established approach to palliate the signs and symptoms related to estrogen deficiency (mainly in women soon after the menopause), as well as for the treatment and prevention of osteoporosis. It is usually prescribed in women without actual risk factors for cardiovascular disease and without a family history of breast cancer. Protection by HRT against osteoporotic fractures is supported by a large meta-analysis of estrogen trials, cohort studies, and trials with bone density outcomes (Cranney et al. 2002). In a large prospective study in 16 608 postmenopausal women (5.2 year follow-up), the relative risk (RR) of a new clinical vertebral fracture as well as hip fracture significantly decreased by 34% (Writing Group for the the Women's Health Initiative 2002). However, long-term estrogen use has to be critically discussed since the results of the Women's Health Initiative and the decision of the data and safety monitoring board which judged that risks exceed benefits in HRT (Writing Grouip for the Women's Health Initiative 2002). Any protective effect of estrogen on bone is dose-related and effective only when the patient is taking estrogen. The benefits of HRT diminish once treatment stops. It was calculated that the risk of fractures would be reduced by 73% in women aged 75-85 years who were on HRT from menopause continuously, and by 23% in women who stopped at age of 65 (Ettinger and Grady 1994).

Alternatively, postmenopausal women can be treated with **tibolone**, a synthetic sex hormone derivative that does not possess side effects of estrogens on breast and cardiovascular system and prevents postmenopausal bone loss. After 2 years of treatment, tibolone appear to have had a more marked effect than raloxifene on bone mineral density at both spine and hip, whilst the effect of tibolone and estradiol plus norethisterone acetate appear to be comparable (Kloosterboer and Edrween 2003). After 10 years of treatment with tibolone, the difference in bone mineral density compared with a placebo group was more than 12% for both lumbar spine and femoral neck (Rymer et al. 2002). Tibolone efficiently prevents hot flushes, has a positive effect on mood, sexuality, and does not induce endometrial proliferation. Preclinical in vivo studies with tibolone have not shown any stimulation of breast tissue, and no trends towards an increase in cardiovascular and/or venous thromboembolism risks have been found (Kloosterboer and Ederween 2003). However, there are no prospective, randomized, clinical trial data available that support the use of tibolone in preventing osteoporosis-related fractures.

The **raloxifene** (RLX), 60 mg per day, has been studied in both the prevention and treatment of osteoporosis. RLX was compared with placebo with respect to radiographically identified vertebral fractures, bone mineral density changes at both the lumbar spine and hip, and biochemical markers of bone turnover. The Multiple Outcomes of Raloxifene Evaluation (MORE) trial evaluated the effects of raloxifene in 7705 postmenopausal women with osteoporosis, of whom about one third had prevalent vertebral fractures at study entry. At the end of one year, the relative risk (RR) of a new clinical vertebral fracture with RLX decreased by 68 %. After 3 years, the RR of an incident vertebral fracture de-

creased by 55 % in women without prevalent vertebral fractures, and by 30 % in women with prevalent vertebral fractures, with RLX. This effect was sustained in the fourth year. All of these reductions were statistically significant. The decrease in the RR of non-vertebral fracture at 3 years with raloxifene was not significant. BMD at the hip and lumbar spine increased by 2.1 % and 2.6 %, respectively, compared with placebo, at 4 years (Ettinger et al. 1999). There were sustained reductions in biochemical markers of bone turnover to the premenopausal range at 3 years with RLX treatment. Examination of bone biopsy specimens from RLX treated women revealed normal bone quality and mineralization.

In the MORE study, at 4 years, treatment with RLX was associated with a significant reduction in the incidence by 72 % of invasive breast cancer (Cauley et al. 2001), with a significant reduction by 40 % in risk of cardiovascular events in the subset of women with osteoporosis and increased cardiovascular risk at baseline (Barrett Connor et al. 2002), and with a significant reduction in the incidence and severity of urinary incontinence. RLX, in contrast to estrogen and tamoxifen, does not induce endometrial tissue proliferation in postmenopausal women, and does not increase the rate of endometrial hyperplasia, and rate of endometrial cancer. The beneficial effects of RLX on tissues other than bone also have to be considered when a long-term treatment of postmenopausal osteoporosis is initiated. Women with osteoporosis without prevalent vertebral fractures, in the MORE Study (mean age, 65 years), had the likelihood of a cardiovascular or breast cancer event (2.2 %) similar to that of a vertebral fracture (4.2 %). Furthermore, the likelihood of a coronary event, invasive breast cancer, or clinical vertebral fracture (1.2 %) was similar (1.4 %, 1.4 %, and 1.2 %, respectively), and each of these events was approximately 5 times more likely than hip fracture (0.25 %). The rate of discontinuation of treatment, that would be deleterious to bone, is lower as compared to other antiresorptive therapies. However, absence of efficacy of RLX in the control of the climacteric syndrome does not support it as a treatment of choice at the beginning of postmenopausal phase.

The largest prospective clinical trial to determine the efficacy of calcitonin in postmenopausal osteoporosis, the prevent recurrence of osteoporotic fractures (PROOF) study, as well as the large metaanalysis, indicated that calcitonin (mainly salmon CT) administered subcutaneously or nasally is effective in prevention of trabecular bone loss in postmenopausal women, despite a minimal change in bone mineral density and only a very modest effect to reduce indices of bone resorption (Cranney et al. 2002; Chesnut et al. 2000). Since calcitonin inhibits osteoclast activity transiently without decreasing osteoblast collagen synthesis (Zikan and Stepan 2002), treatment with calcitonin might have reduced vertebral fracture incidence by maintaining a positive remodeling balance and enabling reparation of microdamage in bone matrix to prevent microdamage accumulation and increased fragility of bone. However, long-term continuous treatments with calcitonin are associated with a progressive decrease in responsiveness. Possible explanations include formation of neutralizing antibodies to calcitonin, a decrease in the number and sensitivity of specific receptors, secondary hyperparathyroidism, as well as local reactions to the nasal administration such rhinitis and nasal mucosal atrophy.

In conclusion, estrogens and raloxifene reduce the risk of vertebral fractures by a physiological mechanism of action on bone metabolism that includes a maintained function of osteoblasts and osteocytes. Calcitonin maintains bone by a physiological mechanism that involves a transient inhibition of osteoclast activity without decreasing osteoblast collagen synthesis. Bisphosphonates decrease bone remodeling by permanent decreases of osteoclast activity and by inducing osteoclast apoptosis. This allows more time for secondary mineralization to proceed to completion in the existing bone tissue mass, and increase of mechanical resistance of bone to load per area. During the treatment, an adequate bone remodeling, reparation of microdamage of bone, and increase of bone strength should be guaranteed. Further investigation is needed to evaluate the different modes of action, and the long-term effects and safety of antiresorptive agents for the long-term management of osteoporosis. The therapeutic management of postmenopausal osteoporosis requires long-term treatment with antiresorption drugs. Selection of treatment should weigh the relative occurrence rates for osteoporosis, cardiovascular events, and breast cancer, the

three major health concerns affecting postmenopausal women. Therefore, along with effects of antiresorption therapies on bone, effects of tissues other than bone also have to be considered when a long-term treatment of postmenopausal osteoporosis is initiated.

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