

Hormonal Therapies and Osteoporosis

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Abstract

Osteoporosis, now defined as a disease characterized by low bone mass and a microarchitectural deterioration of bone tissue leading to enhanced bone fragility and fracture risk, is a major public health problem. Classic hormonal therapies to prevent and treat osteoporosis associated with menopause have recently been questioned due to the risk/benefit ratio of prolonged treatment. There is a critical need for safe and effective alternative therapeutics for this disease. Nonhuman primates have been used as models to assess bone changes associated with estrogen deficiency because their trabecular and cortical bone remodeling processes, monthly menstrual cycles, and reproductive-hormone patterns are similar to those of humans. The ovariectomized nonhuman primate has become the preferred model in which to study effects on bone remodeling, particularly with regard to bone mass, architecture, and strength, in fulfillment of studies required by international guidelines for the development of antiosteoporotic drugs. The nonhuman primate is amenable to several methodologies that assess bone quantity and quality, including dual energy x-ray absorptiometry (DXA), quantitative computed tomography (QCT), histology, static and dynamic histomorphometry, and biomechanical testing, as well as assays developed for clinical use, which serve as biomarkers of bone metabolic processes. The use of the nonhuman primate model in the assessment of osteoporosis therapeutics, both hormonal (sex steroids and their analogues, parathyroid hormone) and nonhormonal (bisphosphonates), has provided valuable information on the safety and efficacy as well as the mechanisms of bone loss associated with estrogen deficiency that is directly applicable to the human situation.

Key Words: bisphosphonates; bone loss; monkey; ovariectomy; PTH; SERMs; sex steroids

Introduction

Osteoporosis, the most prevalent metabolic bone disease in the United States and other developed countries, is characterized by low-energy fractures resulting from inadequate bone mass and compromised microarchitecture (Wasnich 1996). The skeleton comprises cortical bone (the dense bone found, for example, in the

shafts of the long bones) and cancellous or trabecular bone (the network of plates and rods surrounded by bone marrow in the ends of the long bones and vertebrae). In adult humans and other large species, both cortical and trabecular bone are continually remodeled by the coupled activity of osteoclasts (which resorb bone) and osteoblasts (which form new bone to replace that resorbed). Age-related bone loss seen in both sexes is the result of gradual thinning and ultimately loss of trabeculae due primarily to declining osteoblast function, and cortical thinning due to endosteal expansion (Ruff and Hayes 1988). In women, osteoporosis associated with the disappearance of estrogen at menopause is viewed as osteoclast mediated. The end result is perforation of trabecular plates, uncoupling of bone formation and bone resorption, and loss of bone mass (Body 2002).

The agents currently approved by the Food and Drug Administration (FDA¹) for the prevention and/or treatment of osteoporosis are calcium, hormone replacement therapy (HRT¹), the selective estrogen receptor modulator (SERM¹) raloxifene, the bisphosphonates alendronate and risedronate, and calcitonin. The comparative efficacy and safety of these agents, all of which block the activity of bone-resorbing osteoclasts, have been well documented (Body 2002; Eichner et al. 2003; Tuck and Francis 2002). Although HRT has been the main line of defense against osteoporosis associated with menopause, concerns have recently arisen regarding the long-term risk of breast cancer, coronary disease, and pulmonary embolism despite the antifracture efficacy of HRT (Rossouw et al. 2002). A promising new addition to agents used for the treatment of osteoporosis is recombinant human parathyroid hormone 1-34 (rhPTH(1-34)¹). This anabolic agent, which stimulates bone turnover with a substantial net increase in bone formation, is currently being reviewed by the FDA for marketing approval (Tashjian and Chabner 2002; Whitfield et al. 2002).

The FDA requires data from both the rat and a larger species in the preclinical testing of agents for treatment of osteoporosis (FDA 1994). These studies should include

¹Abbreviations used in this article: BMC, bone mineral content; BMD, bone mineral density; CEE, conjugated equine estrogens; DXA, dual energy x-ray absorptiometry; ER, estrogen receptor; ERT, estrogen replacement therapy; FDA, Food and Drug Administration; FSH, follicle-stimulating hormone; GnRH-Ag, gonadotropin-releasing hormone antagonist; HRT, hormone replacement therapy; MPA, medroxyprogesterone acetate; rhPTH(1-34), recombinant human parathyroid hormone 1-34; PTH, parathyroid hormone; SERM, selective estrogen receptor modulator.

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measurement of bone mineral density, histomorphometric analysis of bone architecture, and assessment of bone biomechanical qualities (bone strength) and biochemical markers of bone metabolism. The appropriateness of various small and large animal models for osteoporosis research has been previously reviewed (Bellino 2000; Grynypas et al. 2000; Kimmel 1996; Mosekilde 1995; Newman et al. 1995; Thompson et al. 1995). A large species is required in addition to the rat because the rat skeleton undergoes modeling (change in size and shape) throughout life, a process that by definition involves uncoupled activity of osteoclasts and osteoblasts, whereas the larger species are considered to have a remodeling skeleton more similar to that of humans. In addition, the rat does not usually remodel cortical bone, whereas humans and other large species do.

The ovariectomized rat model is convenient and inexpensive, and has provided information relevant to humans on the effects of test compounds on trabecular bone (Grynypas et al. 2000; Kalu 1991; Wronski and Yen 1991). However, ovariectomy-induced changes are largely confined to cancellous bone in rats, whereas bone strength in humans is known to be dependent also on cortical bone, even in vertebrae, which have a large trabecular bone component (Mosekilde 1998).

Compared with rodents, nonhuman primates are much more similar in terms of their reproductive and skeletal physiology to humans. Old World monkeys, including macaques (*Macaca* sp.) and baboons (*Papio* sp.), are unique among large animal models used in bone research in having menstrual cycles with estrogen and progesterone levels and patterns similar to women (see Hendrickx and Dukelow 1995 and references therein). Nonhuman primates not only have cortical bone Haversian remodeling comparable to humans, but also the response of cortical bone of skeletally mature monkeys to ovariectomy and other stimuli closely parallels that observed clinically. For these reasons, the nonhuman primate has been increasingly used to evaluate the efficacy and safety of new therapies for osteoporosis. Other large species may also be good models for the human adult skeleton, but relatively few studies have been published. Studies in minipigs suggest that they lose bone in response to ovariectomy under certain circumstances (Boyce et al. 1995), studies in dogs document a response to ovariectomy that appears to differ in mechanism from that observed in menopausal women (Malluche et al. 1986), and studies in sheep have had inconsistent results perhaps due to breeding seasonality (Hornby et al. 1995). For species other than primates, estrus cycles or induced ovulation reproductive patterns, and breeding seasonality, create physiological and logistical obstacles to modeling the estrogen deficiency of the human menopause.

The primary focus of this article is the role of the most commonly used nonhuman primates in development of hormonal therapies for menopause-related osteopenia. Additional topics described below include the affects of aging and estrogen deficiency on normal bone biology in nonhuman primates, evaluation of nonhormonal therapies, and the

development of primate models for short-term efficacy screening of bone-active compounds.

Skeletal Biology in Nonhuman Primates

An important feature of nonhuman primate bone is the presence of Haversian osteonal remodeling in cortical bone, as observed in humans (Zoetis et al. 2003). Haversian remodeling in macaques was first described by Burr (1992). Haversian osteons occupy approximately 40% of the mid-femur cortex and 60% of the mid-radius cortex in intact, skeletally mature female cynomolgus monkeys (unpublished data). It is most prominent adjacent to the endocortical surfaces, but can extend throughout the cortical width. Burr and colleagues (2001) have recently reviewed this subject.

In female cynomolgus (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) monkeys, almost all growth plates close by 6 to 7 yr of age, and peak bone mass is achieved by 9 to 11 yr (Champ et al. 1996; Jayo et al. 1994). Young skeletally immature (3- to 4-yr) cynomolgus monkeys have high bone turnover rates, and skeletally mature (10- to 15-yr) monkeys have decreasing bone biomarkers and bone formation rates that reflect an overall decrease in bone turnover with age (Lees and Ramsay 1999).

Age-related changes in bone mass and bone structure, particularly in older females, have been documented in several Old World monkey species. Radiographic analysis of long bone growth in rhesus monkeys reveals increased mean subperiosteal and medullary width and increased mean cortical thickness with age (Pathmanathan et al. 2002). Osteopenia, evidenced by a decreased percentage of cortical bone in radiographs of the second metacarpals, occurs naturally with age in locomoting rhesus monkeys (DeRousseau 1985), in domestically housed pigtail (*Macaca nemestrina*) and cynomolgus monkeys (Bowden et al. 1979), and in baboons (*Papio hamadryas*) (Kammerer et al. 1995). A trend toward vertebral bone loss of 3 to 4% per year was reported by dual energy x-ray absorptiometry (DXA¹) in colony-housed female cynomolgus monkeys >10.5 yr old (Jayo et al. 1994). Spinal bone mineral density (BMD¹) in free-ranging rhesus monkeys remains constant between 9.5 yr (peak bone mass) and ~17 yr, after which it steadily declines (Cerroni et al. 2000). Age-related declines in forearm BMD and total body bone mineral content (BMC¹) were also observed in male (8- to 27-yr) and female (8- to 23-yr) rhesus monkeys in a laboratory setting (Black et al. 2001). Colman and Binkley (2002) have recently reviewed the benefit of using macaques as models of skeletal aging in humans.

Estrogen Deficiency in the Nonhuman Primate Model

Estrogen depletion has one of the most profound effects on skeletal physiology in both humans and nonhuman pri-

mates. Declining ovarian function and irregular menstrual cycles similar to those reported in peri- and postmenopausal women, studied most extensively in the rhesus monkey, occur during the third decade in macaques (Walker 1995) and in >15-yr baboons (Chen et al. 1998). This natural menopause, with low serum estradiol and high follicle-stimulating hormone (FSH¹) levels, is accompanied by decreased bone mass and increased bone turnover in rhesus females (Colman et al. 1999). Decreased radial bone density by DXA and reduced vertebral bone volume accompanied ovarian dysfunction in old female baboons (Aufdemorte et al. 1993).

In addition to natural estrogen depletion, ovariectomy (surgical or chemical) in the monkey model also has profound effects on bone quality and quantity. The ovariectomized monkey has become a widely recognized model of human osteoporosis to assess therapeutics in fulfillment of FDA requirements for “large animal” model data (FDA 1994). It should be emphasized that these models mimic early postmenopausal changes in skeletal biology and not the disease, postmenopausal osteoporosis. Although reduced bone strength has been demonstrated in the ovariectomized monkey model, there are no reports of spontaneous fractures after these experimental manipulations.

Concerns have been expressed that the ovariectomized monkey model does not produce consistent, statistically significant osteopenia and bone fragility, and frequently exhibits a “failure to gain” bone, rather than the clear bone loss expected of a model for human postmenopausal osteopenia. Recently analyzed data from a variety of published and unpublished studies show that experimental outcomes in the ovariectomized monkey model have, nevertheless, been quite consistent (Jerome and Peterson 2001). In this analysis, intact and ovariectomized groups were compared for several endpoints (spine BMD, femoral neck cantilever compression test failure load, and vertebral compression test failure load), in studies ranging in duration from 9 to 24 mo. Ovariectomized animals consistently had lower failure loads and spine BMD than intact animals, with mean differences of 9% for BMD and 13% for load. A similar evaluation of histomorphometric data revealed a relatively consistent trend toward lower values for cancellous bone volume (Cn.BV/TV) in ovariectomized versus intact animals, but this difference was usually nonsignificant in most studies due to a higher variance for histomorphometric data than for BMD or biomechanical endpoints (Jerome and Peterson 2001).

It is important to note that although BMD was consistently lower in ovariectomized than in intact animals, this outcome was due to bone loss in ovariectomized animals in some studies, but was due to failure to gain bone in others. The circumstances under which these different findings occur are now reasonably well documented, and have recently been discussed in detail (Brommage 2002). In most cases, bone gain in nonovariectomized animals and failure to gain in ovariectomized animals have not been due to use of young animals that have not yet achieved peak bone mass.

Rather, they occur when wild-caught animals are ovariectomized within a few months of importation into the laboratory. When domestically reared animals or animals that have been laboratory housed and nonpregnant for approximately 1 yr are used, then bone loss is seen in ovariectomized animals, particularly if a diet lower in calcium than standard laboratory diets is fed (Hotchkiss et al. 2001). Skeletally mature, wild-caught animals gain bone after importation due to the combined effect of recovery from reproductive demands on skeletal mineral stores, dietary changes, and perhaps consumption of fluoridated water. This gain plateaus after approximately 1 yr, or is abrogated by ovariectomy if it is performed earlier.

The degree of osteopenia in ovariectomized monkeys is small compared with the profound cancellous osteopenia observed in ovariectomized rats, but the monkey data are very comparable to data from women. A recent and so far unique study of perimenopausal women documented longitudinal changes in spinal bone mass (Recker et al. 2000). Women lost 10.5% of spinal BMD in a sigmoid pattern of bone loss ending 3 to 4 yr after menopause. As shown in the analysis described above (Jerome and Peterson 2001), ovariectomized monkeys exhibited on average 9% spinal osteopenia. Moreover, the development of osteopenia follows a sigmoid pattern in monkeys, with most of the bone loss occurring during the first year after ovariectomy (Jerome et al. 1997b). Thus, ovariectomized monkeys undergo changes in spinal bone mass comparable to those of postmenopausal women, within a 3- to 4-fold compressed time frame.

In all of the studies cited above, bone mass was measured using DXA, which measures BMC and projected area, calculating a so-called “areal density” or BMD as BMC/area. DXA-derived BMD is not a true volumetric density, and its value depends on the unknown depth of bone in the plane perpendicular to the projected area, as well as on the volumetric density. The interpretation of DXA data can be complex, especially when bone growth occurs during the course of the experiment. Methods are now available for determination of volumetric density of bone in animals, including monkeys (Hotchkiss 1999), and these methods might be better predictors of bone strength in animal studies.

The development of osteopenia and loss of bone strength in ovariectomized monkeys are accompanied by changes in bone dynamics (Legrand et al. 2003). Serum and urine biomarkers of bone turnover increase within a few months, peak at 6 to 12 mo, and remain elevated for 18 mo or longer, while increased bone formation rates are observed at multiple sites by histomorphometry (Balena et al. 1993; Jerome et al. 1986, 1994, 1997a,b; Thompson et al. 1992). Consistent with the pattern of bone loss, bone turnover returns to control levels between 18 and 24 mo in the spine; but interestingly, the high bone turnover response is more robust and persists longer in cortical bone of the femur, radius, and humerus than at cancellous sites, and is accompanied by an increase in porosity (Burr et al. 2001; Jerome

et al. 1997b; Landon and Grynepas 1993). Such changes are poorly documented in women.

In a recent study using fluorescence-assisted synchrotron infrared microspectroscopy, the chemical composition of subchondral bone (knee joint) from ovariectomized cynomolgus monkeys was examined (Miller et al. 2000). The results indicated a reduced rate of mineralization (higher acid phosphate content) with time after ovariectomy as well as abnormal collagen content (increased cross-linking) in remodeled bone from ovariectomized animals relative to intact animals. These ultrastructural factors may play a contributing role in the increased bone fragility in osteoporosis.

The chemical induction of ovariectomy using gonadotropin-releasing hormone agonist (GnRH-Ag¹) was first examined in rhesus and cynomolgus monkeys in the early 1990s (Mann et al. 1990, 1992). These studies demonstrated reduced ovarian steroidogenesis accompanied by reductions in lumbar vertebra, caudal vertebra, and humerus BMD over a 9- to 10-mo period, and elevated serum osteocalcin levels after 6 mo of treatment. These effects were reversible after the termination of GnRH-Ag treatment.

The medical estrogen-depletion model has been used primarily for short-term osteoporosis research. Stroup and colleagues (2001a) used biochemical markers of bone resorption in the cynomolgus monkey to show significant increases in bone resorption, as early as 4 wk after GnRH-Ag treatment, and the return to control levels occurred within 2 wk of estrogen treatment. The short duration of these responses allows reuse of study animals and provides a valuable tool for investigation of bone turnover, especially when evaluating antiresorptive treatments (Stroup et al. 2001a).

Skeletal Research in the Estrogen-deficient Macaque Model

Hormonal Therapies

Until recently, HRT has been the standard treatment for osteoporosis based on the fact that sex steroids play a critical role in bone homeostasis. Both estrogens and androgens suppress bone remodeling by decreasing the number of resorption/formation cycles (Manolagas et al. 2002). Estrogens and androgens also control the rate of programmed cell death (apoptosis) of mature bone cells, having pro-apoptotic effects on resorptive osteoclasts and antiapoptotic effects on osteoblasts. Thus, loss of sex steroids with aging or menopause would favor increased life span of osteoclasts and decreased life span of osteoblasts, resulting in a prevalence of resorption over formation and a net loss of bone mass and strength (Moggs et al. 2003). These activities occur via classical sex steroid receptor-mediated transcription events as well as novel extranuclear actions on signaling cascades (Kousteni et al. 2001). The increased risks over benefits of HRT reported in a large prospective trial by the Women's Health Initiative (Rossouw et al. 2002) have fueled interest in therapy with SERMs, because of their potential to retain

most of the beneficial effects of estrogen on bone while avoiding most of its adverse effects on the reproductive and cardiovascular system (Riggs and Hartmann 2003). The effectiveness of sex steroid therapies and SERMs (tamoxifen, raloxifene, toremifene) for clinical treatment of osteoporosis has been recently reviewed (Eichner et al. 2003; Riggs and Hartmann 2003).

Most studies using sex steroids in the nonhuman primate model have been performed in ovariectomized monkeys, yet in one study, triphasic oral contraceptive effects were studied in intact, young, adult, female cynomolgus monkeys (Register et al. 1997). Significant depressive effects of contraceptive treatment on gains in spinal and whole body BMC and BMD were observed over the course of the 20-mo study, and bone metabolism was inhibited as reflected by reductions in serum osteocalcin, acid and alkaline phosphatase, and calcium levels. The results suggest that oral contraceptive treatment of young adult female monkeys that have not reached peak bone mass inhibits net bone acquisition and/or growth by reducing bone metabolism.

Several sex steroids have been evaluated in the ovariectomized monkey model. Two-year treatment with estrogen replacement therapy (ERT¹) using 17 β -estradiol alone or combined with cyclic progesterone protected against osteopenia, and reduced serum biomarkers and bone formation rates, with the effect of ERT being enhanced by cyclic progesterone (Jayo et al. 1990; Jerome et al. 1994). Administration of ERT (conjugated equine estrogens [CEE¹]), alone or in combination with continuous medroxyprogesterone acetate (MPA¹), for 28 mo also increased spinal bone mass and decreased bone turnover in the cynomolgus model (Jayo et al. 1998). The addition of MPA to the oral CEE regimen provided no additional benefit to bone.

Tibolone ([7 α ,17 α]-17-hydroxy-7-methyl-19-norpregn-5[10]-en-20-yn-3-one), a steroid that combines estrogenic, progestogenic, and androgenic properties, increased spine BMD and body weight when administered to ovariectomized cynomolgus monkeys for 2 yr (Clarkson et al. 2001). Whether the bone protective effects attributed to the estrogenic 3 α - and 3 β - metabolites of tibolone were secondary to increased load-bearing caused by increased body weight in these monkeys could not be determined. However, bone strength of the midshaft femur was also greater in tibolone-treated groups relative to ovariectomized controls (Lees et al. 2001). Neither spinal BMD nor midshaft femur strength was improved after CEE (0.042 mg/kg) treatment with or without MPA (0.167 mg/kg) in this study (Clarkson et al. 2001).

In the search for the ideal molecule that is an estrogen agonist in bone and cardiovascular tissue, but not in breast or uterine tissue, several SERMs have been developed and tested in nonhuman primates. Among these are raloxifene, lasofoxifene, and levormeloxifene, which inhibited bone turnover and maintained spine BMD in ovariectomized cynomolgus monkeys after 1 to 2 yr of treatment (Brommage et al. 2001; Hotchkiss et al. 2001; Lees et al. 2002). Tamoxi-

fen acted as a partial agonist in estrogen-depleted cynomolgus bone by reducing bone turnover (alkaline phosphatase) with no effect on overall spine BMD after 30 mo of treatment. Although it reduced the bone formation rate in the endocortical compartment, there were no effects of treatment on the cancellous compartment of lumbar vertebra. (Lees and Jerome 1996; Lees et al. 1998).

Nevertheless, none of these SERMs proved to be as effective as the estrogenic compound (17 β -estradiol or CEE) used as a positive control in these studies. Estrogen was superior in preventing ovariectomy-induced spinal bone loss (Brommage et al. 2001; Hotchkiss et al. 2001; Lees et al. 2001). Unlike the SERM (levormeloxifene) tested in one study, estrogen also had a beneficial effect at cortical sites (Hotchkiss et al. 2001). Moreover, the mechanisms underlying the effects on bone appear to be different in the estrogenic versus the SERM compounds. Estrogen acts on bone via estrogen receptors (ERs¹) α and β as well as nonreceptor-mediated mechanisms. However, the interaction of SERMs with ER α is distinct for each compound, including ER β -ligand conformation and interaction with transcriptional machinery (Wijayarathne et al. 1999). This characteristic could explain the qualitative and quantitative differences between the bone effects of SERMs and of pure estrogenic compounds.

Treatment with the synthetic, anabolic androgen nandrolone decanoate for 1 or for 2 yr increased vertebral mechanical strength, prevented osteopenia, and decreased turnover in the estrogen-depleted cynomolgus macaque (Gadeleta et al. 2000; Jerome et al. 1997a). Histomorphometric analysis indicated that nandrolone prevented bone loss in the transitional region between cortical and cancellous bone of the vertebra when treatment was initiated at the time of ovariectomy (Jerome et al. 1997c). Recent studies employing Fourier-transform infrared microscopy have also shown significant positive effects of nandrolone on bone mineralization in subchondral and cortical bone, but not trabecular bone, of the proximal tibia of cynomolgus macaques (Huang et al. 2002).

The bone formation-enhancing effects of parathyroid hormone (PTH¹) and its 31- to 38-amino acid fragments have been examined extensively in the ovariectomized monkey model. These compounds have shown great promise as bone anabolic agents in clinical trials (Tashjian and Chabner 2002; Whitfield et al. 2002), with observed increases in bone density of the lumbar spine and hip as well as reduced vertebral and nonvertebral fractures (Neer et al. 2001). Iliac crest bone biopsies have also confirmed that PTH stimulates skeletal remodeling, resulting in an increased percentage of newly formed bone matrix of lower mineral density (Misof et al. 2003). Concerns have also been allayed that PTH may cause cortical thinning by histomorphometric demonstration of increased cortical thickness and improvements in indices of trabecular connectivity with PTH treatment (Dempster et al. 2001).

The consequences of long-term (12- and 18-mo) treatment with rhPTH(1-34) at 1 μ g/kg or 5 μ g/kg daily as well

as the effects of withdrawal of treatment have been studied in the cynomolgus monkey using several endpoints of bone mass, turnover, and strength (Brommage et al. 1999; Burr et al. 2001; Jerome et al. 2001; Turner et al. 1999). Compared with the ovariectomized control group, the high dose (5 μ g/kg) increased spinal BMD by 14.3% and whole body BMC by 8.6% after 12 mo of daily rhPTH(1-34) treatment, with the greatest gains in both parameters during the first 6 mo of treatment (Brommage et al. 1999). Total BMD of the proximal tibia measured by quantitative computed tomography was also elevated by 10.8% after 12 mo. No significant decreases in cortical bone mass were demonstrated densitometrically (Brommage et al. 1999), and it was shown that cortical bone strength was not compromised, despite an increase in porosity (Burr et al. 2001), and lumbar vertebra and femoral neck were significantly increased in strength (Turner et al. 1999). Consistent with BMD changes, histomorphometry of lumbar vertebra, femoral neck, distal radius, and iliac crest indicated that 18 mo of rhPTH(1-34) treatment markedly increased cancellous bone volume at all sites relative to values in ovariectomized animals (Jerome et al. 2001). Moreover, PTH treatment significantly improved trabecular architecture as evidenced by increased trabecular number and decreased trabecular separation, with no significant change in trabecular thickness. The proposed mechanism for these structural changes is initial thickening of trabeculae by PTH followed by longitudinal tunneling, which returns trabecular thickness to normal levels while increasing trabecular number (Jerome et al. 2001). The positive effects of PTH on bone volume, architecture, and strength were still apparent 6 mo after withdrawal following 12 mo of treatment (Sato et al. 2000).

Similar results were obtained in a short-term study in which ovariectomized cynomolgus monkeys received 10 μ g/kg of rhPTH(1-34) by subcutaneous injection 3 days/wk for 6 mo. Significant increases in BMD were observed at cancellous sites, while cortical bone mass was unaffected by treatment, and bone strength was increased in both vertebra and femoral neck (Jerome et al. 1999). Treatment with 5 to 25 μ g/kg of rhPTH for 16 mo in ovariectomized rhesus monkeys caused a shift in the relative amounts of higher density cortical bone and lower density trabecular bone to a greater area of lower density cortical bone and higher density trabecular bone, which did not adversely affect bone strength (Smith et al. 2001).

Nonhormonal Therapies

The efficacy, safety, and mode of action of bisphosphonates have been studied in estrogen-depleted rhesus macaques and baboons. These compounds have played an increasingly prominent role in the treatment of metabolic bone disorders due to their strong affinity for bone and effective inhibition of bone resorption (Fleisch 2000). Many bisphosphonates act by inhibiting the mevalonate pathway and protein prenylation in osteoclasts, resulting in the inhibition of cel-

ular functions and the induction of apoptosis in these bone-resorbing cells (Green 2001). Efficacy data for clinically available bisphosphonates (e.g., alendronate and risedronate) have recently been reviewed (Body 2002; Eichner et al. 2003; Tuck and Francis 2002).

In the rhesus monkey, treatment with zoledronate for 16 mo prevented the increased skeletal turnover and bone loss induced by ovariectomy in a dose-dependent manner (Binkley et al. 1998). Biomechanical testing showed a corresponding preservation of femoral neck stiffness with zoledronate treatment (Grynpas et al. 1998). Similarly, 2 yr of treatment with the aminobisphosphonate alendronate dose-dependently reversed the effects of ovariectomy in baboons (Balena et al. 1993; Thompson et al. 1992). Using a 16-mo dosing schedule of ibandronate, which mimics the clinical regimen, Smith and colleagues (2003) demonstrated suppression of bone turnover and preservation of bone mass, architecture, and strength in ovariectomized cynomolgus monkeys. The greater ability of ibandronate to preserve BMD at trabecular versus cortical sites was attributed to the higher incorporation of ibandronate in trabecular bone, which has a higher surface area available for drug binding (Smith et al. 2003). However, treatment of cynomolgus macaques for 16 mo with clodronate was most effective in preventing cortical bone loss (e.g., tibia) secondary to estrogen deficiency (Itoh et al. 2002). Spinal BMD was dramatically increased in ovariectomized cynomolgus monkeys treated for 54 wk with MCC-565, a bone selective 17β -estradiol-bisphosphonate conjugate (Akihito et al. 2000). In contrast to CEE (25 μ g/kg/day), no adverse uterine effects (i.e., endometrial hyperplasia) were observed in the MCC-565 groups.

Novel Compounds

The ongoing search for osteoporosis therapeutics with improved risk/benefit features in addition to increased understanding of the molecular mechanisms of bone biology have produced novel compounds evaluated for safety and efficacy in the nonhuman primate model. Among these compounds is strontium ranelate (S12911), which has a strong affinity for bone, into which it is incorporated by surface exchange or ionic substitution. *In vitro* studies have shown that strontium increases the number of osteoblasts and decreases the number and activity of osteoclasts (see Dahl et al. 2001 and references therein). In young adult (3- to 4-yr) cynomolgus monkeys, 6-mo treatment with strontium ranelate decreased histomorphometric indices of bone resorption while maintaining those of bone formation in alveolar bone, a site of high bone turnover (Buehler et al. 2001).

Another novel compound is an inhibitor (SB-357114) of cathepsin K, a cysteine protease known to play an essential role in osteoclast-mediated degradation of the organic matrix of bone (Stroup et al. 2001b). In cynomolgus monkeys rendered estrogen deficient by GnRH-Ag, treatment with this inhibitor resulted in an immediate (within 1.5 hr) re-

duction in serum markers of bone resorption that was still evident 5 days after dosing. This sustained decrease in resorption was accompanied by a decrease in osteoblast activity over the same time period, indicative of a decrease in bone turnover. Although this short-term study shows that this inhibitor has promise as a future therapeutic, one issue that requires resolution is its specificity because cathepsin K is also expressed in cells other than osteoclasts (Zaidi et al. 2001). Another cathepsin K inhibitor caused profound reductions in serum markers of bone resorption after administration to GnRH-Ag-treated cynomolgus monkeys; markers were reduced within 1 day of administration, remained depressed throughout treatment over a 4-wk period, and returned to normal within 1 day after dosing ended (Missbach et al. 2002).

Short-term studies have also been performed in cynomolgus macaques to evaluate two other compounds involved in osteoclast physiology, an osteoprotegerin analogue (Martin et al. 2000) and a potent antagonist of $\alpha_v\beta_3$ integrin (vitronectin receptor) (Rodan et al. 2001; Stroup et al. 1999). Osteoprotegerin is a cytokine that inhibits osteoclast activity, whereas the $\alpha_v\beta_3$ integrin antagonist prevents osteoclast-mediated bone resorption *in vitro*. The results of both studies indicated significant reductions in serum resorption markers (type I collagen C-telopeptides [CTX] and/or N-telopeptide cross-links [NTX]), indicating their potential as antiresorptive therapies in postmenopausal osteoporosis.

Current Trends in Nonhuman Primate Bone Research Models

These recent studies in novel compounds highlight an ongoing trend in nonhuman primate bone research, toward relatively short-term studies to establish proof-of-concept or preliminary efficacy dose-ranging with novel compounds. The demand for these studies has been driven in part by the development of therapeutic agents from the human genome, which cannot readily be tested in nonprimates. For example, the compound tested by Missbach and colleagues (2002) is approximately 60-fold less potent in rats than in primates. Evaluation of the skeletal actions of glucocorticoids also illustrates the potential value of short-duration monkey studies (Perez et al. 2003).

The short-term models have been made possible by the availability of a range of serum and urine biomarkers for bone resorption and formation that have become available for clinical use, and most of these assays can be applied to nonhuman primate samples. Due to relatively limited experience to date, it is not yet established how predictive a short-term biomarker dose-response relation is of the longer-term bone mass or strength dose-response relation, and it is likely that it will be at least partly related to the mode of action of the therapeutic agent. A second and even less well understood difficulty is that the biomarkers provide a measure of global (whole body) bone resorption and

formation rates, whereas some therapeutic agents may depend on site-specific effects for their efficacy.

Prospects for Future Research in Primate Models

Nonhuman primates, particularly the macaques, have now been more extensively characterized than any other large animal species as animal models for human skeletal biology. They have been used extensively to evaluate hormonal as well as nonhormonal therapies for osteoporosis, with results that are entirely consistent with clinical data. In the process, they have also provided insights into cortical bone responses to estrogen deficiency and experimental treatments, and into cancellous architectural responses to treatment with PTH, which cannot readily be investigated in humans. Although postmenopausal changes occur more rapidly in the monkey surgical ovariectomy model than in humans, they are of comparable magnitude, and experiments must be appropriately designed and powered to be successful.

In the future, primates will become more important as models for the evaluation of new osteoporotic strategies with combined antiresorptive/anabolic capabilities (e.g., bisphosphonates plus PTH) as well as primate-specific therapeutics developed from the human genome. Continued elucidation of molecular mechanisms for sex steroids and their receptors will drive production of new SERMs and other novel compounds with superagonist protective actions on the cardiovascular and skeletal system without detrimental effects on reproductive organs. The continued use of primates for evaluation of these new therapeutics that affect multiple organ systems will remain important because primates have also been well characterized as models for human cardiovascular, reproductive, and central nervous system biology.

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