Original Full Length Article

High-resolution peripheral quantitative computed tomography and finite element analysis of bone strength at the distal radius in ovariectomized adult rhesus monkey demonstrate efficacy of odanacatib and differentiation from alendronate

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ABSTRACT

Translational evaluation of disease progression and treatment response is critical to the development of therapies for osteoporosis. In this study, longitudinal *in-vivo* monitoring of odanacatib (ODN) treatment efficacy was compared to alendronate (ALN) in ovariectomized (OVX) non-human primates (NHPs) using high-resolution peripheral computed tomography (HR-pQCT). Treatment effects were evaluated using several determinants of bone strength, density and quality, including volumetric bone mineral density (vBMD), three-dimensional structure, finite element analysis (FEA) estimated peak force and biomechanical properties at the ultradistal (UD) radius at baseline, 3, 6, 9, 12, and 18 months of dosing in three treatment groups: vehicle (VEH), low ODN (2 mg/kg/day, L-ODN), and ALN (30 μg/kg/week).

Biomechanical axial compression tests were performed at the end of the study. Bone strength estimates using FEA were validated by *ex-vivo* mechanical compression testing experiments. After 18 months of dosing, L-ODN demonstrated significant increases from baseline in integral vBMD (13.5%), cortical thickness (24.4%), total bone volume fraction BV/TV (13.5%), FEA-estimated peak force (26.6%) and peak stress (17.1%), respectively. Increases from baseline for L-ODN at 18 months were significantly higher than that for ALN in DXA-based aBMD (7.6%), cortical thickness (22.9%), integral vBMD (12.2%), total BV/TV (10.1%), FEA peak force (17.7%) and FEA peak stress (11.5%), respectively. These results demonstrate a superior efficacy of ODN treatment compared to ALN at the UD radii in ovariectomized NHPs.

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Introduction

The hallmarks of osteoporosis include low bone mineral density (BMD), poor bone architecture and decreased bone strength leading to eventual bone fragility and increased fracture risk. Globally, osteoporosis affects more than 70 million people [1–5], and as populations aged, the incidence of osteoporosis-related fragility fractures is anticipated to significantly increase.

Oral bisphosphonates are commonly used to treat osteoporosis and have demonstrated fracture risk reduction. However, their mechanism of action is limited to prevention of bone loss primarily through reduction of bone resorption and formation. Increased understanding of the regulation of the bone remodeling process has led to the development of newer therapies that offer the possibility of improved therapeutic profiles and the potential to reduce the risk of fracture [6]. Cathepsin K (CatK) is a lysosomal cysteine proteinase

that is abundantly expressed in osteoclasts [7]. During bone resorption, CatK is secreted by osteoclasts and accumulates in the acidified resorption lacunae where it degrades matrix proteins [8]. Odanacatib (ODN) is a potent, orally-active selective CatK inhibitor, which is currently being developed for the treatment of postmenopausal osteoporosis [6].

Investigating how a CatK inhibitor might affect the structure and density of specific bone sites that subsequently influence bone strength is critical to understanding the benefits of such an agent in reducing fracture risk. In contrast to assessing bone mass by traditionally used dual-energy X-ray absorptiometry (DXA), in-vivo imaging techniques to better characterize the bone with more accurate measures of bone density and descriptors of geometry have been developed in the past decade [9,10]. High-resolution peripheral quantitative computed tomography (HR-pQCT) is an in-vivo imaging technique for evaluating bone density as well as bone micro-architecture in peripheral bone sites [11–13]. Although restricted to peripheral skeletal bone sites, this technology currently provides the best resolution attainable in humans (82 μm isotropic voxel size). The technique has the added advantage of providing images with a relatively low effective dose of radiation (<3 μSv for one standard measurement). These combined factors make HR-pQCT the best choice at present to determine the trabecular architecture and cortical thickness in-vivo [12]. In addition to allowing density and microarchitectural measurements of bone, data obtained by HR-pQCT capture essential structural descriptors of bone to enable creation of mathematical models for estimation of bone strength through finite element analysis (FEA). These models take the trabecular structure information and cortical geometry together with bone density to estimate the bone mechanical response under specified boundary conditions [10,14–16].

Ovariectomized (OVX) non-human primates (NHPs) are commonly used as animal models for post-menopausal bone loss in osteoporosis drug development [17,18]. In a previous study, the feasibility of performing longitudinal HR-pQCT imaging and FEA in NHP for monitoring estrogen deficiency-induced bone loss and response to osteoporosis treatment was demonstrated [13]. That study, which included HR-pQCT measurements in the last 9 months of a 20-month study of ODN treated OVX rhesus monkeys, established the acquisition and analysis protocols for HR-pQCT, and validated FEA estimation of strength in monkeys [13]. Though lacking the baseline measurements, the study allowed precision of various HR-pQCT parameters and bone strength to be evaluated and the response of these measures to treatment with ODN to be surveyed [13].

The study details and broad objectives of the current study are reported in an accompanying manuscript in this issue (Williams et al., 2013). The study was designed using an improved ODN formulation with enhanced gastrointestinal absorption in order to support two separate objectives; first to further our understandings on the long-term effects of ODN dosed at levels comparable to, and greater than the clinical exposure, on bone strength and biomechanical properties in OVX-rhesus monkeys. Additionally, a head-to-head comparison of the efficacy of ODN versus a bisphosphonate, alendronate (ALN) at equivalent clinical exposures was also evaluated in this model of bone loss. The accompanying manuscript reports results on bone turnover markers, and DXA and quantitative computed tomography (QCT) of the spine and hip (Williams et al., 2013). Ex vivo analyses of ODN vs. ALN on bone site specific remodeling and modeling, as well as the long-term impact of high ODN exposure on bone quality of the OVX-monkeys will be reported separately. Here we present longitudinal DXA and HR-pQCT imaging data of the ultradistal radius of ODN-treated ovariectomized rhesus monkeys in comparison to vehicle-treated (VEH) animals, and ALN treated animals, respectively at baseline and up to 18 months of treatment. Treatment was initiated immediately after ovariectomy, i.e., in bone-loss prevention mode. Note that a group treated with high dose ODN only received HR-pQCT at baseline and at month 18 prior to the end of the study. Hence the limited high resolution

imaging results from this ODN high dose group are not included in the analyses. The in-vivo high-resolution images were used to longitudinally monitor density and micro-architectural parameters of the bone. From the same HR-pQCT images, FE models were generated, and in-vivo estimates of bone strength were obtained. The actual strength of the bones was measured by ex-vivo mechanical testing at the end of the study for comparison with estimated strength from FEA.

This study had two key objectives: (i) to evaluate the efficacy of ODN at approximate clinical therapeutic exposure on peripheral bone microarchitecture and strength and (ii) to investigate the differences in treatment response of ODN compared to that of the bisphosphonate alendronate (ALN) in an estrogen-deficient model of bone loss in NHP. Specific evaluations included the following on the ultradistal radius: (i) longitudinal measurements of density and microarchitectural parameters with DXA and HR-pQCT; (ii) FEA using in-vivo HR-pQCT images for estimation of bone strength; and (iii) validation of FEA-estimated strength against experimentally measured strength through mechanical testing of excised bones.

Methods

Study design

All procedures were approved by the Institutional Animal Care and Use Committee of Merck Research Laboratories. Sixty-four female rhesus monkeys (Macaca mulatta 12–22 years of age) were randomized into four groups according to femoral neck aBMD and 1/3 distal radial Ct.Th. Following bilateral OVX, the four groups were assigned to receive the following treatments: (1) vehicle (VEH) containing hydroxypropyl methyl cellulose acetate succinate (HPMC-AS) polymer; (2) ODN at 2 mg/kg, (L-ODN, p.o., q.d.); (3) ODN at 8 mg/kg (H-ODN, p.o., q.d.); (4) ALN at 30 μg/kg/week (15 μg/kg twice weekly, s.c.). The ALN subcutaneous dose was selected to produce an approximate plasma concentration in the monkeys equaling that of ALN 70-mg oral and once-weekly clinical dose, taking in account the oral bioavailability of 0.75% in humans. ALN at 15 μg/kg twice weekly, s.c. was approximately the i.v. dose of 50 μg/kg every two weeks, which was previously demonstrated to fully protect estrogen-deficiency induced bone loss in OVX baboons [19]. Drug treatment was initiated in prevention mode, approximately 10-days post-surgery. H-ODN dose was subsequently reduced at month 5.5 from 8 mg/kg to 4 mg/kg to maintain plasma exposure at approximately 7-fold clinical daily exposure. The H-ODN group was included for bone safety investigations and data relating to this group are not presented in this report which focuses on results related to exploratory high resolution imaging. Individually housed animals received access to drinking water and enrichment activities ad libitum. All study groups were maintained on a high protein diet (19.8%) containing 1.17% Ca, 0.7% P, 8I U/g vitamin D3 diet (Harlan Teklad 8773 NIB primate diet ~200 g/d). The animals were euthanized at 20 months, and the right radius was harvested for ex-vivo imaging and biomechanical testing.

Bone densitometry by DXA

Areal bone mineral density (aBMD) measurements were performed at baseline, 3, 6, 9, 12, 16, and 18 months (GE Lunar iDXA, General Electric Healthcare, Waukesha, WI, USA). Quality control (QC) procedures were carried out daily with a phantom consisting of four compartments of densities 0.5, 1.4, 2.4, and 3.3 g/cm^2, respectively. The phantom was scanned with the antero-posterior (AP) spine protocol, and analyzed for manual subregions. The daily variability of BMD measured in each phantom compartment was maintained within two standard deviations of the initial baseline measurements. AP right radius scans were performed and analyzed according to the manufacturer's operating procedures with the tissue classification (point typing) and region of interest (ROI) definition