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Effects of minodronic acid and alendronate on bone remodeling, microdamage accumulation, degree of mineralization and bone mechanical properties in ovariectomized cynomolgus monkeys

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ABSTRACT

Suppression of bone remodeling by bisphosphonates leads to accumulation of microdamage in bone. If this microdamage develops due to suppressed repair of remodeling only, more potent bisphosphonates should cause more damage. In this study, we evaluated the effects of reduced bone turnover produced by a potent bisphosphonate, minodronic acid, on microdamage accumulation, the degree of mineralization and mechanical properties of bone in ovariectomized cynomolgus monkeys, and compared these effects with those of alendronate. Sixty female monkeys aged 9–17 years old were divided into five groups. The sham group and the ovariectomized group were treated daily for 17 months with lactose vehicle. The other three groups were treated daily with minodronic acid at a dose of 0.015 mg/kg or 0.15 mg/kg, or alendronate at 0.5 mg/kg orally. After sacrifice, lumbar vertebrae and left femurs were subjected to histomorphometry, microdamage, mineralization analyses, and mechanical testing. Minodronic acid suppressed bone remodeling of cancellous and cortical bone in a dose-dependent manner and the higher dose of minodronic acid suppressed bone remodeling more strongly than alendronate. The lower dose of minodronic acid did not increase microdamage accumulation and compressive strength, but the higher dose of minodronic acid and alendronate resulted in similar increases in cancellous microdamage accumulation and ultimate load in lumbar vertebra. There were no significant differences among the groups in microdamage, degree of mineralization and mechanical properties in cortical bone of the femoral shaft; however, only alendronate showed a tendency to increase highly mineralized osteons and microdamage. These findings suggest that microdamage caused by minodronic acid is less than that expected based on the extent of remodeling suppression, in comparison with alendronate although this was not reflected in any significant change of mechanical properties.

Introduction

Bisphosphonates are strong inhibitors of bone resorption and are widely used as therapeutic agents for bone diseases with high bone resorption, including metastatic bone disease, Paget disease, and osteoporosis [1–5]. Currently, bisphosphonates are the most common therapeutic agents used for osteoporosis because they consistently decrease the incidence of osteoporotic fragility fractures [6–10]. Minodronic acid is a new third-generation bisphosphonate with potent pharmacological activity that has been developed in Japan. Nonclinical studies have shown that this agent is a strong inhibitor of bone resorption at low doses [11], with inhibitory effects on reduction of bone density or strength that are comparable with those of other bisphosphonates [12,13]. A clinical study showed that administration of minodronic acid at 1 mg/day for 12 months produced increases of 5.9% in average lumbar bone density (L2–L4) and 3.5% in total bone density of the proximal femur in Japanese postmenopausal female patients with osteoporosis [14]. Another trial provided evidence that administration of minodronic acid for 2 years reduced vertebral body fracture by 59%.

Potentially harmful effects of reduced bone turnover have been suggested in conditions such as atypical femoral fracture or osteonecrosis of the jaw, although the pathophysiological mechanisms of these conditions are poorly defined [15]. The mechanisms through which suppression of bone remodeling may reduce bone strength include increased mineralization of bone and reduced heterogeneity of mineralization, changes in collagen composition [16], and accumulation of microdamage

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[17–20]. These causes have been proposed for the reduced biomechanical properties of bone observed in preclinical studies in dogs treated with bisphosphonates. Theoretically, the extent of remodeling suppression by bisphosphonates may depend on the drug potency. Thus, the potency of minodronic acid [11] suggests that it may suppress bone remodeling to a greater extent than other bisphosphonates. However, it is unclear whether detrimental effects on bone material differ among bisphosphonates, and the effects of suppressed bone turnover caused by minodronic acid on microdamage accumulation and the degree of mineralization have not been examined.

The purpose of this study is to evaluate the effects of suppression of bone turnover by minodronic acid and alendronate on microdamage, degree of mineralization, heterogeneity of mineralization, and bone mechanical properties in a non-human primate ovariectomized (OVX) animal. Cynomolgus monkeys have similar cancellous and cortical bone remodeling processes to those of humans, as well as similar reproductive physiology [21]. OVX cynomolgus monkeys also exhibit increased bone turnover and loss of bone mass, resembling the changes that occur in women after menopause [22,23]. Therefore, the OVX cynomolgus monkey is the preferred large animal model for evaluation of therapeutic agents for osteoporosis.

Materials and methods

Animals

This study was performed as an additional analysis of bone quality in a study of minodronic acid in primates; the design and results of the core primate study have been reported previously [13]. The core study was approved by the Internal Animal Care and Use Committee of Ina Research, Philippines, Inc. (Laguna, Philippines). Sixty female cynomolgus monkeys aged approximately 9 to 17 years old, as judged from dental conditions and individual records, were acquired from SICONBREC Inc. (Manila, Philippines) and A.T. Viri Primate Breeding Corp. (Manila, Philippines). Epiphyseal closure and adult skeletal status were confirmed by X-ray in each animal.

Animals were individually housed in stainless steel cages in an animal room maintained at a temperature of 23.2–26.4 °C, relative humidity 39.1–79.8%, ventilation rate 12–16 air changes per hour, and artificial lighting for 12 h/day. Each animal was provided daily with approximately 100 g of Certified Primate Diet No. 5048 (PMI Feeds, Richmond, IN, USA), containing 1.0% calcium, 0.6% phosphorus, and 6.6 IU/g vitamin D. Chlorinated deep-well drinking water was provided ad libitum via a drinking nozzle. Body weight was recorded at weekly intervals throughout the study period.

Monkeys were randomized by lumbar spine (L3–L5) BMD data measured by dual energy X-ray absorptiometry (DXA) into five groups: sham (SHAM), OVX (OVX), and treatment with low or high dose minodronic acid (MIN-L, MIN-H, 0.015 and 0.15 mg/kg po, respectively) or alendronate (ALN, 0.5 mg/kg po) ($n = 12$ per group). These doses of bisphosphonates used in the study were set based on the data obtained from osteoporosis models [24,25], and higher than the clinical doses for human osteoporosis treatment by 0.75 and 7.5 times for minodronic acid and by 2.5 times for alendronate. The mean ages of the groups were 11.3, 12.7, 12.3, 11.3 and 11.6 years old, respectively. Before the operation, atropine (0.05 mg/kg) was administered as pre-anesthetic medication. Animals were either ovariectomized or a sham operation was performed. Both ovaries, together with the oviducts, were exposed through a midline incision and removed. The ovaries in the SHAM group were exposed, but not removed. Ampicillin (10 mg/kg) administration was continued for 7 days post-surgery. To evaluate ovarian function, the serum estradiol concentration was measured pre- and post-surgery. At the end of the treatment period, each animal was sacrificed by exsanguination of axillary blood vessels, after which skeletal samples were isolated.

Anesthesia

Surgical procedures were performed under anesthesia with intramuscular injection of ketamine hydrochloride (10 mg/kg, Ketaject; Astrapin Pharma, Hameln Niedersachsen, Germany) and xylazine (0.5 mg/kg, Xylail-20; Troy Laboratories, Glendenning, NSW, Australia). At necropsy, animals were anesthetized with ketamine hydrochloride and xylazine, followed by intravenous injection of sodium pentobarbital (30 mg/kg, Pentoxyn; Parnell Laboratories, Alexandria, NSW, Australia).

Pharmaceutical treatment

Dosing substances were administered once daily for 17 months, starting from the day after surgery. Animals were fasted overnight before and for at least 2 h after drug administration. While the animal was held in a restrainer, about 1 mL of distilled water was flushed in the mouth to lubricate the esophagus. Gelatin capsules containing only lactose were administered orally to the SHAM and OVX groups. Capsules containing minodronic acid or alendronate mixed with lactose were administered. The gastric tube with a capsule attached to the tip was inserted into the esophagus and then the capsule was dislodged with about 5 mL of distilled water.

Tissue preparation for bone histomorphometry

Calcein (2 mg/kg) was administered intravenously to animals 18 and 7 days before necropsy. The L2 and L7 vertebrae and left femur were isolated at necropsy for conventional histomorphometry and microdamage measurement. Tissues surrounding the bones were removed, the L2 vertebra was fixed in 5% paraformaldehyde and embedded in methylmethacrylate (MMA) after Villanueva bone staining. A 3-μm thick sagittal section of the mid portion of L2 vertebra was cut for cancellous bone histomorphometry.

The L7 vertebra and shaft portion of the left femur were fixed and stained en bloc with 1% basic fuchsin in 70% ethanol, dehydrated in an increasing series of ethanol, defatted in xylene, and embedded without decalcification in MMA. Sagittal sections of L7 vertebra and transverse sections of the femur (each 150 μm) were cut and further ground to 80-μm thickness for microdamage analysis.

Histomorphometry

Histomorphometric measurements were performed using a semi-automated digitizing image analyzer, consisting of a light or epifluorescent microscope and a digitizing pad connected to a computer with histomorphometric software (System Supply Co., Nagano, Japan). Polarized light was applied to identify lamellar structure for detecting trabecular packets. All measurements were carried out blindly by one histomorphometrist.

Cancellous bone histomorphometry

Cancellous bone measurement was performed at a magnification of ×100. A square (5×5 mm) area of the L2 vertebra 1 mm above the caudal endplate was measured. The activation frequency (Ac.f, #/year), the secondary parameter of bone turnover, was calculated from the measurements of primary parameters, as described by Parfitt et al. [26].

Cortical bone histomorphometry

Cortical histomorphometric measurements were performed for the whole cross-sectional area of the femur at a magnification of ×100. The activation frequency (Ac.f, #/year) was calculated from the primary parameters, as described by Mashiba et al. [27].