Journal of Bone and Mineral Metabolism (2019) 37:636–647
https://doi.org/10.1007/s00774-018-0968-2

ORIGINAL ARTICLE

Cortical bone mineral density is increased by the cathepsin K inhibitor ONO-5334, which leads to a robust increase in bone strength: results from a 16-month study in ovariectomised cynomolgus monkeys

Hiroyuki Yamada[1] · Yasuo Ochi[1] · Hiroshi Mori[1] · Satoshi Nishikawa[1] · Yasuaki Hashimoto[1] · Makoto Tanaka[1] · Steve Deacon[2] · Kazuhito Kawabata[1]

Received: 15 May 2018 / Accepted: 1 October 2018 / Published online: 24 October 2018

Abstract

This study evaluated the long-term effects of the cathepsin K inhibitor ONO-5334 on bone mass and strength in ovariectomised (OVX) cynomolgus monkeys. Animals were assigned to one of the following six groups: Sham (non-OVX), OVX control treated with vehicle, ONO-5334 1.2, 6 or 30 mg/kg/day, p.o., or alendronate (ALN) 0.05 mg/kg/2 weeks, i.v. for 16 months. Peripheral quantitative computed tomography (pQCT) analysis revealed that ONO-5334 increased not only trabecular bone mineral density (BMD) but also cortical BMD in the distal radius and the lumbar vertebra. ONO-5334 and ALN suppressed the deterioration of trabecular architecture by micro-CT analysis in the distal radius. Assessments of bone strength showed that ONO-5334 increased maximum load at the distal and midshaft radius. The linear regression lines between bone mass and strength in the lumbar vertebra were tended to be shifted towards increasing bone strength in the ONO-5334 6 and 30 mg/kg groups compared with the ALN groups. This indicated that bone strength was higher in the ONO-5334 groups than the ALN group, even though bone mineral content (BMC) and BMD were comparable. Subpopulation analysis revealed that, at similar integral BMC or BMD level, cortical bone mass for ONO-5334 was higher than for ALN; the opposite effects were observed for trabecular bone. In conclusion, ONO-5334 preferentially increased cortical bone, which may provide a greater contribution to bone strength. Since these results support a different mode of action for ONO-5334 compared with that of ALN, ONO-5334 may offer new therapeutic options to patients with osteoporosis.

Keywords ONO-5334 · Cathepsin K inhibitor · Cynomolgus monkey

Introduction

Osteoporosis is a skeletal disorder characterised by compromised bone strength that predisposes patients to an increased risk of fracture. Fracture prevention is the primary treatment goal for patients with osteoporosis [1]. Bisphosphonates, including alendronate (ALN), are the major class of drugs used to treat this disease [2, 3]. Although treatment with bisphosphonates has been shown to reduce the risk of vertebral fractures by 40–70%, their ability to reduce the risk of non-vertebral fractures is much lower (approximately 20%) [2, 4, 5]. Furthermore, a number of potential side-effects associated with the administration of bisphosphonates have been identified and include atypical fractures of the femur that occur with minimal or no trauma [3, 6, 7]. It has been suggested that prolonged administration of bisphosphonates leads to a dramatic decrease in bone turnover, which reduces bone remodelling and repair of microdamage and subsequently, decreases bone strength [3, 8].

PTH analogues are the only approved drugs able to stimulate bone formation, but with a restricted treatment period of no more than 2 years. The compounds able to inhibit the activity of osteoclasts, thus decreasing bone resorption, but not their number so that bone formation remains largely unaffected, could be a potential new treatment of osteoporosis [9, 10]. A possible approach to uncouple bone resorption and bone formation is via inhibition of cathepsin K [10, 11].

✉ Hiroyuki Yamada
hi.yamada@ono.co.jp

[1] Discovery Research Laboratories, Ono Pharmaceutical Co., Ltd, 3-1-1 Sakurai Shimamoto-cho Mishima-gun, Osaka 618-8585, Japan

[2] Drug Development, ONO Pharma UK LTD, MidCity Place, 71 High Holborn, London WC1V 6EA, UK

With this in mind, several specific inhibitors of cathepsin K have been developed including balicatib, odanacatib and ONO-5334. Studies with balicatib [12] and odanacatib [11] demonstrated an increase in bone mineral density (BMD) in postmenopausal women. It has been shown that ONO-5334 also leads to an increase in BMD when administered to postmenopausal women [13]. In addition, and in contrast to ALN, ONO-5334 had little or no effect on bone formation markers whilst the effect on bone resorption markers was similar for both compounds [13]. The question arises as to whether the different mechanism of action of ONO-5334 relative to ALN is associated with an increase in bone strength along with an increase in bone mass.

We have previously reported in a 16-month study in ovariectomised (OVX) cynomolgus monkeys that ONO-5334 preferentially suppressed bone resorption over formation and prevented decreases in trabecular and cortical bone mass as assessed by dual-energy X-ray absorptiometry (DXA) and histomorphometry analysis [14]. In particular, DXA assessments revealed that ONO-5334 led to large and statistically significant increases in BMD at the distal radius compared with the OVX control group; however, this result was not observed for ALN. To further explore this finding, we performed additional measurements using peripheral quantitative computed tomography (pQCT), micro-CT and mechanical tests at the distal and midshaft radius. The relationship between bone mass and bone strength in the lumbar spine was analysed. Here, we report the results of these exploratory investigations.

Materials and methods

Animals

Skeletally mature, adult female cynomolgus macaques (*Macaca fascicularis*, $n = 120$) aged between 9 and 12 years were obtained from Universal Fauna (Jakarta, Indonesia). All the experiments with animals were conducted at Shin Nippon Biomedical Laboratories, Ltd. (SNBL, Kagoshima, Japan). This study was approved by the Institutional Animal Care and Use Committee of SNBL (Approval no. C015-091) and performed in accordance with the ethics criteria contained in the bylaws of the committee.

The animals' body weights were 2.32–4.01 kg at grouping. The animals were kept in a room in which the temperature was maintained at 24.4–28.0 °C, relative humidity at approximately 40–71% and ventilation rate at approximately 15 times/h. The artificial lighting was on 12 h/day (07:00–19:00). Each animal was provided daily with approximately 108 g of pellet food (Purina Mills, LLC, MO, USA) and drinking water was provided ad libitum via an automated watering system.

Study design

Monkeys were randomised by lumbar spine (third–fifth, L3–L5) BMD data measured by DXA into six groups ($n = 20$ per group): Sham operation group (not ovariectomised), OVX control group, three ONO-5334-treated groups and one ALN-treated group before surgery (baseline). The Sham and OVX control groups were orally administered 0.5% (w/v) methylcellulose (vehicle); ONO-5334 treatment groups were orally administered ONO-5334 suspension once-daily from the day after ovariectomy for 16 months at the doses of 1.2 mg/kg, 6 mg/kg or 30 mg/kg of body weight with 0.5% methylcellulose. Dose selection of ONO-5334 was based on clinical exposure. The plasma concentration of ONO-5334 in monkeys 24 h after dosing at 1.2 mg/kg and 6 mg/kg was estimated to be equivalent to that of 100 and 300 mg in human, respectively. According to the guidelines of the Food and Drug Administration, to establish bone safety of a compound, a dose five times higher than the optimally effective dose should be tested; thus, in this study, the highest dose evaluated was 30 mg/kg [15].

ALN was used as an active reference in this study because bisphosphonates are used as a first-line treatment for osteoporosis. The ALN group was administered 0.05 mg/kg ALN in physiological saline intravenously every 2 weeks for 16 months. The rationale for the selection of the dose level and the route of administration of ALN was based on an efficacy study in OVX baboons [16] and this dose level is comparable to ALN 70 mg oral and once weekly clinical dose. ALN significantly suppressed OVX-induced increases in bone formation and resorption markers. In addition, ALN increased BMD and bone strength in the lumbar vertebra to a level similar to that in the Sham group. Similar effects were reported in studies with OVX cynomolgus monkeys for relacatib [17] and denosumab [18] in which ALN was used as a positive control.

pQCT and micro-CT analysis

The fourth lumbar and the radius were isolated at 16 months after OVX. Volumetric BMD and bone mineral content (BMC) in the fourth lumbar was measured by pQCT (XCT-RM, Norland Corp., WI, USA). A cross-sectional pQCT scan of 0.5 mm in thickness was obtained at the midpoint of each vertebral body segment and analysed (pQCT RM/Norland software version 5.40). Volumetric BMD in the radius (distal and midshaft) was measured by pQCT (XCT Research SA+, Stratec Medizintechnik GmbH, Pforzheim, Germany). A cross-sectional pQCT

scan of 0.8 mm in thickness was obtained at the site of 5% (distal) and 50% (midshaft) total length from the distal end of the left radius and analysed using pQCT (RM/Norland software version 5.50E). The distal radius was scanned using a micro-CT system (μCT40, SCANCO Medical, Brüttisellen, Switzerland) and two-dimensional images at the site of 5% total length from the distal end were generated.

The following morphometric indices were determined: trabecular bone volume fraction (BV/TV), trabecular thickness (Tb. Th), trabecular separation (Tb. Sp), trabecular number (Tb. N), structure model index (SMI), and connectivity density (Conn-Dens). Structure model index was used as a parameter to quantify the characteristic form of a three-dimensionally described structure in terms of the number of plates and rods composing the structure. Connectivity density is a topological parameter that estimates the number of trabecular connections per cubic millimetre.

Bone strength

Bone strength parameters at the fourth lumbar and the distal radius were determined using a compression test. Mechanical test for the fourth lumbar was previously described [14]. The distal radius (10 mm section) was placed between two plates and a load was applied at a constant displacement rate of 1.2 mm/min until failure in an Instron Mechanical Testing Instrument (Instron 5544, Bucks, UK). The load and displacement curve was recorded by instrument software (Merlin v5.11, Instron, Bucks, UK). Bone strength parameters were determined at the midshaft radius using a three-point bending test. The radius was placed on a three point-bending fixture with the anterior side facing down in an Instron Mechanical Testing Instrument (Instron 5544, Bucks, UK). The span between the two lower supports was set at 60 mm. The upper load device was aligned to the centre of the radius shaft. The load was applied at a constant displacement rate of 12 mm/min until failure. The load and displacement curve was recorded by instrument software (Merlin v5.11, Instron, Bucks, UK). For all bone strength analyses, the locations of maximum load at failure, stiffness and energy absorbed were selected manually from the load and displacement curve.

Subpopulation analysis

To analyse how bone strength was affected by trabecular and cortical bone mass, we selected the ONO-5334 30 mg/kg and the ALN groups as the targets for comparison because in these groups, the linear regression lines between bone mass and bone strength obtained by correlation analysis were shifted towards increasing bone strength. Subpopulations of these groups ($n = 10$) were selected in a way such that mean values of lumbar BMC and BMD were comparable; these subgroups were compared in terms of bone strength and cortical and trabecular BMC, and were categorised into lower and higher BMC and BMD.

Statistical analysis

Statistical analyses were performed by SAS System for Windows, Release 9.1 or higher (SAS Institute Inc., NC, USA). For pQCT, micro-CT and bone strength parameters, comparisons between the Sham group and the OVX control group, and between the OVX control group and the ALN group were performed using the Student *t* test. Comparisons between the OVX group and each ONO-5334 group were performed using the Dunnett's test. Pearson and Spearman correlation analysis were used to assess for a relationship between bone mass (BMC and BMD) and strength (maximum load) at the lumbar vertebra. With regard to the subpopulation analysis, comparisons between the ONO-5334 30 mg/kg and the ALN groups were performed using the Student *t* test. The significance level is presented as either $p < 0.05$ or $p < 0.01$.

Results

pQCT analysis in the lumbar spine (L4)

No statistically significant changes were observed after OVX in integral, trabecular or cortical BMD or in integral and trabecular bone mass parameters at 16 months (Table 1, Fig. 1a). However, OVX led to statistically significant decreases in cortical BMC and cortical bone thickness compared with the Sham group ($p < 0.05$). This decrease in cortical bone thickness was due to an increase in endosteal circumference (both statistically significant compared with Sham, $p < 0.05$, Table 1).

ONO-5334 at 6 and 30 mg/kg significantly increased integral and cortical BMD (Fig. 1a) compared with the OVX control group ($p < 0.05$); an increase in trabecular BMD to a level similar to that in the Sham group was also observed. ONO-5334 at 30 mg/kg significantly increased cortical thickness compared with the OVX control group ($p < 0.05$) (Table 1). ALN increased integral BMD to a level similar to that in the Sham group (Fig. 1a). Increases in integral BMD and cortical bone thickness were observed together with a decrease in endosteal circumference; all these changes achieved statistical significance in comparisons with the OVX group ($p < 0.05$, Table 1).

pQCT analysis in the distal radius (performed at 5% total length)

A significant decrease in trabecular BMD was observed in the OVX control group compared with the Sham group

Table 1 Integral bone, trabecular bone and cortical bone-related parameters assessed by peripheral quantitative computed tomography

	Sham	OVX control	ONO-5334			Alendronate
			1.2 mg/kg	6 mg/kg	30 mg/kg	0.05 mg/kg
Lumbar vertebral body (L4)						
Integral bone content (mg/mm)	51.08 ± 2.15	46.94 ± 1.61	46.62 ± 1.33	52.89 ± 1.65*	53.59 ± 1.76*	49.17 ± 1.36
Integral bone density (mg/cm^3)	482.8 ± 13.7	450.2 ± 12.5	466.0 ± 8.2	496.1 ± 9.4*	504.2 ± 13.5**	485.9 ± 10.6$^{\$}$
Trabecular bone content (mg/mm)	13.92 ± 0.70	12.99 ± 0.52	12.52 ± 0.45	14.33 ± 0.56	14.19 ± 0.63	13.74 ± 0.49
Trabecular bone density (mg/cm^3)	375.6 ± 14.3	356.4 ± 11.9	357.8 ± 8.3	385.0 ± 10.8	381.8 ± 15.0	389.0 ± 12.5
Cortical bone content (mg/mm)	34.93 ± 2.48	27.51 ± 1.98$^{\#}$	29.57 ± 1.32	34.63 ± 1.71*	37.53 ± 2.55**	32.33 ± 1.53
Cortical bone density (mg/cm^3)	604.7 ± 13.2	586.0 ± 13.7	598.2 ± 9.5	633.4 ± 10.2*	642.3 ± 14.2**	599.2 ± 12.1
Endosteal circumference (mm)	24.35 ± 0.76	26.76 ± 0.88$^{\#}$	25.14 ± 0.65	25.48 ± 0.43	24.10 ± 1.11	24.14 ± 0.89$^{\$}$
Periosteal circumference (mm)	36.34 ± 0.42	36.20 ± 0.56	35.45 ± 0.54	36.54 ± 0.38	36.53 ± 0.38	35.65 ± 0.44
Cortical bone thickness (mm)	1.91 ± 0.13	1.50 ± 0.11$^{\#}$	1.64 ± 0.07	1.76 ± 0.08	1.98 ± 0.17*	1.83 ± 0.11$^{\$}$
Distal radius						
Integral bone content (mg/mm)	25.61 ± 0.89	22.80 ± 0.72$^{\#}$	24.08 ± 0.72	31.07 ± 0.90**	32.37 ± 1.01**	21.82 ± 0.47
Integral bone density (mg/cm^3)	422.5 ± 12.2	401.7 ± 14.5	450.9 ± 19.3	564.9 ± 16.3**	593.0 ± 17.4**	410.6 ± 12.7
Trabecular bone content (mg/mm)	7.06 ± 0.40	6.14 ± 0.43	5.51 ± 0.51	4.33 ± 0.30**	4.47 ± 0.38*	6.12 ± 0.46
Trabecular bone density (mg/cm^3)	232.3 ± 7.5	201.0 ± 6.8$^{\#\#}$	198.2 ± 6.8	220.5 ± 8.7	250.5 ± 9.0**	213.8 ± 5.4
Cortical bone content (mg/mm)	14.70 ± 0.86	13.91 ± 0.77	16.11 ± 0.87	23.92 ± 0.86**	24.68 ± 1.17**	12.77 ± 0.62
Cortical bone density (mg/cm^3)	729.0 ± 13.0	715.1 ± 19.4	781.7 ± 21.9*	880.1 ± 6.5**	875.3 ± 10.9**	724.4 ± 19.7
Endosteal circumference (mm)	22.57 ± 0.49	21.76 ± 0.59	20.55 ± 0.74	18.72 ± 0.48**	18.19 ± 0.50**	21.27 ± 0.60
Periosteal circumference (mm)	27.60 ± 0.41	26.81 ± 0.44	26.13 ± 0.55	26.33 ± 0.35	26.20 ± 0.27	25.99 ± 0.47
Cortical bone thickness (mm)	0.80 ± 0.04	0.80 ± 0.04	0.89 ± 0.04	1.21 ± 0.05**	1.28 ± 0.06**	0.75 ± 0.03
Midshaft radius						
Cortical bone content (mg/mm)	21.10 ± 0.52	18.96 ± 0.62$^{\#}$	20.66 ± 0.63	22.13 ± 0.73**	22.08 ± 0.63**	18.73 ± 0.46
Cortical bone density (mg/cm^3)	1124.3 ± 6.7	1061.2 ± 9.8$^{\#\#}$	1109.8 ± 9.8**	1138.7 ± 8.0**	1124.6 ± 5.6**	1063.4 ± 13.8
Endosteal circumference (mm)	9.53 ± 0.30	9.66 ± 0.30	8.97 ± 0.30	8.88 ± 0.27	9.36 ± 0.29	9.59 ± 0.27
Periosteal circumference (mm)	18.08 ± 0.27	17.83 ± 0.24	17.74 ± 0.22	17.97 ± 0.28	18.30 ± 0.24	17.72 ± 0.19
Cortical bone thickness (mm)	1.36 ± 0.03	1.30 ± 0.03	1.40 ± 0.04	1.45 ± 0.04*	1.42 ± 0.04*	1.29 ± 0.03

Data are presented as the mean ± SEM; 19–20 animals per treatment group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ difference from Sham group; $^{*}p < 0.05$, $^{**}p < 0.01$ difference from OVX control group; $^{\$}p < 0.05$ difference from OVX control group

($p < 0.01$; Fig. 1b). ONO-5334 at 30 mg/kg prevented OVX-induced decreases in trabecular BMD. Significant increases in integral BMD were observed for ONO-5334 6 and 30 mg/kg ($p < 0.01$). All doses of ONO-5334 significantly increased cortical BMD compared with the OVX control group ($p < 0.05$; Fig. 1b). For the ONO-5334 6 and 30 mg/kg groups, the increase in cortical BMD was accompanied by a statistically significant increase in cortical thickness and a statistically significant decrease in endosteal perimeter compared with the OVX control group ($p < 0.01$ for both comparisons; Table 1). ALN had no statistically significant effect on integral, trabecular or cortical BMD compared with OVX control (Fig. 1b).

pQCT analysis in the midshaft radius (performed at 50% total length)

After OVX, a statistically significant decrease was observed in cortical BMD for OVX control compared with the Sham group ($p < 0.01$; Table 1). All doses of ONO-5334 significantly increased cortical BMD compared with the OVX control group ($p < 0.01$; Table 1). This increase in cortical BMD was accompanied by an increase in cortical thickness; these changes achieved statistical significance in comparisons of ONO-5334 6 and 30 mg/kg with OVX control ($p < 0.01$ for both comparisons; Table 1). ALN exerted no statistically significant effect on cortical BMD compared with OVX control.

Micro-CT analysis in distal radius

Bone trabecular architecture deteriorated significantly following OVX (Table 2). ONO-5334 suppressed in a dose-dependent fashion the OVX-induced decrease in the trabecular bone-related parameters; statistical significance was confirmed for all these parameters except Tb. Th in the ONO-5334 30 mg/kg group was compared with the OVX control group ($p < 0.05$). ALN significantly suppressed the

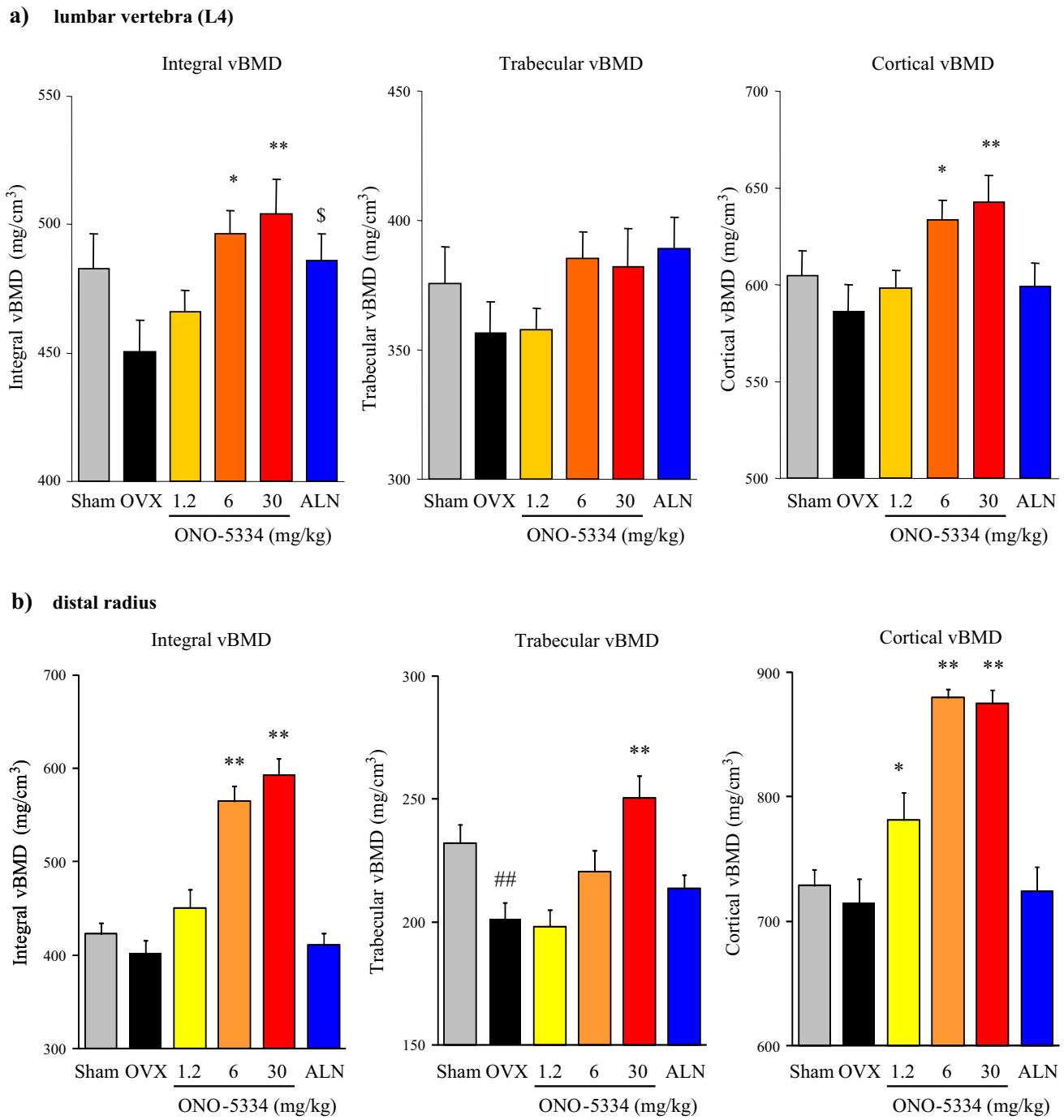

Fig. 1 Bone mineral density assessed by peripheral quantitative computed tomography at **a** the lumbar vertebra (L4) and **b** the distal radius after 16 months of treatment with ONO-5334. Data are presented as mean ± SE ($n = 19$–20 animals). $^{\#\#}p < 0.01$ versus Sham group; $^{*}p < 0.05$, $^{**}p < 0.01$ versus OVX control group; $^{\$}p < 0.05$ versus OVX control group

OVX-induced changes in BV/TV, Tb. N, Tb. Sp, Conn-Dens and SMI compared with the OVX control ($p < 0.05$) but had no effect on Tb. Th (Table 2). Scan images appeared to indicate that ONO-5334 thickens cortical bone by promoting bone formation in the endosteal surface (Fig. 2a). Three-dimensional images of trabecular bone suggested that ONO-5334 may also thicken trabecular bone (Fig. 2b).

Table 2 Effect of ONO-5334 on the trabecular structure of the distal radius assessed by micro-CT

	Sham	OVX control	ONO-5334			Alendronate
			1.2 mg/kg	6 mg/kg	30 mg/kg	0.05 mg/kg
BV/TV (%)	0.200 ± 0.016	0.111 ± 0.011$^{\#\#}$	0.124 ± 0.014	0.169 ± 0.014*	0.200 ± 0.018**	0.157 ± 0.011$^{\$\$}$
Tb. N (1/mm)	1.58 ± 0.08	1.22 ± 0.07$^{\#\#}$	1.25 ± 0.08	1.44 ± 0.07	1.55 ± 0.10*	1.48 ± 0.06$^{\$\$}$
Tb. Th (mm)	0.162 ± 0.004	0.149 ± 0.004$^{\#}$	0.153 ± 0.004	0.158 ± 0.006	0.163 ± 0.007	0.144 ± 0.003
Tb. Sp (mm)	0.63 ± 0.05	0.85 ± 0.05$^{\#\#}$	0.85 ± 0.07	0.68 ± 0.04	0.65 ± 0.05*	0.66 ± 0.03$^{\$\$}$
Conn-Dens (1/mm^3)	6.76 ± 0.90	3.28 ± 0.59$^{\#\#}$	3.44 ± 0.48	4.76 ± 0.58	5.69 ± 0.96*	4.83 ± 0.48$^{\$}$
SMI	1.52 ± 0.14	1.95 ± 0.09$^{\#}$	1.77 ± 0.09	1.64 ± 0.16	1.39 ± 0.17**	1.59 ± 0.13$^{\$}$

BV/TV bone volume per tissue volume, *Tb. N* trabecular number, *Tb. Th* trabecular thickness, *Tb. Sp* trabecular separation, *Conn-Dens* connectivity density, *SMI* structure model index

Data are presented as the mean ± SEM; 19–20 animals per treatment group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ difference from Sham group; $*p < 0.05$, $**p < 0.01$ difference from OVX control group; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ difference from OVX control group

Bone strength in distal and midshaft radius

Maximum load decreased in the OVX control group at the distal ($p < 0.01$) and midshaft radius ($p < 0.05$) compared with the Sham group (Fig. 3). ONO-5334 at 6 and 30 mg/kg significantly increased maximum load at the distal and midshaft radius compared with the OVX control group ($p < 0.05$, Fig. 3). This increase in maximum load was accompanied by an increase in stiffness and energy absorption in distal and midshaft radius (Table 3), respectively. ALN had no effect on maximum load at the distal and midshaft radius (Fig. 3).

Relationship between bone mass and strength in the lumbar vertebra

In the lumbar vertebra, a statistically significant correlation was observed between bone mass and bone strength in all groups except for the BMD and maximum load in the ONO-5334 1.2 mg/kg group (Table 4). The linear regression lines between bone mass and bone strength seemed to be shifted towards increasing bone strength in the ONO-5334 6 and 30 mg/kg groups compared with the OVX control, ONO-5334 1.2 mg/kg and the ALN groups (Fig. 4a, b). Subgroup analysis between maximum load and BMC revealed that bone strength was significantly higher in the ONO-5334 subgroup than in the ALN subgroup ($p < 0.05$), although mean values of integral BMC were similar between the ONO-5334 subgroup and the ALN subgroup (Fig. 5a). At the same integral BMD level, trabecular BMD in the ALN subgroup was higher than in the ONO-5334 subgroup. Conversely, maximum load and cortical BMC in the ONO-5334 subgroup were higher than in the ALN subgroup; these changes achieved statistical significance in the lower BMC or BMD group ($p < 0.05$; Fig. 5b).

Discussion

The current analysis was undertaken for several reasons. As previous investigations assessed BMD in the distal radius and the lumbar vertebra only using DXA, it was necessary to assess the effects of ONO-5334 on different bone compartments (i.e. cortical and trabecular) using pQCT. Also, ONO-5334 had previously demonstrated an apparent larger effect on non-vertebral bone (i.e. the radius) than in vertebral bone (i.e. lumbar vertebra). Therefore, in this study, we explored this observation further. Finally, we investigated whether the increase in bone mass produced by ONO-5334, in comparison with ALN, leads to an increase in bone strength, since both compounds have a different mode of action, as shown by differences in the bone turnover profile.

Studies on pQCT have confirmed that ONO-5334 increased not only trabecular BMD but also cortical BMD in the distal radius and the lumbar vertebra; the effect on cortical BMD was more pronounced. ONO-5334 also led to increases in cortical BMD in the midshaft radius. Cortical bone is more predominant in non-vertebral bones. Also, ONO-5334 is distributed through the blood stream to both cortical and trabecular bone and this may be the main reason why the effect of ONO-5334 on cortical bone was greater than ALN. In fact, more than 70% of ONO-5334 was distributed to non-vertebral bones considering the concentration of ONO-5334 in vertebral bone as 100% in a monkey study using radio-labelled ONO-5334 [19]. Compared with OVX control, ALN increased integral BMD at L4; however, the effects on the distal and midshaft radius were not statistically significant. This is consistent with a previous clinical study that showed a relatively lower effect of ALN on non-vertebral bone compared with vertebral bone [20, 21]. One possible reason as to why the effect of ALN on cortical rich bones, e.g. radius, was lower than that on trabecular-rich bones, e.g. lumbar vertebra, may be due to a character of bisphosphonates. Bisphosphonates

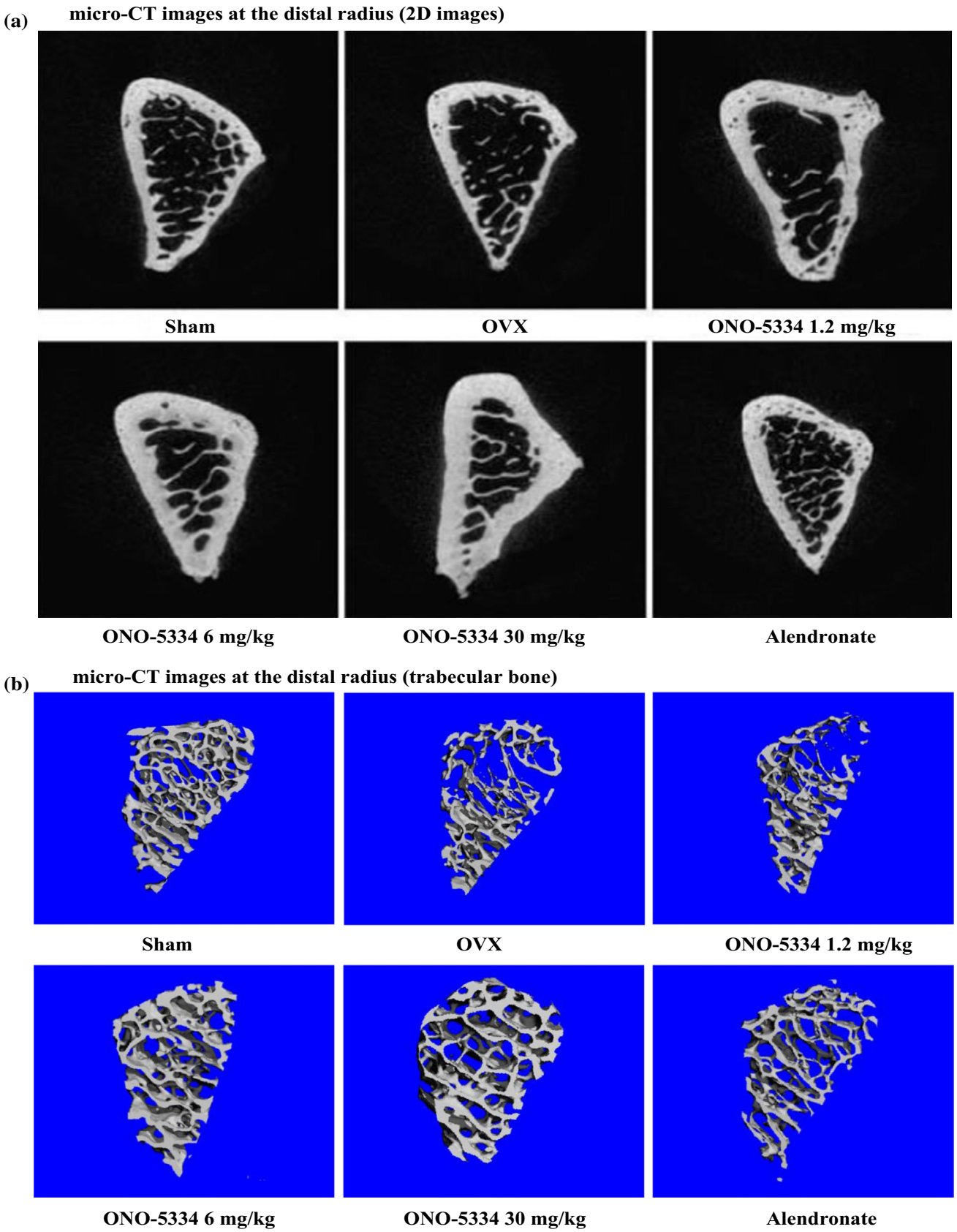

Fig. 2 Representative micro-CT images at the distal radius. **a** Two-dimensional image; individuals having median cortical thickness in each group are shown, **b** three-dimensional images of trabecular bone; individuals having median trabecular thickness in each group are shown

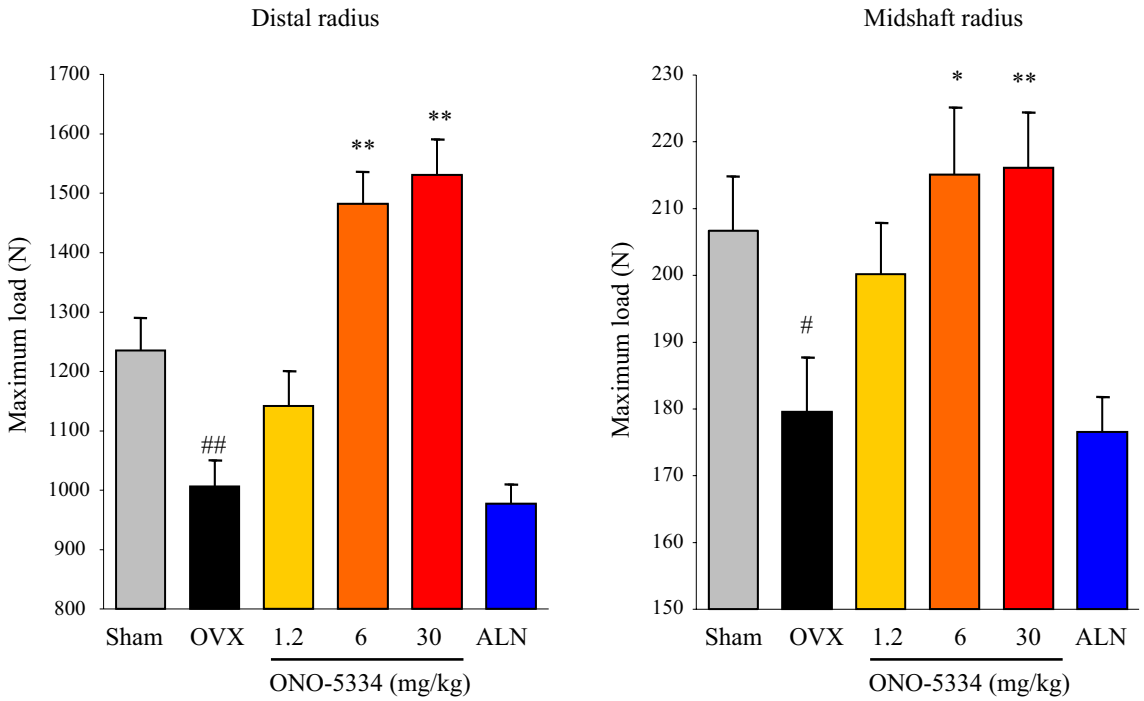

Fig. 3 Bone strength as assessed by maximum load at the distal and midshaft radius after 16 months of treatment with ONO-5334. Data are presented as mean ± SE ($n = 19$–20 animals). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ versus Sham group; $^{*}p < 0.05$, $^{**}p < 0.01$ versus OVX control group

Table 3 Effect of ONO-5334 on bone strength at the distal and midshaft radius

	Sham	OVX control	ONO-5334			Alendronate
			1.2 mg/kg	6 mg/kg	30 mg/kg	0.05 mg/kg
Distal radius						
Maximum load (N)	1236.5 ± 54.9	1006.1 ± 43.8$^{\#\#}$	1142.0 ± 58.5	1483.2 ± 53.4**	1530.5 ± 59.5**	977.4 ± 32.5
Stiffness (N/mm)	2286.4 ± 28.3	2233.6 ± 24.6	2262.8 ± 20.9	2343.3 ± 20.4**	2365.5 ± 24.1**	2232.9 ± 23.9
Energy absorption (mJ)	418.3 ± 33.5	297.6 ± 24.2$^{\#\#}$	369.4 ± 31.8	573.7 ± 34.7**	615.9 ± 40.6**	268.2 ± 14.4
Midshaft radius						
Maximum load (N)	206.6 ± 8.2	179.5 ± 8.2$^{\#}$	200.1 ± 7.7	215.0 ± 10.1*	216.0 ± 8.3**	176.4 ± 5.3
Stiffness (N/mm)	120.9 ± 6.8	100.3 ± 5.9$^{\#}$	109.4 ± 5.1	116.5 ± 7.1	116.7 ± 6.1	96.3 ± 3.8
Energy absorption (mJ)	339.8 ± 23.7	307.1 ± 19.3	368.3 ± 22.4	414.2 ± 35.0*	423.7 ± 29.0**	326.6 ± 20.1

Data are presented as the mean ± SEM; 19–20 animals per treatment group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ difference from Sham group; $^{*}p < 0.05$, $^{**}p < 0.01$ difference from OVX control group

Table 4 Correlation analysis between bone mass and bone strength

Group	Number	BMC versus ML Pearson correlation coefficient	*p* value	BMD versus ML Spearman correlation coefficient	*p* value
Sham	19	0.84	< 0.01	0.81	< 0.01
OVX control	20	0.83	< 0.01	0.65	< 0.01
ONO-5334 1.2 mg/kg	20	0.62	< 0.01	0.20	0.39
ONO-5334 6 mg/kg	19	0.75	< 0.01	0.81	< 0.01
ONO-5334 30 mg/kg	20	0.75	< 0.01	0.75	< 0.01
Alendronate	20	0.61	< 0.01	0.46	< 0.05

BMC integral bone mineral content, *BMD* integral bone mineral density, *ML* maximum load

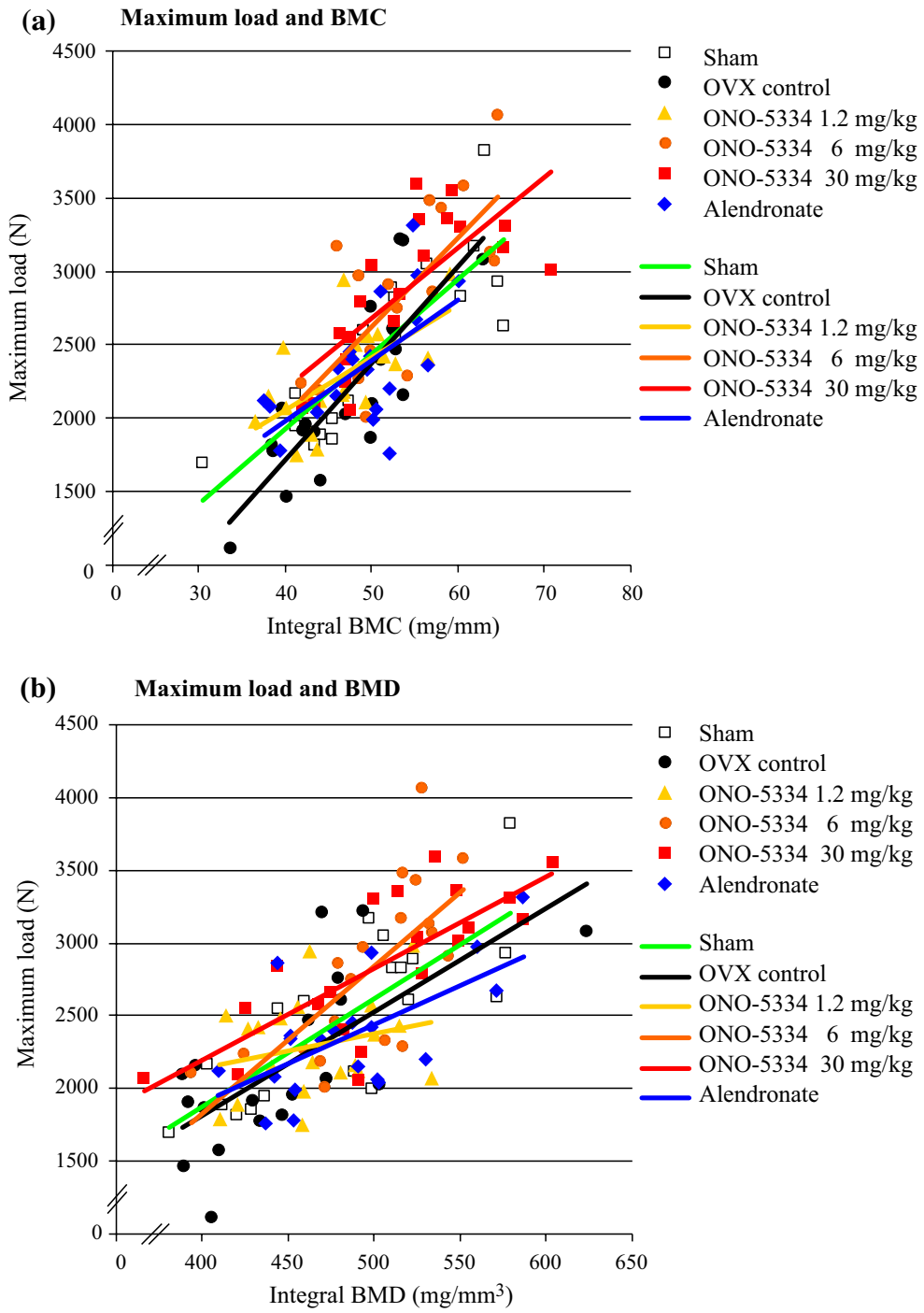

Fig. 4 Relationship between bone mass and strength at the lumbar vertebra (L4). Scatter plot and regression line were drawn by regression analysis. **a** Maximum load and BMC and **b** maximum load and BMD

are considered to be incorporated into the bone via bone turnover. Actually, ALN was tended to accumulate in trabecular bone in rats [22]. Similarly, the concentrations of ibandronate in non-vertebral bones were less than 30% of that in vertebral bone in monkeys [23].

In a previous study, we reported that changes in trabecular structure were observed more accurately by three-dimensional analysis using micro-CT than by conventional two-dimensional histomorphometry [24]. Thus, in the current study, we used micro-CT to assess the effect of ONO-5334

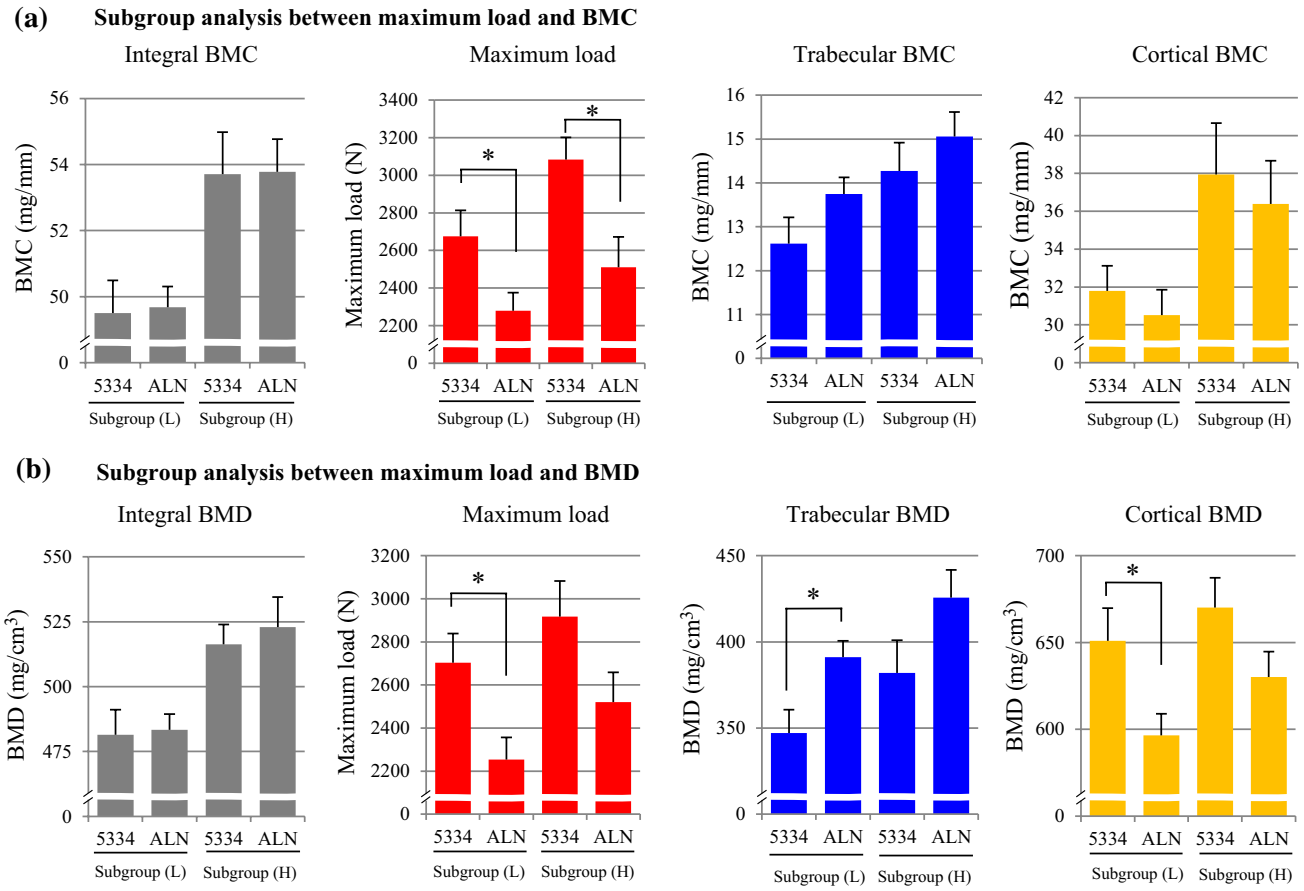

Fig. 5 Subgroup analysis between bone mass and bone strength at the lumbar vertebra (L4). **a** Subgroup analysis between maximum load and BMC and **b** subgroup analysis between maximum load and BMD. Subgroup (L): lower BMC or BMD group, Subgroup (H): higher BMC or BMD group. Data are expressed as mean ± SE ($n = 10$ animals). ONO-5334: 30 mg/kg $^*p < 0.05$, Significantly different from the ALN group

and ALN on trabecular bone-related parameters. Both ONO-5334 and ALN suppressed OVX-induced deterioration on BV/TV, Tb. N, Tb. Sp, Conn-Dens and SMI; however, there were no remarkable differences in trabecular structure between these two compounds. These findings are consistent with the results obtained with structurally different cathepsin K inhibitors, i.e. odanacatib [25].

Bone quality and bone mass are considered as very important factors in determining bone strength [1, 26]. For instance, in previous studies with sodium fluoride, a decrease in bone strength was found despite increases in bone formation and bone mass [27, 28]. This was mainly due to deterioration of bone quality and increased bone fragility. In the present study, the effect of ONO-5334 on the relationship between bone mass and strength was assessed. In these analyses, there was a statistically significant correlation between BMC, BMD and bone strength indicating that ONO-5334 increases bone strength along with increases in bone mass. Furthermore, the correlation analysis between bone mass and bone strength showed that the linear regression lines were shifted towards increasing bone strength in the ONO-5334 groups compared with the ALN groups. These data suggest that bone strength was higher in the ONO-5334 groups than in the ALN group, even though BMC and BMD values were similar for both groups.

To further explore this finding, we analysed how bone strength was affected by the trabecular and cortical bone mass. To be fair comparison, subpopulations of ONO-5334 and ALN groups were selected to ensure that mean values of lumbar BMC were comparable. A subgroup analysis showed that higher bone strength was observed for ONO-5334 compared with ALN when assessing for the same BMC level in the lumbar vertebra. In the ONO-5334 subgroup, cortical BMC were higher than in the ALN group, whilst the converse was observed for trabecular bone. Similar tendency was observed when analysed the data using BMD. These results suggest that cortical bone provides a greater contribution to bone strength than trabecular bone. Nonetheless, bone strength may vary depending on the proportion between cortical and trabecular bone.

In a Phase II study with ONO-5334, no clear differences in BMD between ONO-5334 and ALN were found when assessed by DXA at 24 months [13]. This contrasts with our results. One explanation for this discrepancy may be that although DXA is widely available and has been used in Phase III studies, this technique has several limitations. For example, it does not allow assessment of bone geometry or differentiation between cortical and trabecular bone [29]. To overcome these limitations, we used pQCT in the Phase II study. In the course of 2 years, ONO-5334 led to a significant and persistent increase of trabecular and integral BMD at the spine and the hip. Cortical BMD also progressively increased but at a lower rate [30]. It is also possible that as newly generated bone is not yet fully mineralized, BMD assessment may underestimate bone content and thus, only in longer-term assessments would any differences between both compounds be observable. This is even more relevant considering that the bone remodelling rate in monkeys is about one-third lower than that in humans and thus, the duration of the treatment period in the current study, 16 months, corresponds to approximately 4 years in humans [31]. Clinical trials with odanacatib in postmenopausal women showed that its administration led to continuous increases in the BMD of the lumbar spine up to 8 years of treatment [32–34]. This odanacatib-induced continuous increase in BMD may be due to its ability to increase cortical bone, i.e. odanacatib-treated monkeys showed an increase in the bone formation rate at the periosteal surface where considered modelling site [35]. Conservation of modelling-based bone formation has been observed in denosumab-treated cynomolgus monkeys, which may contribute to continuous BMD increase [36].

In conclusion, no negative effects of ONO-5334 on the relationship between bone mass and strength were detected in OVX cynomolgus monkeys. We found that ONO-5334 preferentially increased cortical bone over trabecular bone, whilst the converse was observed for ALN. Cortical bone may provide a greater contribution to bone strength than trabecular bone. As existing treatments for osteoporosis have a limited ability to reduce fracture risk in non-vertebral bone, cathepsin K inhibition by ONO-5334 may offer new therapeutic options to patients with osteoporosis.

Acknowledgements The authors would like to thank Naoto Horai (Shin Nippon Biomedical Laboratories, Ltd.) for his expertise and technical support in animal experiments and care. We also thank our colleagues Yasutomo Nakanishi and Mark Bruce for preparing the manuscript.

Compliance with ethical standards

Conflict of interest All authors are employed by Ono Pharmaceutical Co., Ltd.

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