Serum and urine bone resorption markers and pharmacokinetics of the cathepsin K inhibitor ONO-5334 after ascending single doses in post menopausal women

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Cathepsin K plays a critical role in bone resorption where it degrades the major organic components of bone and cartilage.
- Therefore inhibitors of cathepsin K may offer clinical benefit in metabolic bone diseases characterized by accelerated bone resorption including osteoporosis.

WHAT THIS STUDY ADDS

- This phase I study in post menopausal women provides the first safety, tolerability, pharmacokinetic and pharmacodynamic data for single doses of the cathepsin K inhibitor, ONO-5334.
- Morning administration of ONO-5334 showed rapid and dose-dependent suppression in bone resorption.
- This study shows that ONO-5334 is safe, well tolerated and suppresses bone resorption and therefore shows potential as a new treatment for osteoporosis.

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AIMS

To investigate the safety, pharmacokinetics and pharmacodynamics of the new cathepsin K inhibitor, ONO-5334.

METHODS

A double-blind, placebo-controlled, randomized study was carried out in 52 healthy post menopausal females. Single ascending doses of ONO-5334 (3–600 mg) were evaluated in six cohorts. The effect of food was studied at ONO-5334 100 mg.

RESULTS

Across the doses tested, mean ONO-5334 C_{max} occurred 0.5–1.0 h after dosing and the $t_{1/2}$ ranged from 9.1 to 22 h. Linear increases in C_{max} and $AUC(0, \infty)$ were observed in the 3–300 mg and 3–600 mg dose range, respectively. After food, the geometric mean ratio (95% CI) C_{max} and $AUC(0, \infty)$ for ONO-5334 were 0.78 (0.31, 1.94) and 0.95 (0.67, 1.35)-fold greater than fasted, respectively. ONO-5334 significantly reduced serum bone resorption markers within 4 h vs. placebo. Statistical significance was achieved for ONO-5334 doses ≥ 30 mg for C-terminal telopeptide of type 1 collagen (CTX) and ≥ 300 mg for N-terminal telopeptide of type 1 collagen (NTX). Statistical significance was still evident at 24 h for ONO-5334 100 mg with serum CTX and 600 mg with serum NTX. The maximum suppression in serum CTX occurred at 4 h post dose with difference compared with placebo of –32%, –59%, –60% and –66% for 30, 100, 300 and 600 mg ONO-5334, respectively. Second morning urine void 24 h post dose showed statistically significant suppression of urinary CTX and NTX at 100 mg and above vs. placebo. ONO-5334 600 mg showed statistically significant suppression up to 72 h for serum CTX, urinary CTX and urinary NTX and 48 h for serum NTX vs. placebo. Adverse events were transient with no evidence of dose relationship.

CONCLUSIONS

ONO-5334 displayed linear plasma pharmacokinetics over the (predicted therapeutic) dose range, 3–300 mg, with clear suppression of urinary bone resorption markers at doses ≥ 100 mg for serum markers at 24 h. ONO-5334 was well tolerated up to 600 mg day⁻¹ when administered to healthy post menopausal women.

Introduction

Fractures resulting from osteoporosis are a major public health concern. Osteoporosis is characterized by increased bone turnover that leads to low bone mineral density and an increased risk of fractures. One approach to reducing the risk of fractures is to reduce bone resorption. Antiresorptive agents such as the bisphosphonates are effective in the treatment of vertebral fractures but the response in non-vertebral fractures is significantly lower [1]. Tolerability issues and concern over the long term suppression of bone turnover which may give rise to events like atypical fractures of the femur [1] has led to interest in drugs with different mechanisms of action. Drugs that influence osteoclast activity rather than just the osteoclast number may permit the modification of bone formation without having long term effects on bone formation [2].

Cathepsin K is a member of the papain cysteine proteinase superfamily [3] and has a critical role in the bone resorption process. Cathepsin K is released by osteoclasts and degrades the organic matrix of bone including that of collagen type I and II. Peptide products of the degradation include C-terminal telopeptide of type I collagen (CTX-I) and N-terminal telopeptide of type I collagen (NTX). These have been used as biochemical markers of bone resorption and treatment efficacy in bone metabolic diseases [4]. Humans lacking cathepsin K exhibit pycnodysostosis, which is characterized by abnormally dense bones (osteosclerosis) [5]. Cathepsin K deficient mice show osteopetrosis and display features characteristic of pycnodysostosis [6, 7]. Specific inhibitors of cathepsin K may therefore have potential as a new type of medication for metabolic bone diseases that involve elevated bone resorption.

Although no cathepsin K inhibitor is currently licensed for treatment, several candidate molecules have entered clinical development including balicatib, relacatib and odanacatib. Balicatib and odanacatib suppress markers of bone resorption and are associated with increases in bone mineral density in humans [8–10]. The clinical development of several cathepsin K inhibitors has been discontinued [11] and currently only odanacatib and ONO-5334 are in clinical development.

ONO-5334 (N-((1S)-3-((2Z)-2-((4R)-3,4-Dimethyl-1,3-thiazolidin-2-ylidene)hydrazino)-2,3-dioxo-1-(tetrahydro-2H-pyran-4-yl)propyl)cycloheptanecarboxamide, Ono Pharmaceutical Co. Ltd, Japan) is a low molecular weight synthetic inhibitor of cathepsin K and is being developed as an oral therapeutic agent for bone metabolic diseases including osteoporosis. ONO-5334 exhibits a potent and selective inhibitory effect on cathepsin K *in vitro* [12–14] and is associated with improvements in both bone mineral density and bone strength in the ovariectomized monkey osteoporosis model [15].

This phase I clinical study was designed to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of single doses of the new cathepsin K inhibitor

ONO-5334 (with/without food) in healthy post menopausal women.

Methods

Study design

This double-blind, placebo-controlled, single dose escalation study in healthy post menopausal female subjects was conducted at the Clinical Pharmacology Unit, Kendle International B.V., the Netherlands. Fifty-two subjects were planned for enrolment in five groups of eight subjects each and in one group of 12 subjects to determine the safety, tolerability and pharmacokinetics of ONO-5334. The pharmacodynamic effects of ONO-5334 on the bone resorption markers, serum CTX and serum NTX, were also assessed. Eligible subjects attended the unit on the morning of the day before dosing and remained for at least 72 h post dose. In each group of eight subjects, six subjects were randomized to receive single oral doses of ONO-5334 tablets (3, 10, 30, 300, or 600 mg) and two subjects matching placebo tablets. In the group of 12 subjects, six were randomized to receive 100 mg ONO-5334 and six matching placebo. Each group of eight subjects was dosed in the fasted state during one treatment period only. The group of 12 subjects was dosed in the fasted state during the first treatment period (period 1) and received the same treatment a week later in the fed condition, after receiving a standard breakfast, consisting of three slices of bread, a cracker, diet margarine, jam, cheese, ham, semi-skimmed milk and fruit juice (period 2), as part of a preliminary investigation into the effect of food on the pharmacokinetic profile of ONO-5334. Subjects attended a follow-up visit 5 days after the final dose.

The rationale for the starting dose of ONO-5334 in this study was based upon the maximum recommended starting dose (MRSD) determined by the no observed adverse effect level (NOAEL) and the pharmacologically active dose (PAD). The MRSD determined by NOAEL was 3 mg/person, which was derived by converting the 30 mg kg⁻¹ rat NOAEL dose to a value of 2.916 mg kg⁻¹ for humans. A safety factor of 100 was used in the calculation instead of the standard factor of 10 to take into consideration safety concerns associated with administering new medications to post menopausal females. The MRSD determined from the PAD was 6 mg/person, which was derived by converting the 0.6 mg kg⁻¹ dose, which was the lowest pharmacologically active dose in the rat model, to a value of 0.096 mg kg⁻¹ for humans. The lowest dose of 3 mg was therefore chosen as a suitable starting dose. The rationale for selecting the highest dose of ONO-5334 was based on the NOAEL of 100 mg kg⁻¹, which translated to a maximum dose (on a mg kg⁻¹ basis) of 6000 mg day⁻¹ for humans. The highest dose of ONO-5334 in this study was 600 mg (one tenth of the maximum dose). Dose intervals between the minimum and maximum were based on a standard

pharmacokinetic-safety risk approach. The need to obtain sufficient data to evaluate pharmacokinetic linearity was balanced against risk and the number of subjects required to meet the aims of the study. No safety concerns were identified in any of the pre-clinical studies. Thus, increases of approximately three-fold were considered to be justified. Dose escalation decisions followed review of safety and tolerability data from the preceding group by the principal investigator and the sponsor.

Study subjects

Healthy post menopausal females aged 45–75 years with a body mass index of 19 to 32 kg m⁻² were eligible to take part in the study. Post menopausal was defined as women who had been amenorrhoeic for more than 1 year if over 50 years of age. If aged 45–50 years women were considered post menopausal if amenorrhoeic for more than 2 years if and they demonstrated an appropriate clinical profile that was confirmed at screening by oestradiol and follicle stimulating hormone (FSH) concentrations consistent with menopause (FSH >30 IU l⁻¹, oestradiol <92 pmol l⁻¹). Subjects were excluded from the study if they had any relevant medical history including osteoporosis, history of severe allergic reaction, drug or alcohol abuse or other clinically significant abnormalities at screening. Subjects were also excluded if they smoked (even within last 6 months), drank more than 14 units of alcohol week⁻¹ or received prior to dosing, prescription drugs within 28 days (St Johns' Wort within 6 weeks), non-prescription drugs or over the counter drugs within 48 h unless deemed necessary by the principal investigator and where they did not interfere with study procedures or the subject's safety. Strenuous exercise, caffeine and alcohol were prohibited for 48 h prior to study start and during the study. All subjects gave written informed consent and the study was approved by the Stichting Therapeutische Evaluatie Geneesmiddelen Ethics Committee.

Assessments

Blood samples (7.5 ml/sample) for the determination of ONO-5334 concentration were collected prior to dosing, 10, 20 and 30 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 h after dosing. The plasma samples obtained were divided into three sample tubes and stored at -20°C. The samples were analyzed by Sumika Chemical Analysis Service, Ltd (Japan). Plasma ONO-5334 was isolated using solid phase extraction and a validated liquid chromatography/tandem mass spectrometry method (with a lower limit of quantification (LOQ) of 0.02 ng ml⁻¹) was used to quantify concentrations of ONO-5334. The precision of the ONO-5334 assay was <15% (coefficient of variation) and the accuracy of the assay was within 15% of the actual value for ONO-5334.

Serum samples for the analysis of CTX and NTX were obtained at baseline (pre-dose), 4, 6, 12, 24, 48 and 72 h after administration and second morning void urine samples were collected before breakfast at baseline (pre-

dose), 24, 48 and 72 h after administration. Analysis of CTX was undertaken using Serum CrossLaps® ELISA and Urine CrossLaps® EIA, Immunodiagnostic Systems Ltd, Boldon, UK and of NTX using Osteomark® NTX, Osteometrics, Decatur, GA, USA.

Standard safety assessments were performed throughout the study including vital signs, physical examinations, 12-lead electrocardiogram (ECG), continuous ECG, haematology, biochemistry, urinalysis and adverse event (AE) monitoring.

Statistical analysis

No formal power calculation was performed to estimate the appropriate number of subjects to be studied but the sample size was considered adequate to fulfil the study aims.

Pharmacokinetic parameters were calculated using WinNonlin® Ver. 4.0.1 software (Pharsight Corp., Mountain View, California). The area under the plasma concentration vs. time curve from time zero to infinity (AUC(0,∞)) was estimated using the linear trapezoidal rule. The peak plasma concentration (C_{max}) values and time associated with the maximal concentration (t_{max}) were obtained from the observed data. Oral clearance (CL/F) was calculated as dose/AUC(0,∞). Dose dependency of C_{max} and AUC(0,∞) was assessed by the power model (point estimate and 95% confidence interval [95% CI] of the slope (β) of the regression formula log (Y) = α + β × log (X)). Dose proportionality was confirmed if the 95% CI of β included 1 and fell within the range of 0.7, 1.3. Appropriate tests were used to determine any statistically significant differences between doses for half-life (t_{1/2}, parametric, Tukey–Kramer test) and t_{max} (non-parametric, Steel–Dwass test) using a two-sided 5% level of significance. To evaluate the effect of food on ONO-5334 pharmacokinetics, C_{max} and AUC(0,∞) were compared between the fasted and fed states in the 100 mg of ONO-5334 group. The ratio of the fed to the fasted state was calculated for individual subjects and the geometric mean ratios and 95% CIs determined using log-transformed data. When the 95% CI of the geometric mean did not include 1, it was considered that food had affected the pharmacokinetics of ONO-5334. Half-life was analyzed in a similar way to AUC(0,∞) and C_{max}. t_{max} was analyzed by Wilcoxon signed rank test at a two-sided 5% level of significance.

For bone resorption markers, any values below the LOQ were assigned the LOQ for the assay (serum CTX 0.173 µg l⁻¹, serum NTX 5 µg l⁻¹, urine CTX 99 µg l⁻¹ and urine NTX 20 nM BCE). For urine CTX and urine NTX, values were corrected for creatinine. Percentage changes from baseline (pre dose) could not be evaluated where a subject's bone resorption marker at baseline was below the LOQ. Any subjects with a baseline value below the LOQ were therefore excluded from the analyses. Percentage changes from baseline (pre-dose) were analyzed at each time point using analysis of covariance (ANCOVA) with log-

transformed pre dose value as a covariate and treatment as a fixed effect. ONO-5334 treatment groups were compared with placebo at each post dose time point.

There was no formal statistical analysis of safety data. Treatment emergent adverse events (TEAE) were defined as AEs that occurred or worsened after the first investigational product intake.

Results

Fifty-two subjects were randomized to the study and all completed. There were no specific differences in baseline characteristics between treatment groups (Table 1). No subjects were excluded from the safety or the pharmacokinetic analysis populations, although estimates for $t_{1/2}$, $AUC(0,\infty)$ and CL/F could not be derived for one subject because concentrations of ONO-5334 were too low.

Pharmacokinetic assessments

Within the tested dose range (3–600 mg), fasted plasma ONO-5334 concentrations reached a mean C_{max} (5.69 to 941 ng ml⁻¹) within 0.33 to 1.5 h of dosing and thereafter decreased in a biphasic fashion (Table 2, Figure 1). In the initial phase, ONO-5334 concentrations fell rapidly,

decreasing to less than one-tenth of the mean C_{max} 4 h after dosing. Mean $t_{1/2}$ ranged from 9.1 to 22 h and mean $AUC(0,\infty)$ from 7.70 to 2400 ng ml⁻¹ h over the dose range. In the latter phase of elimination in the 600 mg dose group, plasma ONO-5334 concentrations decreased at a slower rate relative to other dose groups.

Mean $AUC(0,\infty)$ increased linearly with dose from 3 to 600 mg and mean C_{max} increased linearly with dose from 3 to 300 mg, but not from 300 to 600 mg. Dose dependency of C_{max} (3–300 mg) and $AUC(0,\infty)$ (3–600 mg) were assessed and the point estimate (95% CI) of β was calculated to be 1.04 (0.89, 1.20) for C_{max} and 1.12 (1.05, 1.19) for $AUC(0,\infty)$. Maximum plasma concentration met the dose proportionality criteria. The parameter $AUC(0,\infty)$ did not meet the dose proportionality criteria, although the point estimate for β was close to 1 and its 95% CI fell within 0.70, 1.30.

At 600 mg, the t_{max} was slightly delayed compared with that in the 3 and 10 mg dose groups (1.0 h vs. 0.5 h and 0.5 h, respectively). These differences achieved statistical significance (both $P < 0.05$). For mean $t_{1/2}$, differences between ONO-5334 doses from 3 to 600 mg did not achieve statistical significance.

The geometric means (95% CIs) for C_{max} and $AUC(0,\infty)$ after post prandial administration were 0.78 (0.31, 1.94)

Table 1

Summary of baseline characteristics

		Placebo	ONO-5334						
Variable		n = 16	3 mg n = 6	10 mg n = 6	30 mg n = 6	100 mg n = 6	300 mg n = 6	600 mg n = 6	All n = 52
Age (years)	mean	62.3	61.8	63.3	60.5	60.0	59.8	61.2	61.5
	SD	4.70	3.97	7.34	2.07	5.69	6.37	6.05	5.08
Height (cm)	mean	167.5	166.0	158.8	164.8	165.0	166.5	163.5	165.1
	SD	5.52	5.96	4.13	5.16	7.66	6.80	3.78	5.96
Weight (kg)	mean	72.1	71.1	65.0	69.9	75.4	75.5	71.3	71.6
	SD	9.25	5.59	6.57	6.99	6.47	10.75	5.80	8.06
BMI (kg m ⁻²)	mean	25.7	25.8	25.8	25.8	27.7	27.2	26.6	26.2
	SD	2.90	2.27	2.42	3.26	1.15	3.43	1.47	2.57

n, number of subjects in specified group; SD, standard deviation.

Table 2

Pharmacokinetic parameters after administration of single oral doses of ONO-5334 to post menopausal women (fasted and both fed/fasted for 100 mg)

Dose (mg)	C_{max} (ng ml ⁻¹)	t_{max} (h)	$AUC(0,\infty)$ (ng ml ⁻¹ h)	$t_{1/2}$ (h)	CL/F (ml min ⁻¹)	CL/F (ml min ⁻¹ kg ⁻¹)
3	5.69 (1.84)	0.50 (0.33–0.50)	7.70 ^{a)} (3.44)	11 ^{a)} (3)	7270 ^{a)} (2230)	102 ^{a)} (32)
10	24.0 (13.9)	0.50 (0.33–0.50)	27.0 (8.9)	17 (12)	6690 (1980)	102 (26)
30	90.6 (68.3)	0.50 (0.50–1.5)	96.4 (43.4)	22 (14)	6020 (2370)	86.4 (33.2)
100 (fasted)	339 (259)	1.0 (0.50–1.0)	422 (118)	17 (3)	4210 (1150)	55.7 (13.1)
100 (fed)	255 (165)	1.5 (1.0–3.0)	419 (196)	18 (2)	4530 (1480)	59.8 (16.0)
300	832 (738)	0.75 (0.33–1.5)	1480 (860)	13 (5)	4390 (2520)	59.5 (36.3)
600	941 (500)	1.0 (0.50–1.5)	2400 (530)	9.1 (1.8)	4320 (850)	60.9 (10.6)

[n = 6, ^{a)} n = 5]. C_{max} , AUC , $t_{1/2}$, CL/F : mean (SD). t_{max} : median (range).

Figure 1

Log-transformed mean ($\pm$ SE) change in plasma concentration of ONO-5334 with time after single oral administration at doses of 3–600 mg in a fasted condition. Changes in plasma concentration are presented for 0–72 h (A) and 0–24 h (B) with different time scale. $n = 5$ –6. $\circ$ ONO-5334 3 mg; $\triangle$ ONO-5334 10 mg; $\square$ ONO-5334 30 mg; $\bullet$ ONO-5334 100 mg; $\blacktriangle$ ONO-5334 300 mg; $\blacksquare$ ONO-5334 600 mg

and 0.95 (0.67, 1.35) fold those obtained in fasting subjects, respectively. Median $t_{\max}$ after post prandial administration was 1.5 h (range 1.0–3.0), which was slightly but significantly delayed (by ~ 0.5 h) compared with the fasted condition ($P = 0.039$). Mean $t_{1/2}$ was similar for the fasted and fed states.

Pharmacodynamic assessments

One subject in the 600 mg dose group for serum CTX and one subject in the 100 mg dose group for urine NTX had pre dose (baseline) concentrations below the LOQ. Analyses of serum CTX and urine NTX were produced excluding these subjects.

There was a notable variation in concentrations of both serum CTX and NTX throughout the day (placebo group) with a minimum concentration occurring at 6 h post dose followed by a return to baseline values at 24 h post dose. The pre dose sample was collected at approximately 09.00 h.

After dosing with ONO-5334, serum CTX was suppressed within 4 h (first time point measured post dose) but only consistently (in all subjects) for ≥ 30 mg. The exception was one subject in the 600 mg dose group who showed no apparent suppression for the reasons explained above. The maximum suppression (% change from baseline) in serum CTX for each dose occurred 6 h post dose (-64% for 30 mg, -68% for 100 mg, -66% for 300 mg, -72% for 600 mg compared with -50% for placebo). A number of subjects had values below the LOQ

post dose (Table 3) indicating the actual magnitude of effect may be slightly greater than can be measured by the assay. This was notable from the 100 mg dose level where five, six and two (of the six subjects) had values below LOQ at 4, 6 and 12 h post dose, respectively. In the 600 mg group, all five evaluable subjects had values below the LOQ at 4, 6, 12 and 24 h post dose. When taking into account the normal variation in serum CTX concentrations across the day (as observed under placebo treatment), the maximum suppression occurred earlier at 4 h post dose (difference to placebo: -32% for 30 mg, -59% for 100 mg, -60% for 300 mg, -66% for 600 mg; 6 h post dose: -14% for 30 mg, -18% for 100 mg, -16% for 300 mg, -22% for 600 mg). This difference from placebo achieved statistical significance at the 4 h and 6 h post dose time points for doses of 30 mg and above. Serum CTX suppression continued at 12 h and 24 h post dose for doses above 100 mg, and for 600 mg serum CTX was statistically significantly suppressed vs. placebo at 48 h and 72 h post dose.

After dosing with ONO-5334, serum NTX was suppressed within 4 h (first time point measured post dose) and significantly vs. placebo for ≥ 300 mg (Figure 2). One subject in the 100 mg dose group showed greater than 200% increases relative to baseline at 4, 6 and 12 h post-dose (Table 4). The next highest within the 100 mg group was 28% (at 12 h). The maximum suppression compared with baseline in serum NTX for each dose generally occurred 6 h post dose (notwithstanding 100 mg) (-40% for 30 mg, 65% for 300 mg and -62% for 600 mg compared

Table 3
Analysis of percentage changes in serum CTX compared with baseline

	Placebo (n = 16)	ONO-5334 3 mg (n = 6)	10 mg (n = 6)	30 mg (n = 6)	100 mg (n = 6)	300 mg (n = 6)	600 mg (n = 5)
Pre-dose (Absolute CTX value) ($\mu\text{g l}^{-1}$)	0.7 (0.58, 0.81)	0.73 (0.55, 0.92)	0.66 (0.47, 0.85)	0.71 (0.52, 0.89)	0.53 (0.34, 0.72)	0.61 (0.42, 0.8)	0.65 (0.45, 0.86)
4 h (% CFB)	NC	-3.34 (-13.73, 7.06)	-10.18 (-20.55, 0.19)	-31.64 (-42.02, -21.25)	-58.95 (-69.56, -48.35)	-60.37 (-70.84, -49.9)	-66.07 (-77.17, -54.97)
6 h (% CFB)	NC	18.83 (6.32, 31.34)	-11.64 (-24.12, 0.84)	-14.08 (-26.58, -1.58)	-18.32 (-31.08, -5.56)	-16.42 (-29.02, -3.82)	-22.47 (-35.83, -9.11)
12 h (% CFB)	NC	8.86 (-2.87, 20.58)	-5.6 (-17.3, 6.11)	-8.71 (-20.43, 3.01)	-31.65 (-43.62, -19.68)	-29.18 (-40.99, -17.36)	-37.76 (-50.29, -25.24)
24 h (% CFB)	NC	8.18 (-14.03, 30.38)	-6.43 (-28.58, 15.73)	8.62 (-13.56, 30.81)	-24.54 (-47.2, -1.88)	-25.45 (-47.82, -3.08)	-74.18 (-97.89, -50.46)
48 h (% CFB)	NC	1.1 (-19.07, 21.28)	-7 (-27.13, 13.13)	9.07 (-11.09, 29.22)	-9.26 (-29.85, 11.33)	-0.44 (-20.76, 19.88)	-56.03 (-77.58, -34.49)
72 h (% CFB)	NC	-14.8 (-32.88, 3.28)	-7.29 (-25.33, 10.75)	5.34 (-12.72, 23.4)	-11.37 (-29.82, 7.08)	11.6 (-6.62, 29.81)	-25.77 (-45.08, -6.46)

CFB, change from baseline; n, number of subjects with evaluable data within group. Model = log(pre dose) + Treatment, except for pre dose where Model = Treatment. NC, Not calculated. (1), (2), (3), (4), (5) and (6) = 1, 2, 3, 4, 5 and 6 values at that time point were below quantification limit, respectively. These values were included in analyses using the quantification limit.

with -23% for placebo). The mean suppression for 100 mg with and without data from the subject with the outstanding increase was 14% and -24%, respectively. There was no apparent reason (e.g. procedural or AEs) why the 100 mg subject group or the one outstanding subject in particular failed to show results expected to be more consistent with a dose response effect. Serum NTX was significantly suppressed compared with placebo at 12 h to 48 h post dose, inclusive, only for 600 mg although there was a tendency ($P < 0.1$) for 300 mg to stay suppressed at 12 h and 24 h.

Secondary void urine CTX at 24 h showed statistically significant suppression compared with placebo at doses ≥ 100 mg (-38% for 100 mg, -96% for 300 mg and -104% for 600 mg; Table 5, Figure 3). For the 600 mg dose group, there was clear, statistically significant suppression of urine CTX compared with placebo that continued for 3 days (-52% at 72 h).

For urinary NTX, one 100 mg subject had pre dose (baseline) concentration below the LOQ and so these results exclude this subject. Statistically significant suppression in urine NTX at 24 h compared with placebo was observed for ≥ 100 mg (-63% for 100 mg, -75% for 300 mg and -113% for 600 mg; Table 6, Figure 3). For 600 mg, significant suppression continued for 3 days (-57% at 72 h, $P < 0.01$).

Safety assessments

There were no serious adverse events or withdrawals/discontinuations from the study treatment due to adverse events (AEs). Fifty-five TEAEs were reported by 30 of the 52 subjects (57.7%) when dosed under fasted conditions. Ten out of 16 subjects (62.5%) reported TEAEs after placebo treatment whereas 20 of 36 (55.5%) subjects reported TEAEs after ONO-5334 (fasted states; Table 7). Eight TEAEs were reported by six out of 12 subjects who were dosed after receiving a standard breakfast. One subject on placebo treatment had one TEAE, whereas five subjects dosed with 100 mg ONO-5334 had seven TEAEs. All TEAEs were transient. Thirty-two of 55 TEAEs occurred within the first day of dosing. The duration of the TEAEs did not appear to be related to treatment or dose. Four subjects experienced TEAEs that were graded as severe in intensity: vomiting and back pain in the placebo group, renal colic after ONO-5334 300 mg and headache after ONO-5334 600 mg. All these events resolved within 1 day. The most frequently reported TEAEs according to System Organ Class were gastro-intestinal disorders and nervous system disorders (Table 7). There were no other clinically relevant changes in ECG, laboratory assessments, vital signs or physical examinations.

Discussion

When ONO-5334 was orally administered to post menopausal Caucasian women in a fasted state at doses of 3 to

Figure 2

Effects of single ascending doses of ONO-5334 on A) serum CTX and B) serum NTX throughout 72 h post dose. Mean (SE) percentage change from baseline, $n = 6$ for all treatment groups ($n = 5$ for 600 mg sCTX), $n = 16$ for placebo. ◆ placebo; ● ONO-5334 3 mg; ▲ ONO-5334 10 mg; ■ ONO-5334 30 mg; ○ ONO-5334 100 mg; △ ONO-5334 300 mg; □ ONO-5334 600 mg

600 mg, its $C_{\max}$ was proportional to dose within the range of 3 to 300 mg. However, it was less than proportional between 300 and 600 mg. Although the point estimate of β for $AUC(0,\infty)$ (3–600 mg) did not meet pre-defined criteria which define dose-proportionality, the point estimate of β was close to 1 and the 95% CI was within pre-defined levels. Therefore, it was considered that $AUC(0,\infty)$ of ONO-5334 was nearly proportional to dose from 3 to 600 mg. The $t_{\max}$ value for ONO-5334 at 600 mg was slightly delayed compared with those at 3 and 10 mg but there was no difference among the doses 3–300 mg. $t_{1/2}$ was not significantly different between any doses. Taking into account the available plasma pharmacokinetic data, it appears that ONO-5334 demonstrated linear pharmacokinetics over the expected therapeutic dose range 3 to 300 mg after single dosing.

The delayed $t_{\max}$ at 600 mg and the less than proportional $C_{\max}$ between 300 and 600 mg may have been a consequence of the saturated dissolution rate of the ONO-5334 tablet. Consequently, the absorption rate decreased with increasing dose. However, as $AUC(0,\infty)$ was almost proportional to dose even between 300 and 600 mg, the total absorption ratio may not be affected by the increase in dose. This suggests that the gastrointestinal region involved in ONO-5334 absorption may not be limited just to the upper tract, and that the lower tract may play some role in absorption. Hence controlling the dissolution rate of the ONO-5334 tablet may achieve a more sustained ONO-5334 plasma profile.

When comparing the pharmacokinetic profile between the fasted and fed conditions at a dose of 100 mg, $C_{\max}$ after post prandial administration was slightly lower (0.78-fold)

than in the fasted state. However, changes in $C_{\max}$ were not consistent across all subjects tested and the ratio of the geometric mean for $C_{\max}$ after post prandial administration compared with fasting was also wide (0.31 to 1.94). Hence, $C_{\max}$ may be more under the influence of intra-individual variability than food. As the ratio of the geometric mean for $AUC(0,\infty)$ after post prandial administration compared with the fasted state was 0.95 and its 95% CI was 0.67, 1.35, it was concluded that total exposure to ONO-5334 was unaffected by food, although it should be noted that $t_{\max}$ was slightly delayed. Given the nature of the target disease (osteoporosis) and the mechanism of action of anti-resorptive agents (cathepsin K inhibitors), we conclude that this small effect of food on $t_{\max}$ will be of little clinical significance.

ONO-5334 decreased serum CTX in a dose-dependent manner. As serum CTX values of several subjects decreased below the limit of detection, the observed dose effect of ONO-5334 on serum CTX is slightly underestimated. The serum NTX concentrations in the 100 mg dose group changed in a different manner over time to other dose groups. The difference in percentage change was affected by a subject in the 100 mg group who had a low pre dose (baseline) value and relatively high post dose values up to 12 h (200%). However, even without this subject the 100 mg results were not consistent in following the expected dose–response effect. These findings cannot be explained by changes in study conduct (e.g. sample viability or assay procedures) in this dose group compared with others and may have been due to the limited number of subjects in the current study. As the impact of natural variability among subjects on study endpoints increases with

Table 4
Analysis of percentage changes in serum NTX compared with baseline

ONO-5334								
	Placebo (n = 16)	3 mg (n = 6)	10 mg (n = 6)	30 mg (n = 6)	100 mg (n = 6)	300 mg (n = 6)	600 mg (n = 6)	
Pre-dose (Absolute NTX value) (nm BCE)	18.02 (15.11, 20.93)	20.23 (15.48, 24.99)	22.58 (17.83, 27.34)	16.62 (11.86, 21.37)	14.48 (9.73, 19.24)	12.57 (7.81, 17.32)	12.88 (8.13, 17.64)	
4 h (% CFB)	NC	6.76 (-27.86, 41.38)	9.39 (-25.31, 44.09)	-15 (-49.54, 19.53)	-6.15 (-41.44, 29.14)	-57.24 (-94.06, -20.42)	-51.32 (-87.54, -15.11)	
6 h (% CFB)	NC	8.42 (-25.25, 42.09)	14.12 (-19.63, 47.86)	-17.1 (-50.68, 16.49)	36.92 (2.6, 71.24)	-41.54 (-77.35, -5.73)	-38.91 (-74.13, -3.69)	
12 h (% CFB)	NC	3.03 (-34.47, 40.52)	14.37 (-23.21, 51.94)	-12.85 (-50.25, 24.54)	53 (14.79, 91.21)	-38.84 (-78.71, 1.03)	-44.55 (-83.77, -5.34)	
24 h (% CFB)	NC	1.31 (-26.01, 28.64)	0.86 (-26.52, 28.25)	4.97 (-22.29, 32.23)	26.32 (-1.53, 54.17)	-26.63 (-55.69, 2.43)	-63.88 (-92.46, -35.29)	
48 h (% CFB)	NC	5.4 (-19.73, 30.54)	1.87 (-23.32, 27.07)	-3.75 (-28.82, 21.32)	28.73 (3.11, 54.35)	-4.45 (-31.18, 22.29)	-39.79 (-66.08, -13.5)	
72 h (% CFB)	NC	0.28 (-22.86, 23.42)	-6.38 (-29.57, 16.81)	-4.3 (-27.38, 18.78)	14.28 (-9.3, 37.87)	-9.59 (-34.19, 15.02)	-13.7 (-37.9, 10.5)	

CFB, change from baseline; n, number of subjects with evaluable data within group. Model = log (pre dose) + Treatment, except for pre dose where Model = Treatment. NC, Not calculated. (1), (2), (3), (4), (5) and (6) = 1, 2, 3, 4, 5 and 6 values at that time point were below quantification limit, respectively. These values were included in analyses using the quantification limit.

Table 5
Analysis of percentage changes in urine CTX compared with baseline

		ONO-5334						
		Placebo (n = 16)	3 mg (n = 6)	10 mg (n = 6)	30 mg (n = 6)	100 mg (n = 6)	300 mg (n = 6)*	600 mg (n = 6)
Pre-dose (Absolute CTX value) ($\mu\text{g mmol}^{-1}$ creatinine)	LSMean	344.88 (295.08, 394.67)	298.67 (217.35, 379.98)	308.83 (227.52, 390.15)	328.33 (247.02, 409.65)	378 (296.68, 459.32)	200 (110.92, 289.08)	280 (198.68, 361.32)
	95% CI for LSMean Difference vs. Placebo	NC	4.6 (-16.95, 26.16)	-8.58 (-29.81, 12.66)	0.58 (-20.61, 21.77)	-38.2 (-59.59, -16.82)	-96.38 (-121.6, -71.16)	-103.7 (-125.3, -82.13)
24 h (% CFB)	95% CI for difference	NC	12.19 (-21.42, 45.8)	-5.38 (-38.5, 27.74)	17.94 (-15.11, 50.99)	1.42 (-31.93, 34.78)	13.76 (-25.57, 53.09)	-73.38 (-107.1, -39.68)
	Difference vs. Placebo	NC	-1.85 (-36.84, 33.15)	-11.58 (-46.05, 22.9)	2.98 (-31.42, 37.39)	25.56 (-9.16, 60.28)	21.17 (-19.77, 62.11)	-52.01 (-87.09, -16.93)

n, number of subjects with evaluable data within group. Model = log (pre dose) + Treatment, except for pre dose where Model = Treatment. NC, Not calculated. (1), (5) = 1 and 5 values at that time point were below quantification limit, respectively. These values were included in analyses using the quantification limit. *one subject had missing pre-dose.

Figure 3

Effects of single ascending doses of ONO-5334 on (A) urinary CTX and (B) urinary NTX throughout 72 h post dose. Second morning urine void collected. Mean (SE) percentage change from baseline, $n = 5-6$ for all treatment groups and $n = 16$ for placebo. ◆ placebo; ● ONO-5334 3 mg; ▲ ONO-5334 10 mg; ■ ONO-5334 30 mg; ○ ONO-5334 100 mg; △ ONO-5334 300 mg; □ ONO-5334 600 mg

decreasing sample size, effects of natural subject variability were a possibility inherent in the sample size planned for the current study.

Serum and urine CTX and NTX are recognized markers of bone resorption. After collagen is degraded by proteases such as cathepsin K and matrix metalloproteases, these peptides of collagen are released into the bloodstream and metabolized in the kidney and liver. These peptides are then excreted into urine. However, the preference for which of the two markers provides the most useful indicator of ONO-5334 activity is unclear. It appeared that serum CTX concentrations were more sensitive to single doses of ONO-5334 than serum NTX even after natural diurnal variation had been taken into account. Compared with placebo, mean maximum values of suppression were -66% for serum CTX and -57% for NTX.

Placebo data suggest that the diurnal variation in serum NTX is less marked than that of serum CTX. The suppressive effect of ONO-5334, with the diurnal variation taken into account, was more easily detected for serum CTX than for NTX at lower dose levels due to a greater magnitude of decrease. Suppression at doses of 100 mg and above was detectable for serum CTX and ≥ 300 mg for serum NTX. However, at dose levels of 100 mg and above, accurate serum CTX values could not be measured due to the quantification limit of the assay. This occurred much less frequently for serum NTX and, therefore, serum NTX may be more reliable as a marker of bone turnover in evaluating the pharmacodynamics of ONO-5334 (notwithstanding the issues with the 100 mg dose group). At lower dose levels, however, more data points may be required for serum NTX than CTX to detect significant suppression due

to the smaller magnitude of the suppression for serum NTX.

When considering the administration of a once daily dosing schedule, it is important that suppression of bone markers is maintained for 24 h and that suppression is observed in both serum and urine samples. In the current study, suppression of serum CTX appeared to be ongoing at 24 h at ONO-5334 doses of 100 mg and above. Suppression of serum NTX was observed at doses of ONO-5334 300 mg and above, although statistical significance was achieved only at the 600 mg dose. Statistically significant suppression of urine markers was observed at 24 h for both CTX and NTX at doses of ONO-5334 100 mg and above. The CTX marker appeared to show slightly greater sensitivity to ONO-5334 than NTX, thus highlighting the importance of selecting the correct bone turnover marker, especially when making decisions on dose selection.

In a fashion similar to that of serum CTX, urinary CTX appeared to exhibit greater suppression than NTX. It is not known whether such differences in these bone resorption markers are due to the mechanism of action of ONO-5334 or some impact on metabolism. Since the cleavage sites of cathepsin K are located close to the sequence of type I collagen, low molecular weight CTX/NTX may be considered to be a more sensitive marker for cathepsin K inhibitors than total CTX/NTX. Interestingly, Kawada *et al.* measured plasma CTX in rats before and after centrifugation with a filter to remove molecules with a molecular weight of 30 000 or more [16]. The results showed a maximum suppression of about 80% in change from baseline using the filter compared with 40% without the filter. Unfortunately, the authors did not measure serum NTX or

Table 6
Analysis of percentage changes in urine NTX compared with baseline

		ONO-5334 3 mg (n = 6)	10 mg (n = 6)*	30 mg (n = 6)	100 mg (n = 5)†	300 mg (n = 6)	600 mg (n = 6)
Pre-dose (Absolute NTX value) (nm BCE mmol ⁻¹ creatinine)	LSMean	63.5 (48.69, 78.31)	66.67 (42.48, 90.85)	62.67 (38.48, 86.85)	32.2 (5.71, 58.69)	59.17 (34.98, 83.35)	50.5 (26.32, 74.68)
	95% CI for LSMean						
	Difference vs. Placebo	NC	24.34 (-19.06, 67.75)	-25.08 (-68.51, 18.34)	-62.81 (-115.7, -9.97)	-75.23 (-118.6, -31.83)	-112.6 (-156.2, -69.08)
	95% CI for difference	NC					
48 h (% CFB)	LSMean	NC	17.09 (-70.82, 105)	92.26 (10.09, 174.44)	-65.59 (-165.1, 33.97)	-10.31 (-92.43, 71.82)	-85.24 (-167.6, -2.84)
	95% CI for LSMean						
	Difference vs. Placebo	NC	-35.07 (-77.09, 6.95)	38.3 (-3.74, 80.35)	-20.85 (-67.99, 26.28)	-12.95 (-54.97, 29.07)	-56.92 (-99.08, -14.76)
	95% CI for difference	NC					
72 h (% CFB)	LSMean	NC	NC	NC	NC	NC	NC
	95% CI for LSMean						
	Difference vs. Placebo	NC	NC	NC	NC	NC	NC
	95% CI for difference	NC					

n, number of subjects with evaluable data within group. Model = log (pre dose) + Treatment, except for pre dose where Model = Treatment. NC, Not calculated. *one subject had missing value at 48 h. †two subjects had missing values, one at 24 h and one at 48 h.

urinary CTX. Large molecular weight fragments are not filtered through the kidney. It remains to be confirmed whether this means the assays are likely to detect predominantly low molecular weight CTX in urine and thus magnify changes in low molecular weight CTX in urine compared with that in serum.

The immunoassays employed in this study used different antibodies to detect CTX in serum and urine. In serum, the antibody was specific for the β (isomerized) form of CTX only, whereas, in urine the antibody was specific for both the α (non-isomerized) and β forms of CTX. In post menopausal women α -CTX represents a smaller proportion of total urinary CTX than β -CTX [17–19]. Differences in the detection of the α and β forms may provide an explanation for the observed difference in apparent increased suppression of urine CTX compared with serum CTX following administration of ONO-5334, although this is not certain. Differences were observed between serum and urinary NTX even though NTX does not exist as isomers. Therefore, it is prudent to use more than one marker of bone resorption and further evaluation is warranted to identify the most appropriate marker of bone turnover for ONO-5334 and cathepsin K inhibitors.

With regards to the safety and tolerability of ONO-5334, no serious adverse events were reported in the study. TEAEs were reported in all treatment groups and the nature of AEs with active treatment was not clearly different compared with placebo. There was no clear dose-dependent relationship in the timing or duration of AEs. In fact, the lowest dose group (3 mg) reported the greatest number of AEs. Increasing the dose did not result in an increase in the frequency, incidence or intensity of the reported AEs. All AEs were transient and predominantly mild in nature. There were no other clinically significant findings in any other safety assessment. Therefore, ONO-5334 was considered to be well-tolerated after single administration in postmenopausal women.

In summary, ONO-5334 was well tolerated in post menopausal women at doses up to 600 mg per day. ONO-5334 pharmacokinetics were linear up to 300 mg. Food may have delayed the absorption of, but not the exposure to, ONO-5334. At doses of ONO-5334 100 mg and above, decreases were observed in serum CTX, urine CTX and urine NTX for at least 24 h. At the 600 mg dose, suppression of serum NTX was observed for 48 h and suppression of serum CTX, urine CTX and urine NTX continued until 72 h post dose. Further investigation into the pharmacodynamic effect on bone turnover markers and pharmacokinetic effects of repeated dosing would be a justified next step in the development of ONO-5334 for osteoporosis.

Competing Interests

Shinichi Nagase, Yoshitaka Hashimoto, Michiyo Ohyama, Maria Small, Tomohiro Kuwayama and Dr. Steve Deacon are

Table 7

Summary of treatment-emergent adverse events (by relationship and severity/seriousness) and frequently reported TEAEs (two or more subjects in any dose group) after administration of single oral doses of ONO-5334 to healthy post-menopausal women

	Placebo Fasted n = 16	ONO-5334 3 mg n = 6	10 mg n = 6	30 mg n = 6	100 mg n = 6	300 mg n = 6	600 mg n = 6	100 mg Fed n = 6	Placebo n = 6
At least one AE	10	6	2	2	4	4	2	5	1
At least one drug-related AE*	6	4	1	–	1	3	2	3	1
AEs of moderate intensity	3	–	–	–	–	1	–	–	–
AEs of severe intensity	–	–	–	–	–	–	–	–	–
Serious AEs	–	–	–	–	–	–	–	–	–
Gastrointestinal disorders	4	3	2	2	4	4	2	5	1
Hard faeces	0	0	0	0	0	0	0	0	0
Flatulence	0	0	0	0	0	2	0	0	0
Nausea	2	1	0	1	0	1	0	1	0
Vomiting	2	0	0	0	0	0	0	0	0
Nervous system disorders	5	4	1	2	1	1	1	3	0
Headache	4	2	1	2	0	1	0	2	0
Somnolence	2	2	0	0	1	0	1	0	0

n, number of subjects in specified group. *Relationship to study medication according to the investigator: related = probably, possibly or definitely related.

employees of the sponsor, Ono Pharmaceutical Co., Ltd or ONO PHARMA UK LTD.

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