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Increased cortical porosity is associated with daily, not weekly, administration of equivalent doses of teriparatide

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ABSTRACT

Introduction: The pharmacokinetic profile of parathyroid hormone (PTH) determines its effects on bone resorption and formation. When administered intermittently, anabolic effects are favored in comparison with the continuous treatment. Among the intermittent treatment regimens, lower frequency of administration may have a lower effect on bone remodeling. We therefore hypothesized that weekly administration of teriparatide will produce less increase in intracortical remodeling and porosity than reported using daily treatment.

Methods: We treated 17 female New Zealand white rabbits aged 6 months for 1 month with teriparatide [human PTH(1-34)] as follows. (i) Vehicle-treated Control (n = 4); (ii) 20 μg/kg daily (n = 3); (iii) 40 μg/kg daily (n = 3); (iv) 140 μg/kg weekly (n = 3); (v) 280 μg/kg weekly (n = 4). Proximal femurs were imaged *ex vivo* using micro-CT (Scanco Viva CT-40) at 15 μm voxel size. Areas, pore size, and porosity were analyzed on the total, compact cortex (CC), and transitional zones in a 10 mm length region of interest (ROI) starting at the midshaft using StrAx1.0.

Results: Compared to controls, the 20 μg/kg daily was associated with 3.0% higher porosity in the transitional zone (p = 0.09) while the 40 μg/kg daily was associated with a higher porosity in the cortex (8.7%; p = 0.04) and in the transitional zone (5.7%; p = 0.007). The daily regimens were also associated with a greater proportion of porosity due to pores >15 μm^2; particularly in the transitional zone where 20 and 40 μg/kg daily increased porosity 2 fold (p = 0.06) and 5 fold (p = 0.04) relative controls respectively. The 140 and 280 μg/kg weekly were not associated with an increase in porosity. There was no difference in total, compact or transitional zone cross sectional areas between the groups.

Conclusion: Effects of intermittent teriparatide depend on the dose and frequency of administration. Daily dosing, particularly the higher dose, but not weekly dosing, increased cortical porosity. Work is needed to investigate the effects of the regimens on bone formation.

1. Introduction

Bone fragility is the result of bone loss during aging and following menopause [1,2]. Most of the bone loss (~70%) is cortical because 80% of the skeleton is cortical, and most of this bone loss is the result of intracortical and endocortical remodeling which produce cortical porosity and cortical thinning [3,4]. The loss of cortical bone is important because it reduces the stiffness of bone out of proportion to the amount of bone lost [5].

Currently, most drugs used in clinical practice are anti-resorptive agents, in particular the bisphosphonates and denosumab. These treatments slow, but they do not stop bone loss. Nor do these treatments reverse structural deterioration present at the time of treatment [6]. The initial rise in bone mineral density (BMD) is the net result of refilling of cavities excavated before treatment opposed by fewer new resorptive cavities, and increases in matrix mineral density of the bone no-longer resorbed. Anti-resorptives reduce cortical porosity modestly but do not deposit bone upon the periosteal or endocortical surfaces thickening cortices [7].

Anabolic therapies deposit bone by modeling and remodeling based bone formation [8]. The latter may initially be associated with increased porosity as a volume of older more fully mineralized matrix is resorbed producing a cavity which eventually may be replaced by a larger volume

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of newly formed matrix which undergoes primary, then slower secondary, mineralization. During the delay in refilling of the cavity, porosity may increase, particularly in the inner cortical region adjacent to the medullary canal and this porosity may compromise bone strength [9–11].

Teriparatide is a formulation of endogenous PTH containing a 34 amino acid sequence that is identical to the N-terminal portion of the hormone. The pharmacologic activity of teriparatide is similar to the physiologic activity of PTH. Continuous endogenous production of PTH (as in primary hyperparathyroidism) increases bone remodeling and bone loss but intermittent treatment leads to an increase in the number and activity of osteoblasts, increased bone mass and partial reconstruction of microstructure with cortical thickening and improved trabecular thickness [11]. When administered as a daily subcutaneous injection to human or other remodeling animals, increased porosity has been reported in several studies [9–11].

We focused on the effect of treatment on cortical porosity because teriparatide is used for patients of structural deterioration and increased cortical porosity [3]. Daily teriparatide regimens increase bone formation and resorption markers [12,13]. A study in mice reported that teriparatide administration of a total dose of 20 μg/kg/day but given at a lower frequency has a lesser effect on bone remodeling [14]. Therefore we hypothesized that weekly administration of teriparatide may result in lesser intracortical remodeling and less cortical porosity than daily therapy.

2. Methods

We treated 17 female New Zealand white rabbits aged 6 months for 1 month with teriparatide [human PTH(1-34)] (Asahi Kasei Pharma; Japan) as follows. (i) Vehicle-treated control (n = 4); (ii) Once daily 20 μg/kg (n = 3); (iii) Once daily 40 μg/kg (n = 3); (iv) Once weekly 140 μg/kg (n = 3); and (v.) Once weekly 280 μg/kg (n = 4). Both daily and weekly doses were comparable with respect to the way these drugs are used clinically. The experimental protocol was approved by the Experimental Animal Ethics Committee at Ina Research Inc. and conducted in accordance with guidelines concerning the management and handling of experimental animals.

Proximal femurs were imaged *ex vivo* using micro-computed tomography (CT) (Scanco Viva CT-40) at 15 μm voxel size at region of interest (ROI) spanning 10 mm in length ROI and starting at the midshaft (Fig. 1). The images were analyzed using StrAx1.0 (StraxCorp, Melbourne, Australia. StrAx1.0 segments bone from background and then segments cortical from trabecular compartments. The cortex is further segmented into its compact-appearing, outer and inner transitional zones so that the porosity and cortical fragments produced by unbalanced intracortical remodeling are confined to the transitional zone, not erroneously allocated to the medullary compartment, an error which underestimates cortical porosity and overestimates trabecular density [13]. In brief, the segmentation is based on the density profile analysis. The peak of the profile is on the compact-cortex and the lowest part of the profile is on the trabecular compartment. Between each compartment, is a descending limb which is the transitional zone. The part of this descending limb above the inflexion point is the outer transitional zone, and the lower part is the inner transitional zone [15]. Total porosity and porosity distribution were analyzed in each of the cortical compartments.

Haversian canal size range from 7 to 26 μm in diameter in rabbits [16]. Thus, even at the voxel of 15 μm, voxels containing void will also contain mineralized bone matrix. To accurately quantifying porosity due to pores above and below the voxel dimensions requires that the proportions of void and mineralized bone matrix of each voxel are quantified. This is done using an interpolation function such that voxels with attenuation similar to that produced by background are assigned a value of 0% (empty voxels). Voxels with attenuation similar to that produced by fully mineralized bone (density ≥ 1200 mg HA/cm^3) are assigned 100% (full voxels). The void volume of a voxel = 100 − mineralized bone volume fraction. Porosity in the total cortex and each cortical compartment was calculated as the average of the summed void volume fractions of all voxels in the corresponding compartment. We quantified areas of pores >15 μm^2 and assessed their contribution to porosity in the total cortex, compact and transitional zones. The reproducibility errors for segmentation and quantification of porosity expressed as root mean square coefficients of variation ranged from 0.54 to 3.98% [15].

Mean values and standard errors of the means (SEM) were calculated. Comparisons between groups were made using non-parametric test (Mann-Whitney) because of the non-normal distribution of the data. Significance level was p = 0.05 (two-tails).

3. Results

There was no difference in the total cross sectional areas of the total cortex (void plus pores) the controls, daily and weekly regimens groups

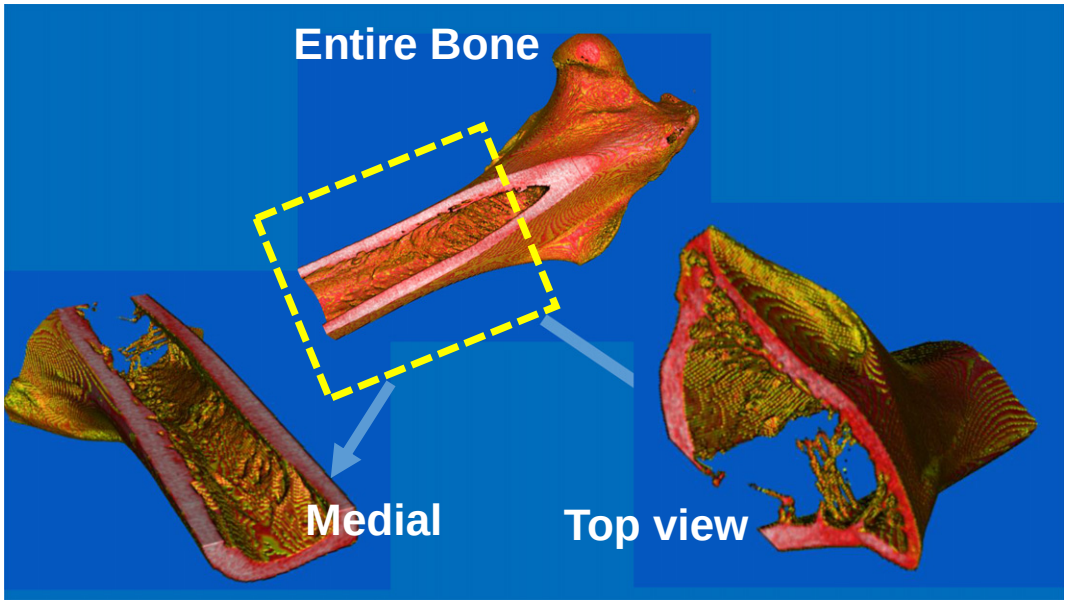

Fig. 1. A 3D view of the proximal femur and the region of interest in which structure was assessed. Medial (bottom left) and top (bottom right) views are also shown.

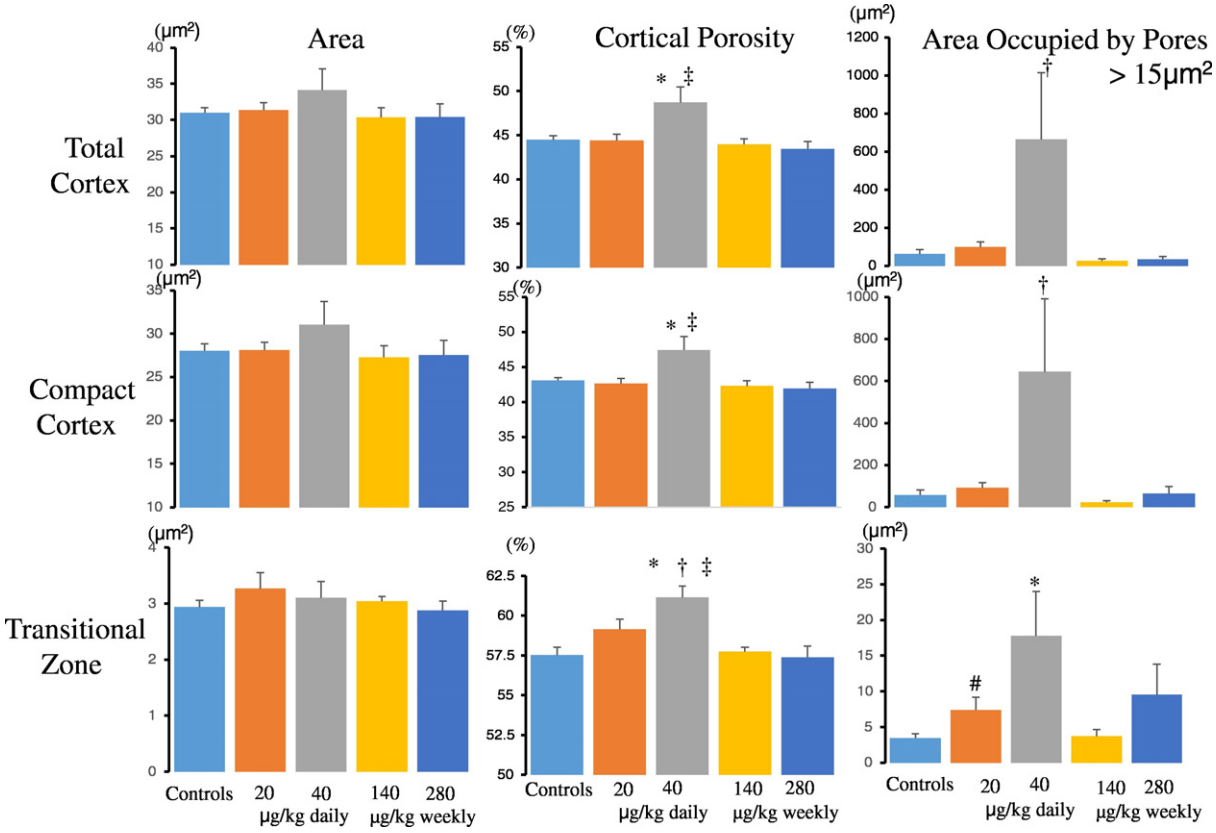

Fig. 2. Upper panels (Total cortex- compact cortex plus transitional zones) There were no group difference in cortical areas. Porosity of the total, was higher with 40 mcg/daily relative controls and 140 mcg/weekly. The area occupied by pores <15 μm^2 was larger in 40 mcg/daily as compared to the W140. Results were similar for the Compact-Cortex (middle panels). Lower Panels There were no group difference in transitional zones areas (outer plus inner). Porosity of the total, was higher with 40 mcg/weekly relative controls and weekly groups. The area occupied by pores <15 μm^2 was larger with daily regimens as compared to controls. This was statistical significant with 40 mcg/daily. Comparing treatment regimens and controls: * $p < 0.05$ and # $p > 0.05$ and <0.1. Comparing rabbits on different treatment regimens: † $p < 0.05$ comparing 40 μg daily to 140 μg weekly and ‡ $p < 0.05$ comparing 40 μg daily to 280 μg weekly.

(Fig. 2, upper left panel). However, total porosity was increased (8.7%; $p = 0.04$) in rabbits treated with 40 μg/daily as compared to controls.

The regimen of 40 μg/daily also resulted in higher cortical porosity as compared to other treatment regimens although this was only statistically significant when compared to the 280 μg/daily. The increment in porosity was 8.8% ($p = 0.09$) when compared to the 20 μg/daily; 9.7% ($p = 0.07$) and 10.7% ($p = 0.03$) when compared to the 140 μg/weekly and 280 μg/weekly respectively (Fig. 2, upper middle panel).

The 40 μg/kg daily dose increased the area occupied by pores with area >15 μm^2 ($p = 0.09$) by 6 fold more than control and by 6 fold >20 μg/kg daily. Weekly regimens did not increase the area occupied by pores with area >15 μm^2 compared with controls (Fig. 2, Upper right panel). Results for the compact appearing cortex were similar (Fig. 2, middle panels).

The transitional zone cross sectional areas did not differ between the vehicle, daily, and weekly groups (Fig. 2, lower left panel). Cortical porosity increased by 3.0% using 20 μg/kg daily ($p = 0.09$), by 5.7% using 40 μg/kg daily ($p = 0.007$) compared to control. The increase in porosity using weekly regimens was numerically less than the daily regimens. Total porosity using 40 μg/kg daily was 5.3% higher than found using 140 μg/kg weekly ($p = 0.01$) and 5.9% higher than using the 280 μg/kg weekly ($p = 0.02$). (Fig. 2, lower, middle panel).

The 20 and 40 μg/kg daily regimens were associated with respectively a two fold ($p = 0.06$) and five folds ($p = 0.04$) larger area occupied by pores of >15 μm^2 relative to control. This was not observed using the weekly regimens. There was about 5 fold more area occupied by pores >15 μm^2 using 40 μg/kg daily than using 140 μg/kg weekly ($p = 0.08$) (Fig. 2, lower right panel). Overall differences in porosity between treatment regimens are illustrated in Fig. 3.

4. Discussion

We report that the effects of teriparatide on cortical bone depend on the dose, frequency of administration, and the cortical compartment. Weekly regimens did not increase porosity. Daily regimens increased porosity, especially in the transitional zone and more so with the 40 μg/kg dose. The higher porosity was more the result of an increase in pores >15 μm^2 in the transitional zone. The daily dose of 40 μg/kg was associated a larger area occupied by pores >15 μm^2 in rabbits treated with weekly 140 μg/kg. There was no difference in areas of the total cortex, compact cortex, or transitional zone between treated and control groups or between treated rabbits.

Many investigators report an increase in cortical porosity with daily regimen of teriparatide. Sato et al. reported that OVX monkeys treated for 18 months with 1 or 5 μg/kg/day teriparatide [PTH (1-34)] had a dose-dependent increase in cortical porosity at the proximal femur [11]. However, bone matrix volume fraction increased so there was no net negative effect on mechanical properties.

We report an increase in cortical porosity with daily regimens of teriparatide, more so with daily 40 μg/kg than daily 20 μg/kg doses. The increase in porosity was observed within the compact cortex and the transitional zone which is the inner portion of the cortex adjacent to the marrow cavity. The higher porosity was due to voxels that contain mostly void volume (50% or more) rather than the 40 to 80% of voxels containing mostly matrix mineral. This suggests that teriparatide produces larger pores by enlarging existing canals which then coalesce.

No increase in porosity was observed with weekly regimens. The clinical implications of these findings are unclear given this is a brief study of a small sample of rabbits but the findings warrant further

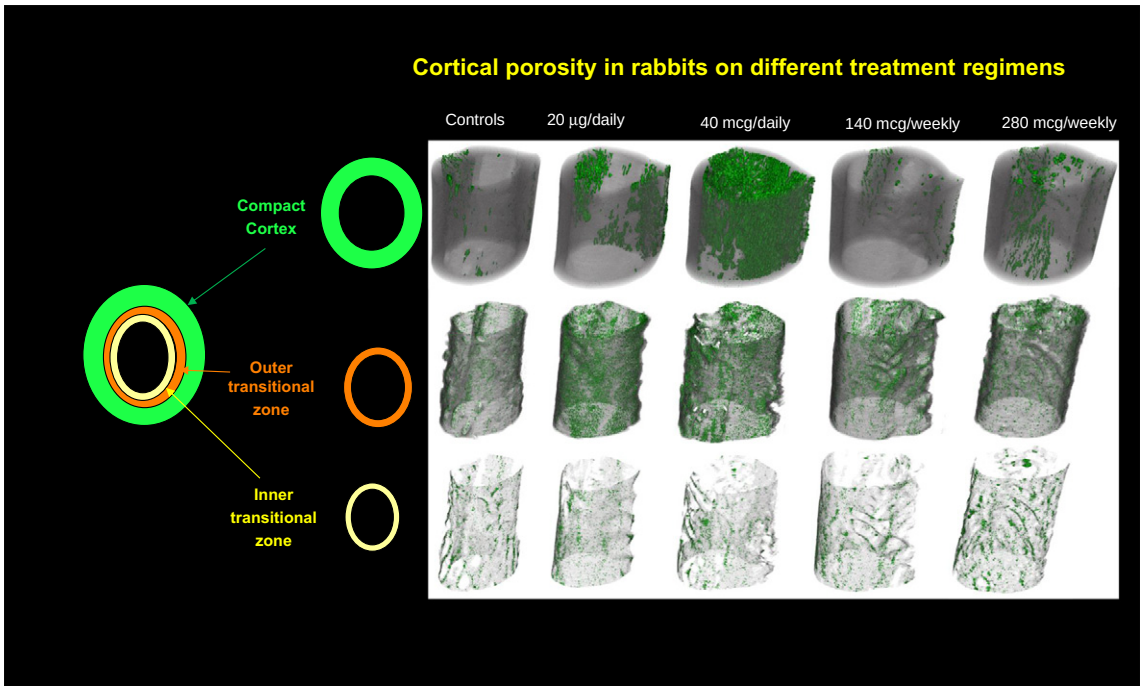

Fig. 3. Effects of treatment on cortical porosity. The cortex is transparent grey and pores are color-coded green. The transitional zone is divided into an outer (adjacent to the compact-cortex) and inner (adjacent to the marrow cavity) zones for clarity.

investigation to evaluate the effects on mechanical properties of the daily and weekly regimens. Both daily and weekly teriparatide reduce fracture risk in studies in human subjects [17,18]. Thus, we are not claiming superiority of one regimen over the other in terms of fracture risk reduction, this would require large comparator trials.

This study has several limitations. The small number of rabbits may have lessened our ability to detect some effects. The study was too brief and the sample size too small to evaluate an anabolic effect of daily or weekly regimens. It remains plausible that a regimen that produces less resorption and cortical porosity might also produce a lesser anabolic effect. The differing effect of both treatment regimens is supported by the study by Yamamoto et al. [14]. Assessment of microstructure was done using imaging only. Pore size was not measured. At the voxel size of 15 µm, the spatial resolution was ≤25 µm, and thus not allowing satisfactory resolution of most pores in rabbits which are below 30 µm [11].

In summary, the effects of intermittent teriparatide are dependent on the mode of administration and are region-specific. Daily regimens increased porosity in the transitional zone, and the larger dose did so throughout the cortex. Weekly regimens did not increase porosity. Whether the porosity of the transitional zone is deleterious to strength requires further study. Clinical studies are needed in patients with osteoporosis to determine whether weekly administration offers advantages over daily administration.

Conflict of interest statements

R Takao-Kawabata, **K Hirano**, **H Yamane**, **A Takakura**, **Y Isogai**, and **T Ishizuya** are employees of Asahi Kasei Pharma Corporation.

RM Zebaze has received grant and/or research support from Amgen, Merck Sharp & Dohme, Servier, Warner-Chilcott, Asahi Kasei Pharma, Genzyme, Sanofi, GSK, and Pfizer. He is a shareholder in StraxCorp, is remunerated by Strax Corp as president of R&D, and is one of the inventors of the StrAx1.0 algorithm. RM Zebaze is also a director on the board of StraxCorp.

E Seeman has received research support and/or speaker fees from Amgen, Asahi Kasei Pharma, Genzyme, and Warner-Chilcott, Merck Sharp & Dohme, and Allergan. He is a director of the board and shareholder in StraxCorp, is remunerated by StraxCorp as chief medical officer, and is one of the inventors of the StrAx1.0 algorithm.

Y Peng is a shareholder and an employee of StraxCorp Pty Ltd.

A Ghasem-Zadeh is one of the inventors of the StrAx1.0 algorithm and receives consulting fees from StraxCorp.

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