Involvement of cellular senescence in bone loss after discontinuation of PTH administration

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INTRODUCTION: Intermittent administration of PTH1-34(PTH) plays a central role as anabolic agent for the treatment of osteoporosis. However, PTH is known to cause rapid bone resorption if untreated after treatment is finished. The mechanism of this rapid bone resorption remains poorly understood. Recently, accumulation of senescent cells has been reported to play a role in age-related osteoporosis. We hypothesized that PTH-induced accelerated metabolic turnover leads to the accumulation of senescent cells and is one of the causes of this rapid bone resorption. In this study, We investigated the association between PTH administration and senescent cells accumulation.

METHODS: Old mice (18 months of age in C57BL/6 mice) were treated with PTH for 4 weeks (5 times/week, 10 times/week, 20 times/week, 200 μg/kg/week). Then, senolytic drugs (dasatinib 5 mg/kg/week + quercetin 50 mg/kg/week) were administered for 8 weeks in parallel with PTH administration for 4 weeks (10 times/week, 200 μg/kg/week). PTH administration more than 10 times a week was defined as high frequency administration. To determine the effect of week-old age on senescent cell accumulation, the same treatment intervention was applied to young mice (3 months of age). Bone mass changes were measured by micro-CT, and each group was evaluated by qPCR and immunohistochemistry for senescent cell characteristics and senescence-associated secretory phenotype (SASP). In order to define the association between PTH treatment and the mTOR pathway in osteoblasts, Western blottinh was performed in Vitro. The ANOVA procedure of significance were used in the statistical analyses. Less than 0.05 P values were considered significant.

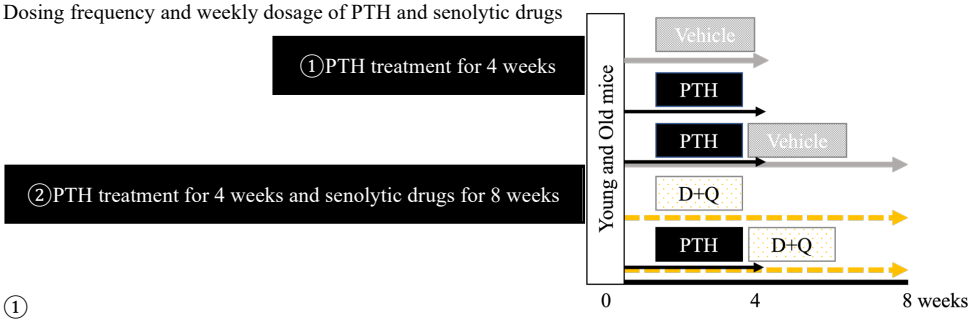
RESULTS SECTION: In the Old group, PTH administration at high frequency resulted in thinning of cortical bone and bone trabeculae and an increase in bone volume (BV/TV). qPCR analysis showed the increased expression of p16 and SASP (IL6, MMP13) and immunostaining showed the increased p16 expression in osteoblasts. In addition, most of the increased bone volume was resorbed at 4 weeks after PTH withdrawal, but the resorption was mitigated in the group receiving the senolytic drugs. This bone resorption mitigation effect by the senolytic drugs was not observed in the young group (N=5 per group).

DISCUSSION: Different pathologies of bone resorption after PTH treatment between the young and old mice was observed. That is, the resorption in the old group was associated with senescent cell accumulation, which was shown by the maintenance of bone mass with senolytic drugs administration. In our study, PTH stimulation induced S6K, 4EBP1 phosphorylation was enhanced, suggesting an effect on osteoblasts via the mTOR pathway. Our results suggest that intervention on senescent cells may be able to inhibit bone resorption after PTH quiescence.

SIGNIFICANCE/CLINICAL RELEVANCE: Accumulation of senescent cells was observed when PTH was administered at high frequency to Old mice. Bone resorption after discontinuation of PTH treatment was mitigated by co-administration of the senolytic drugs.

IMAGES AND TABLES:

Dosing frequency and weekly dosage of PTH and senolytic drugs



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Groups	Treatment	Dosage in 1 Injection (μg/kg per Dose)	Dosing (times a week)	Total Weekly Dose (μg/kg per week)
Control	Vehicle	0	5	0
40 μg/kg PTH per day	PTH PTH PTH	40 20 10	5 10 20	200 200 200

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Groups	Medication	Dosage (mg/kg/day)	Dosing
PTH 200 μg/kg/week (10 times a week)	Vehicle		once a week
PTH 200 μg/kg/week (10 times a week)	Dasatinib Quercetin	5 50	once a week
Vehicle (10 times a week)	Dasatinib Quercetin	5 50	once a week

Micro-CT images or mice lumbar vertebrae