

Effect of intermittent injection of recombinant human parathyroid hormone on bone histomorphometry of ovariectomized rats¹

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ABSTRACT

AIM: To observe the effect of intermittent parathyroid hormone (PTH) administration on bone histomorphology of relatively old ovariectomized rats. **METHODS:** The 6-month-old female SD rats were randomly divided into 5 groups: (1) sham-operated for baseline (ShamB, $n = 5$), (2) ovariectomized for baseline (OVXB, $n = 6$), (3) Sham-operated for end point (ShamE, $n = 6$), (4) ovariectomized for end point (OVXE, $n = 6$), (5) ovariectomized for PTH treatment (OVXEP, $n = 6$). ShamB and OVXB rats were sacrificed 3 months after operation, ShamE, OVXE and OVXEP rats were sacrificed 4.5 months after operation. During 3-4.5 months after operation, OVXEP rats received daily subcutaneous injection of rhPTH₁₋₈₄, while ShamE and OVXE received vehicle injection. The proximal tibiae of all rats were processed without decalcification for quantitative bone histomorphometry. **RESULTS:** The percent trabecular area (TbAr) of OVXEP was significantly greater than that of OVXE ($P < 0.05$), and was similar to that of OVXB ($P > 0.05$), but was smaller than that of ShamE ($P < 0.05$); the trabecular thickness (TbTh) of OVXEP was thicker than any other group (all, $P < 0.05$); the trabecular number (TbN) of OVXEP was only slightly higher than that of OVXE; the percent labeled perimeter (LPm), mineral apposition rate

(MAR) and bone formation rate with bone area as referent (BFR/BV) of OVXEP were all higher than those of ShamE and OVXE respectively ($P < 0.01$), whereas the osteoclast number (N of Oc) of OVXEP was similar to those of ShamE and OVXE ($P > 0.05$). **CONCLUSION:** Short-term intermittent injection of rhPTH₁₋₈₄ can prevent further bone loss in 9-month-old rats 3 months after ovariectomy, the mechanisms of this therapy are that PTH could increase TbTh while not alter TbN, and promote bone-forming activity while not influence bone-resorptive activity.

INTRODUCTION

Patients with osteoporosis often have such manifestations as bone pain, shortened body height, bone deformity, and even pathologic fracture, usually with bone mass lost greatly at this stage. The currently used anti-osteoporotic agents such as estrogen, bisphosphonate, and calcitonin are inhibitors of bone resorption and can not promote bone formation, therefore, their therapeutic efficacies are limited.

Parathyroid hormone (PTH) has traditionally been considered to be a typical bone-resorbing hormone, but after many years' animal and clinical studies, it is now known to be the most promising anabolic agent of bone as it is administered intermittently^[1]. However, most of previous animal studies were carried out on 3-month old or even younger rats^[2-6], while the older rats which have lower bone turnover and less active osteoblasts were seldom used. The clinical situation is that the patients are usually diagnosed and given treatment long after menopause (> 10 years), to mimic this condition, we examined the effect of PTH on bone histomorphometric parameters in relatively older ovariectomized rats.

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MATERIALS AND METHODS

Chemicals The recombinant human parathyroid hormone₁₋₈₄ (rhPTH₁₋₈₄) was produced by Nanjing Military Medical Institute, and was documented in cultured cells to have the same biological activity as synthetic hPTH₁₋₃₄ (Sigma)⁽⁷⁾. rhPTH₁₋₈₄ was dissolved (1 g/L) in phosphate buffered solution (PBS) containing 0.001 % acetic acid and stored at -70 °C. Calcein was dissolved in 2 % NaHCO₃ (10 g/L) and stored at 4 °C in darkness. They were all sterilized by filtration before use.

Animals and research protocol Virgin female Sprague-Dawley rats of 5 months old were purchased from Jiangsu Provincial Center of Experimental Animals (Grade II, Certificate No 97001) and were acclimatized to new environment for 1 month. Rats were housed in 69 cm × 30 cm × 30 cm cages and had *ad libitum* access to water and commercial standard food. The 6-month-old rats were randomly divided into 5 groups: (1) sham-operated for baseline (ShamB, *n* = 5), (2) ovariectomized for baseline (OVXB, *n* = 6), (3) Sham-operated for end point (ShamE, *n* = 6), (4) ovariectomized for end point (OVXE, *n* = 6), (5) ovariectomized for PTH treatment (OVXEP, *n* = 6). ShamB and OVXB rats were sacrificed at 3 months after operation, and ShamE, OVXE and OVXEP were sacrificed at 4.5 months after operation. During 3-4.5 months after operation, OVXEP rats received subcutaneous injection of rhPTH₁₋₈₄ (200 μg/kg), once a day, 6 times a week, while ShamE and OVXE rats were injected with vehicle. Calcein (10 mg/kg) were injected intraperitoneally into each animal 6 and 2 d prior to sacrifice to observe new bone formation.

Bone histomorphometric analysis Excised right hindlimb tibiae were dehydrated, embedded in methylmethacrylate without decalcification and sectioned longitudinally by a microtome (Leica 2155). Sections were stained with Masson-Goldner Trichrome method. Measurements were made with a digitizing system consisting of light and epifluorescent microscope which is connected to a computer with a morphometry program "Osteomeasure Version 2.312" (Osteomeasure Inc). Measuring area in the metaphyseal trabecular bone was between 1.0 and 4.0 mm from the lowest point of the growth plate-metaphyseal junction in the caudal direction. Total tissue area, trabecular area, and trabecular

perimeter as static parameters were measured to calculate the percent trabecular area (TbAr), trabecular thickness (TbTh), trabecular number (TbN), and trabecular separation (TbSp). Single labeled perimeter, double labeled perimeter, interlabeled width, and the number of osteoclast attaching to bone surface as dynamic parameters were measured to calculate the percent labeled perimeter (LPm), mineral apposition rate (MAR), bone formation rate with bone area as referent (BFR/BV), and osteoclast number (N of Oc).

Statistical analysis Data were presented as $\bar{x} \pm s$. For comparisons of multiple groups, one-way analysis of variance (ANOVA) was used for normal distribution data; positive skewness data were logarithmically transformed to normal distribution and further analysed by ANOVA; negative skewness data were analyzed first by Kruskal-Wallis rank sum test and further by Conover's *t* test.

RESULTS

The static bone histomorphometric parameters The TbAr of OVXB was smaller than that of ShamB (*P* < 0.05); that of OVXEP was obviously greater than that of OVXE (*P* < 0.05), and was similar to that of OVXB (*P* > 0.05), but was smaller than that of ShamE (*P* < 0.05). The TbTh of OVXEP was thicker than any other group (*P* < 0.05), those among other 4 groups except for OVXEP were not different from each other. The TbN of OVXB was decreased compared with that of ShamB (*P* < 0.01); that of OVXEP was modestly increased compared with OVXE (0.05 < *P* < 0.1), and obviously lower than that of ShamE, ShamB, and OVXB, respectively (*P* < 0.01). The TbSp of OVXEP was not different from that of OVXE and OVXB respectively (*P* > 0.05), and obviously greater than those of ShamE (*P* < 0.05) and ShamB (*P* < 0.01, Tab 1).

The dynamic bone histomorphometric parameters The LPm of OVXB was greater than that of ShamB (*P* < 0.05); that of OVXEP was similar to that of OVXB (*P* > 0.05), and was greater than any other 3 groups except for OVXB (*P* < 0.01). The MAR of OVXB was higher than that of ShamB (*P* < 0.05), ShamE (*P* < 0.01), and OVXE (*P* < 0.01), respectively; that of OVXEP was similar to that of OVXB (*P* > 0.05), and also higher than that of ShamB (*P* <

Tab 1. The static bone histomorphometric parameters in various groups. $n = 5$ for ShamB group. $n = 6$ for OVXB, Sham E, OVXE, and OVXEP group. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ vs OVXEP. $^dP < 0.05$, $^eP < 0.01$ vs OVXB.

Group	TbAr/%	TbTh/ μm	TbN/ mm^{-1}	TbSp/ μm
ShamB	26 \pm 7 ^{ef}	64 \pm 12 ^b	4.0 \pm 0.4 ^c	191 \pm 38 ^e
OVXB	12 \pm 7	67 \pm 7 ^b	1.8 \pm 0.8 ^c	590 \pm 439
ShamE	18 \pm 6 ^b	74 \pm 9 ^b	2.4 \pm 0.7 ^c	379 \pm 136 ^b
OVXE	4.2 \pm 1.8 ^{bc}	70 \pm 9 ^b	0.59 \pm 0.24 ^f	1871 \pm 759
OVXEP	10 \pm 3	91 \pm 13	0.8 \pm 0.6 ^f	1466 \pm 835

0.05), ShamE ($P < 0.01$) and OVXE ($P < 0.01$), respectively. The BFR/BV of OVXB was modestly higher than that of ShamB ($0.05 < P < 0.1$), and higher than that of ShamE ($P < 0.05$); that of OVXEP was similar to that of OVXB ($P > 0.05$), and was higher than that of OVXE, ShamE, and ShamB, respectively (all, $P < 0.01$). N of Oc: The N of Oc in OVXB was greater than any other group, that of OVXEP was not different from that of OVXE, ShamE, and ShamB, respectively ($P > 0.05$, Tab 2).

Tab 2. The dynamic bone histomorphometric parameters in various groups. $n = 5$ for ShamB group. $n = 6$ for OVXB, Sham E, OVXE, and OVXEP group. $\bar{x} \pm s$. $^cP < 0.01$ vs OVXEP. $^dP < 0.05$, $^eP < 0.01$ vs OVXB.

Groups	LPm (%)	MAR ($\mu\text{m} \cdot \text{d}^{-1}$)	BFR/BV ($\% \cdot \text{year}^{-1}$)	N of Oc (mm^{-1})
ShamB	9.1 \pm 2.2 ^{ce}	0.88 \pm 0.21 ^{ce}	94 \pm 17 ^c	0.26 \pm 0.04 ^f
OVXB	14 \pm 3	1.10 \pm 0.12	130 \pm 38	0.46 \pm 0.11
ShamE	10.5 \pm 2.9 ^c	0.78 \pm 0.18 ^{cd}	86 \pm 15 ^{ce}	0.26 \pm 0.12 ^f
OVXE	12 \pm 5 ^c	0.82 \pm 0.16 ^{cd}	108 \pm 15 ^c	0.31 \pm 0.08 ^c
OVXEP	18 \pm 4	1.18 \pm 0.18	164 \pm 47	0.22 \pm 0.06 ^f

Body weight and general condition The changes of body weight before and after PTH/vehicle injection period in ShamE, OVXE, and OVXEP were not different from each other (Conover's t test, $P > 0.05$). The physical activities in all these three groups were similar (Tab 3).

DISCUSSION

We had observed the TbAr of OVXB reflecting the trabecular bone volume was greatly reduced compared with ShamB, and this result certified our success in

Tab 3. The changes of rat body weight during PTH/vehicle injection period. $n = 5$ for ShamB group. $n = 6$ for OVXB, Sham E, OVXE, and OVXEP group. $\bar{x} \pm s$.

Group	BW/g
ShamE	-8 \pm 18 (8.0)
OVXE	6 \pm 11 (10.5)
OVXEP	3 \pm 12 (10.0)

Note: The number in parenthesis is the mean rank.

making osteopenic rat model. In fact, the osteopenia induced by ovariectomy can be testified only 6 weeks after operation⁽⁸⁾. The bone loss was mainly due to the reduction of TbN while the TbTh was changed little, this is consistent with the previous report⁽⁹⁾. The LPm and MAR which reflected bone-forming activity, and N of Oc which reflected bone-resorptive activity of OVXB were respectively greater than those of ShamB, which suggested that the ovariectomy in rat could cause higher bone turnover, just as the situation in postmenopausal women.

The TbAr of proximal tibia of OVXEP was similar to that of OVXB, and was 151 % higher than that of OVXE. The result implied that a short treatment (1.5 months) with PTH could prevent further bone loss caused by ovariectomy. The histologic basis of the effectiveness of this therapy was increased TbTh while the TbN was only slightly increased, which was similar to Meng's report⁽⁸⁾. Although the TbTh of OVXEP was increased by PTH injection, the increase was too small compared with already existed great TbSp to remarkably increase the TbSp.

The LPm, MAR, and BFR/BV of OVXEP were higher than any other group except for OVXB, while the N of Oc of OVXEP was not different from any other group except for OVXB. These results suggested that intermittent administration of PTH could promote bone-forming activity while not influence bone-resorptive activity, which was the key difference of the mechanisms between PTH as an anabolic agent and inhibitors of bone resorption such as estrogen, bisphosphonate, and calcitonin. Upon the results described above, we might infer that more bone volume could have been achieved if we had treated the OVXEP rats for longer time.

In summary, intermittent PTH injection lasting for 1.5 months can maintain the trabecular area in tibial metaphysis of 9-month-old rats 3 months after ovariectomy, the mechanisms of this therapy was

supposed to increase the TbTh without altering TbN and to promote bone-forming activity without influencing bone-resorptive activity.

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间歇性注射重组甲状旁腺激素对去卵巢大鼠骨组织形态计量学的影响¹

Rfb A

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关键词 甲状旁腺激素类; Sprague-Dawley 大鼠; 骨质疏松; 骨骼; 组织学

目的: 观察甲状旁腺激素(PTH)间歇注射对月龄较老的去卵巢大鼠骨组织形态计量学的影响. **方法:** 6月龄雌性SD大鼠被随机分成5组:(1)假手术基础组(ShamB), (2)去卵巢基础组(OVXB), (3)假手术终点组(ShamE), (4)去卵巢终点组(OVXE), (5)去卵巢后PTH治疗组(OVXEP). ShamB和OVXB组大鼠于术后3个月、ShamE及OVXE和OVXEP组大鼠在术后4.5个月时处死并取骨. 在术后3-4.5个月时给OVXEP组大鼠每日皮下注射1次(6次/周)rhPTH₁₋₈₄, 给ShamE和OVXE组大鼠则注射rhPTH₁₋₈₄溶剂. 对大鼠近端胫骨进行不脱钙切片以观察其骨组织形态计量学指标的变化. **结果:** OVXEP组骨小梁面积百分比(TbAr)明显高于OVXE组($P < 0.05$), 与OVXB组相似($P > 0.05$), 但仍低于ShamE组($P < 0.05$); OVXEP组的骨小梁厚度(TbTh)高于任何其他各组($P < 0.05$), 其骨小梁数量(TbN)仅比OVXE组略高, 其标记周长百分比(LPm)、矿化沉积率(MAR)和骨形成率(BFR/BV)均分别高于OVXE和ShamE组($P < 0.01$), 而其破骨细胞数量(N of Oc)与后二组相似($P > 0.05$). **结论:** 去卵巢后3个月的9月龄大鼠短期间歇注射rhPTH₁₋₈₄能阻止其骨量进一步减少, 其作用机制是间歇注射PTH能增加TbTh而不明显改变TbN, 能促进成骨活性而不影响破骨活性.

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