Parathyroid Hormone Enhances Osteoblast Differentiation: An in Vitro Study

Chung Shih

Institute of Biology and Anatomy, National Defense Medical Center Division of Oral and Maxillofacial Surgery, Department of Dentistry, Tri-Sevrice General Hospital

Abstract

With aging, the decrease in bone mass (or bone mineral density) could cause osteoporosis or increase the occurrence of fracture. It ultimately involves all the soft tissues in the injured area as well as the bone, resulting in loss of function or even death. Current evidences showed that there is an increase in bone mass in both osteoporotic patients and animals treated with low dose of parathyroid hormone (PTH). In addition, PTH could increase cortical bone volume and prevent postovariectomized osteopenia. However, the precise effect of PTH on bone cell differentiation is still unclear. The purpose of this study was to investigate the precise effect of low dose PTH on osteoblast differentiation. Cell culture system, 3H-thymidine incorporation assay, alkaline phosphatase activity assay, type I collagen synthesis assay, static and dynamic bone histomorphometry and light microscopy were performed on MC3T3-E1 cells. Low dose of hPTH (human PTH) significantly increased osteoprogenitor cell proliferation (p<0.01 and 0.005) and osteoblastic alkaline phosphatase activity (p<0.05 and 0.01) while no evidence of significant effect on collagen synthesis. In conclusion, low dose of PTH effectively enhances osteoblast differentiation.

Key words: Bone mineral density, Osteoblast, Osteoporosis, Parathyroid hormone.

Introduction

Bone mass increases during the early years of life, adolescence, and early adulthood. There is evidence that trabecular bone loss precedes cortical loss and undergoes a greater acceleration during the menopause leading to the possibility of occurrence of osteoporosis(1-4). Osteoporosis is a common bone disorder defined as a decrease in bone mass and a deterioration of the bone tissue architecture which increases fracture risk in elderly(5-7). In the United States, approximately one-half of the women and one-third of the men will develop osteoporotic fractures over the course of their lifetimes. Between 10% and 15% of hip fractures, victims die in the first year after the fracture, and 50% of the survivors are unable to return to independent
living\(^8,9\). Therefore, investigation on keeping bone tissue and cells functionally and prevention of osteoporosis is important in preventive medicine today. However, clinically, osteoporosis could only be treated with calcitonin or estrogen to inhibit osteoclastic bone resorption\(^{10,11}\). Parathyroid hormone (PTH) is synthesized and secreted by chief cells in parathyroid gland, and able to increase bone resorption\(^{12}\). Reeve \textit{et al} reported that PTH could increase trabecular bone mass in postmenopausal osteoporotic patients\(^{13}\). Recent evidences also demonstrated that PTH could augument trabecular bone volume and prevent postovariectomized osteopenia\(^{14-18}\). However, the precise effect and mechanism of PTH on stimulation of bone cell differentiation are still not clear. The purpose of this study was to investigate the precise effect and mechanism of human PTH (hPTH) on osteoblast differentiation using in vitro MC3T3-E\(_1\) cell culture system.

**Materials and Methods**

**Cell Culture**

MC3T3-E\(_1\) cells were plated into 35 mm Petri dishes in DMEM\(^{19}\) (GIBCO BRL, Grand Island, NY, USA) containing 10% fetal calf serum, 0.1\%(v/v) of a 29.2 mg/ml solution of L-lutamine, 0.1\%(v/v) of a 10000 units/ml solution of penicillin-streptomycin and 0.1\%(v/v) of a 250 \(\mu\)g/ml solution of Fungizone (GIBCO BRL, Grand Island, NY, USA). Three days after plating, various concentration (0, 0.1 or 1 nM) of human parathyroid hormone (hPTH) was daily added.

**\(^3\)H-thymidine Incorporation Assay\(^{20,21}\)**

Cells were pulse-labeled with \(^3\)H-thymidine (0.5 \(\mu\)Ci/ml, Amersham Teading, Ltd., UK) for 16 hours. After incubation, the medium was removed, the monolayers were rinsed twice with PBS and the cell layer was collected.

Then, 6\% of cold trichoracetic acid (TCA) was added and the samples were kept at 4\(^\circ\)C for 30 mins. After centrifugation, the TCA-insoluble precipitate was dissolved in 0.1 N NaOH and the solubilized radioactive material was collected in polypropyl vials. Aliquots of 4 ml were counted in a liquid scintillation counter (\(\beta\)-counter). The data were expressed as cpm/\(\mu\)g DNA.

**Alkaline Phosphatase Activity Assay\(^{20,21}\)**

Bone cell/tissue in Petri dish was washed with phosphate-buffered saline (PBS), pH 7.4, and then incubated for 20 mins at 37\(^\circ\)C in 0.05\% trypsin (Sigma, St. Louis, MO, USA) and 400 units/ml collagenase (Sigma, USA). Cells were harvested by centrifugation and washed with DMEM. Cells were resuspended in 1 ml of PBS and disrupted sonication. The homogenate was added to substrate (10 mM \(\rho\)-nitrophenyl phosphate) and reacted for 3 minutes at 25\(^\circ\)C. The absorbance of the yellow product (4-nitrophenol) at 405 nm was quantitated using Varian spectrophotometer. The amount of ALP activity present was determined by reference to a standard curve generated using various concentrations of a stock preparation of ALP (Qualitrol precision, Merck, Germany).

Protein concentration was determined by reference to a standard curve generated using various concentrations of bovine serum albumin (0.001–0.1 mg/ml, Sigma, USA). ALP activity was expressed as unit/mg protein.

**Type I Collagen Synthesis Assay\(^{20,21}\)**

After treatment with hPTH (0, 0.1 or 1 nM), cells were labeled with 5 \(\mu\)Ci/ml of L\([5-\text{H}\)] proline in DMEM medium for 24 hours. After
labeling, cell layers were rinsed with 1 ml ice-cold PBS containing proteinase inhibitors and the washing was pooled with the medium. Cell layers were harvested with a rubber policeman, washing, homogenized and dialyzed against 1 mM sodium sulfate for 3 days. The labeled proline incorporated into collagenase-digestible protein was measured and expressed as %.

**Statistical Analysis**
All the data obtained in this study were presented as mean ± S.D.. One way analysis of variance (ANOVA) followed by Dunn’s test for multiple comparison was used for statistical analysis of the data.

**Results**

**Effects of human parathyroid hormone (hPTH) on bone colony formation**

When MC3T3-E1 cells were plated in Petri dishes and left settled for 2 days, the viable cells adhered to the bottom of the dishes while the nonviable cells remained in suspension and were completely removed at the first feeding. MC3T3-E1 cells were cultured in DMEM with or without low dose of hPTH for further studies.

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Number of Petri dishes</th>
<th>(^3)H-thymidine incorporation (10 cpm/µg DNA)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>1.2013±0.1011</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>2.5197±0.1253*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>2.8013±0.1081**</td>
<td></td>
</tr>
</tbody>
</table>

* p<0.01, ** p<0.005 significantly different from the control.
ANOVA followed by Dunn’s test.

**Effect of hPTH on osteoprogenitor cell proliferation**

The effect of various concentrations (0, 0.1 or 1 nM) of hPTH on osteoblast proliferation was investigated 16 hours after labeled with \(^3\)H-thymidine. With hPTH, osteoprogenitors cell proliferation (cpm/µg DNA) was significantly increased (0.1 nM, 2.5197 ± 0.1253; 1 nM, 2.8013 ± 0.1081) as compared to the control (1.2013±0.1011) (table 1).

**Effect of hPTH on osteoblastic alkaline phosphatase activity**

After treatment with different concentrations of hPTH (0, 0.1 or 1 nM), alkaline phosphatase activity in MC3T3-E1 cells (osteoblasts) was analyzed and forward significantly increased as compared to the control (p<0.05, p<0.01, table 2).

**Effect of hPTH on type I collagen synthesis in osteoblast**

In bone tissue, collagen synthesis is a biochemical marker associated with differentiated osteoblasts. In this study, there were no significant differences in type I collagen synthesis between the control and hPTH-added groups (table 3).
Human parathyroid hormone (hPTH) is a linear polypeptide with a molecular weight of 9500 that contains 84 amino acid residues. It is synthesized and secreted by chief cells in parathyroid gland. PTH acts directly on bone to increase bone resorption and mobilize calcium. In addition to increasing the plasma calcium and depressing the plasma phosphate, PTH increases phosphate excretion in the urine. PTH also increases reabsorption of calcium in the distal tubules of the kidney, formation of 1,25 (OH) 2 vitamin D₃, and calcium absorption from the intestine. PTH is an active bone resorber by effectively increasing osteoclast formation and activity. In fact, early in 1976, Reeve demonstrated that low dose of PTH could increase trabecular bone mass in postmenopausal osteoporotic patients. Recent evidences showed that PTH was able to prevent postovariectomy osteopenia. An understanding of bone cell proliferation and differentiation would be greatly facilitated by an immortalized cell culture system that permits independent investigation of proliferation and differentiation of osteoprogenitors cells and differentiated osteoblast function. The MC3T3-E1 cells, derived from newborn murine calvariae, display a time dependent and sequential expression of osteoprogenitors cell and osteoblast characteristics analogous to in vivo bone formation, bone cell proliferation and differentiation, and have been widely used in our laboratory since 1996. In this study, we investigated the precise effect of hPTH on osteoblast differentiation and demonstrated that intermittent administration of low dose of hPTH (0.1 or 1 nM) could effectively enhance bone cell

### Table 2. Effect of parathyroid hormone on osteoblastic alkaline phosphatase (ALP) activity

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Number of Petri dishes</th>
<th>ALP activity (units/ng protein) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>0.097±0.022</td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>0.191±0.011*</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>0.289±0.017**</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01 significantly different from the control.
ANOVA followed by Dunn’s test.

### Table 3. Effect of parathyroid hormone on type I collagen synthesis in osteoblasts

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Number of Petri dishes</th>
<th>Type I collagen synthesis (%) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>11.2633±1.3522</td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>10.9648±1.0250</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>11.8215±1.5211</td>
</tr>
</tbody>
</table>

ANOVA followed by Dunn’s test.

### Discussion

Human parathyroid hormone (hPTH) is a linear polypeptide with a molecular weight of 9500 that contains 84 amino acid residues. It is synthesized and secreted by chief cells in parathyroid gland. PTH acts directly on bone to increase bone resorption and mobilize calcium. In addition to increasing the plasma calcium and depressing the plasma phosphate, PTH increases phosphate excretion in the urine. PTH also increases reabsorption of calcium in the distal tubules of the kidney, formation of 1,25 (OH) 2 vitamin D₃, and calcium absorption from the intestine. PTH is an active bone resorber by effectively increasing osteoclast formation and activity. In fact, early in 1976, Reeve demonstrated that low dose of PTH could increase trabecular bone mass in postmenopausal osteoporotic patients. Recent evidences showed that PTH was able to prevent postovariectomy osteopenia. An understanding of bone cell proliferation and differentiation would be greatly facilitated by an immortalized cell culture system that permits independent investigation of proliferation and differentiation of osteoprogenitors cells and differentiated osteoblast function. The MC3T3-E1 cells, derived from newborn murine calvariae, display a time dependent and sequential expression of osteoprogenitors cell and osteoblast characteristics analogous to in vivo bone formation, bone cell proliferation and differentiation, and have been widely used in our laboratory since 1996. In this study, we investigated the precise effect of hPTH on osteoblast differentiation and demonstrated that intermittent administration of low dose of hPTH (0.1 or 1 nM) could effectively enhance bone cell
differentiation by increasing osteoprogenitor cell proliferation and osteoblastic alkaline phosphatase activity while no obvious effect on type I collagen synthesis. Therefore, low dose hPTH could possibly act as a bone former (by enhancing osteoblastic differentiation and activity) in the prevention of estrogen deficiency-induced osteopenia or osteoporosis.

References

21. Shih C: Effects of interleukin-1 receptor antagonist and tumor necrosis factor binding...


副甲狀腺素增進造骨細胞分化的體外研究

史 中
國防醫學院 生物及解剖學研究所
三軍總醫院牙科部 口腔顱面外科

摘 要

年齡增長後，骨量(或骨質密度)的下降易造成骨質疏鬆的發生，進而增加骨折發生的機會。骨折後除了影響骨組織外，同時可能涉及周圍的軟組織，最後導致局部組織功能喪失甚至危及生命。由最新文獻得知，骨質疏鬆患者或動物若施予低劑量副甲狀腺素可以增加骨量。另外，副甲狀腺素可以增加皮質骨體積和預防卵巢切除後骨質流失的情形。但是，到目前為止，副甲狀腺素對骨中細胞(bone cell)分化的實際作用為何仍不清楚。因此，本研究旨在探討低劑量副甲狀腺素對造骨細胞分化的實際效應。研究中使用MC3T3-E1細胞，並藉由細胞培養系統、氫三放射性標定胸腺嘧啶評估法、鹼性磷酸酶活性評估法、第一型膠原蛋白合成評估法和骨組織形態學測量法及光源鏡檢來進行實驗結果之計量和分析。結果發現：與對照組比較，低劑量副甲狀腺素顯著地增加造骨細胞增生(p<0.01及0.005)和造骨細胞鹼性磷酸酶活性(p<0.05及0.01)，但對第一型膠原蛋白合成則無明顯影響。因此，本研究結果顯示，低劑量副甲狀腺素有效地增進造骨細胞分化。

關鍵語：骨質密度，造骨細胞，骨質疏鬆，副甲狀腺素。